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Preface

Dear Friends and Colleagues,

The World Congresses on Controversies in Obstetrics, Gynecology & Infertility (COGI) are designed as an umbrella for the ever-growing number of sub-disciplines of our profession and offer clinically oriented solutions to burning practical dilemmas.

The program is specially designed around debates on controversial issues in all fields of Obstetrics, Gynecology and Infertility, and is devised as a meeting forum for world experts from all branches of our profession. COGI provides the best forum for specialists who require detailed updates in the different fields.

The 18th COGI Congress’ Proceedings incorporate the major 3 disciplines that are represented at the Congress.

We would like to thank the authors and co-authors of the articles published in this Proceeding’s edition for their contribution in advancing solutions to problems that we are facing on a daily basis.

Prof. Zion Ben-Rafael
Congress Founder and Co-Chairperson
On behalf of the Organizing Committee
INFERTILITY AND ART
The Relationship between the Number of LH Receptor and the Success of Oocyte Maturity in the Process of in Vitro Maturation

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SUMMARY

Objective: To evaluate the relationship between the number of LH receptor and the success of oocyte maturity in the process of in vitro maturation (IVM).

Methods: Experimental study was conducted in the Permata Hati Infertility Clinical Laboratory, Dr. Sardjito General Hospital, Yogyakarta, Indonesia with 300 oocytes obtained through collecting immature bovine's oocytes from the abattoir and grouped the oocytes into three groups based on the pattern of oocyte cumulus cells on the vesicle germinal stage 2-8 mm with three layers of cumulus cell. Group A which has 100% of cumulus cells covered the oocytes, group B>50% and group C<30%. The number of LH receptor of each group of cumulus cells was examined by immunohistochemistry. The IVM process was performed to the three groups. Oocyte maturity was evaluated by finding the emergence of the first polar body of the three groups and related to the number of LH receptor.

Results: The cumulus cells showed a difference of oocyte maturity in IVM. The maturity rate showed that the number of LH receptor was related to the morphological pattern of oocyte cumulus cells. The maturity of the group A was higher than either group B and C, namely, 74% compared to 60% and 12%. The average number of LH receptors in group A, B, and C was 183.4, 78.8, and 24.0
respectively. A significant difference was found in the three groups (p<0.0001). The bigger number of cumulus cells contains more number LH receptors related to the oocyte maturity.

**Conclusion:** The number of LH receptor can be used as a predictor to determine the success of oocyte maturation in the process of IVM.

**Keywords:** Oocyte Cumulus Cell, LH Receptor, IVM.

**INTRODUCTION**

Ovarium stimulation product in this current IVF program can be replaced by taking the immature oocytes and conducting IVM. The main advantages of conducting the IVM are to prevent the risk of ovarium hyperstimulation, to minimize the cost and to reduce treatment complication [1, 2, 3].

IVM in the immature oocytes has been conducted in the super ovulation cycle, natural cycle, and PCOS patients [2, 5], 10-15% of the oocytes originally from the stimulated cycle are still immature and these oocytes can be processed through IVM to produce good mature oocytes [4].

It was previously reported that the oocytes with various morphologic patterns of oocyte cumulus cell were obtained at the time of oocyte taking, either in the super ovulation cycle, natural cycle or PCO patients, namely, the oocyte at vesicle germinal stage with oocyte cumulus cell pattern [6].

This occurred due to the angle of taking and the pressure of the vacuum during the ovum pick up.

In fact, various morphologic pattern of oocyte cumulus cell in IVM process produced different ability to the oocyte maturity. Currently, there are not many studies done on the oocyte cumulus cell morphology-related oocyte maturity. Oocyte maturity depends on the communication between follicular cell and the existence of FSH and LH receptors. Oocyte cumulus cells respond the FSH and LH and secrete various substances playing an important role in the nucleus and cytoplasm maturation. FSH is important for the development of invivo preovulation folicle and to induce LH receptors [4, 7, 8] GVBD is initiated by the pre-ovulation surge of gonadotropin hormone (LH). Many potential factors have become the mediator controlling the cumulus cell in GVBD. The great number of cyclic adenosine monophosphate (cAMP) and purin hypoxanthine in culture media prevents oocyte GVBD. Oocyte and cumulus cell are connected by gap junctions. The gap junction allows the regulator of molecules such as steroid, calcium ion, IP3 (inositol 1, 4, 5-triphosphate), cAMP, and purin to pass freely between oocyte cytoplasm and cumulus cell [4].

Addition of LH (luteinizing hormone) into the culture media induces GVBD.
The possibility of LH to induce GVBD is by an indirect action mediated by cumulus cells because LH receptor are not found in the oocyte [4]. The LH-involving mechanism induces the loss of communication between oocyte and cumulus cell that the flow of molecule regulator into the oocyte stops. The LH-induced GVBD may also be mediated by IP3/Ca$^{2+}$. This indicates that LH plays an important role in the further stage of follicle development, provides support for final maturation and dominant function of follicle, therefore, the existence of LH in the follicle before ovulation is an important contributor for the optimal follicle development which in the end produces healthy oocytes [4].

**MATERIAL AND METHOD**

Various morphologic patterns of cumulus cell and oocyte are estimated to be providing a different ability to oocyte maturity. After that, LH receptor in the oocyte cumulus cell is a determining factor for oocyte formation with optimal maturity. The experimental study was conducted in the Permata Hati Infertility Clinical Laboratory, Dr. Sardjito General Hospital, Yogyakarta, Indonesia with the samples of 300 oocytes.

The bovine’s immature oocytes used in this study were obtained from the abattoir and then grouped into 3 (three) groups based on the pattern of oocyte cumulus cell at the vesicle germinal stage of 2-8 mm with three layers of cumulus cell. Group A consisted of the cumulus cell which 100% covered the oocyte, Group B consisted of the cumulus cell which > 50% covered the oocyte, and Group C consisted of the cumulus cell which < 30% covered the oocyte. IVM was conducted to the three groups by using TCM plus HMG 0.1 IU/mL plus Bovine’s Folicel Fluid 10%.

To check the number of LH receptors in oocyte cumulus cell, 5 sample oocytes taken from the respective three patterns of oocyte cumulus cell were denuded through repeated mechanical suction by using pipette. Then, the LH receptor was examined by using immunohistochemistry (Figure 1) (Monoclonal Antibody, Termo Scientific).

The quality of oocyte maturity was valued 24 hours after the emergence of IPB. The maturity quality of the oocytes that had reached the MII maturity was evaluated and compared to the other groups and then related to the expression of LH receptors in the three groups. To obtain the valid data, data collection was done through documentation observation.

1. Observation in this study was a direct observation on the IVM procedures.
2. Documentation covered the data collection supporting the result of observation including the development of ovum of each group and image documentation.
The data collected were then processed and analyzed. The level of significance used $p$-value 0.05 (95%). Through univariate analysis, the data obtained were descriptively analyzed to show the characteristics of research population. Bivariate analysis was done to evaluate the relationship between 2 (two) variables, namely, independent and dependent variables. The analysis was done through stata 6.

**RESULTS**

This study was conducted based on the samples of 300 oocytes which were grouped into three groups based on the pattern of oocyte cumulus cell at the vesicle germinal stage of 2-8 mm with three layers of cumulus cell. Group A consisted of the cumulus cell which 100% covered the oocyte, Group B consisted of the cumulus cell which > 50% covered the oocyte, and Group C consisted of the cumulus cell which < 30% covered the oocyte.

The result of this study indicated that the oocyte cumulus cells showed a difference of function during IVM process. The maturity rate in this study showed that the number of LH receptor was related to the morphological pattern of oocyte cumulus cells with oocyte maturity. The maturity of the cumulus cells which 100% covered the oocyte was higher than that of the cumulus cells which > 50% and < 30% covered the oocytes, namely, 74% compared to 60% and 12% (Table 1). A significant difference was found in the three groups ($p < 0.0001$) (Table 2). The result of this study also showed that the average number of LH
receptors in the three groups (A, B, and C) was 183.4, 78.8, and 24.0 respectively (Table 3). When related to IVM maturity, this difference showed that the bigger number of oocyte cumulus cells influenced the oocyte maturity. In group A, the maturity of the cumulus cell which 100% covered oocyte was 74%. In group B, the maturity of the cumulus cell which > 50% covered oocyte was 60%. In group C, the maturity of the cumulus cell which < 50% covered oocyte was 12%. In this study, it can also be proven through immunohistochemistry that oocyte does not have LH receptor (Figure 2).

Tab. 1. Comparison of Oocyte Maturity (MII) of the Three Groups.

| Parameter | Group A | Group B | Group C | Total | p-value*
|-----------|---------|---------|---------|-------|---------
| MII       | 74      | 60      | 12      | 146   | <0.001 |
| Immatur   | 26      | 40      | 88      | 154   |         |
| Total     | 100     | 100     | 100     | 300   |         |

*p-value < 0.001 (very significant)
Percentage of MII in the Three Groups, A : 74%, B : 60%, C : 12%

Tab. 2. The Difference of LH Receptor of Oocyte Cumulus Cell of the Three Groups.

<table>
<thead>
<tr>
<th>Pattern of cumulus cells</th>
<th>N</th>
<th>X ± SD</th>
<th>95% CI for mean</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>5</td>
<td>183.40 ± 38.132</td>
<td>(134.81 ; 231.91)</td>
<td></td>
</tr>
<tr>
<td>Group B</td>
<td>5</td>
<td>78.80 ± 17.138</td>
<td>(57.52 ; 100.08)</td>
<td>0.0001*</td>
</tr>
<tr>
<td>Group C</td>
<td>5</td>
<td>24.00 ± 13.812</td>
<td>(6.7 ; 41.25)</td>
<td></td>
</tr>
</tbody>
</table>

*One way anova test was significant

Tab. 3. The Prediction of the Relationship of the Number of LH Receptor with Maturity Presentation.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of receptor LH</th>
<th>CI 95% Average number of average</th>
<th>Presentation of Oocyte Maturation</th>
<th>CI 95% the Percentage of oocyte maturation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X ± SD</td>
<td>upper and Lower</td>
<td>Upper and Lower</td>
<td>Upper and Lower</td>
</tr>
<tr>
<td>A</td>
<td>183.4 ± 38.132</td>
<td>134.81 and 231.91</td>
<td>74 %</td>
<td>65.3 % and 82.7 %</td>
</tr>
<tr>
<td>B</td>
<td>78.80 ± 17.138</td>
<td>57.52 and 100.08</td>
<td>60 %</td>
<td>50.3 % and 69.7 %</td>
</tr>
<tr>
<td>C</td>
<td>24.00 ± 13.812</td>
<td>6.7 and 41.25</td>
<td>12 %</td>
<td>5.6 % and 18.4 %</td>
</tr>
</tbody>
</table>
Fig. 2. There is no LH receptor in the oocyte.

CONCLUSION

The number of LH receptor can be used as a prediction to determine the success of oocyte maturation in the process of in vitro maturation.

REFERENCES

A Survey of Lymphocyteimmunization Therapy in Repeated Unexplained IVF Failure Patients from 2010-2012 of Sarem Women’s Hospital, Iran

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SUMMARY

The causes for repeated implantation failure (RIF) are various including immunologic abnormalities. Using immunotherapy, likes Lymphocyte Immunization Therapy (LIT) recommended for managing this condition. LIT is a procedure whereby white blood cells from the prospective father are injected intradermal to the prospective mother to prepare the maternal immune system in the development of immunologic tolerance to the genetically foreign pregnancy tissues. This non-experimental study performed to estimate the crude effect of LIT procedure. This study was performed on the 61 cases that have history of multiple (≥ 2) prior IVF failures, over the past 2 years with unexplained infertility and have a negative WBC cross match for Anti-Paternal of Cytotoxic Antibody (APCA). LIT procedure initiated by taking 20 ml of blood from the fathers and immunization was done by the prepared lymphocytes in at least 2 times of 0.5 cc intradermal injection with 3 weeks intervals. APCA rate above the 30% was positive and then cases had been candidate for the subsequent IVF
cycle. Patients followed for their pregnancy by serum ß-HCG testing and the booster injection was done for ß-HCG+ ones in 1st trimester. A total of 61 studied patients have mean age of 34.4 ± 4.6 years and mean infertility duration of 8.07 ± 4.5 years. The pregnancy rate per LIT cycle was 31.1% (19/61), and among these pregnant patients; the «take home baby rate» was 84.2% (16/19). Based on this study it seems that, LIT procedure can be helpful optional treatment choices for the unexplained IVF failure women with negative WBC cross-match.

**Keywords:** LIT, Infertility, Implantation failure, Delivery success rate.

**INTRODUCTION**

Some couples fail repeatedly following the cycles of IVF, which named repeated implantation failure (RIF) or repeated unexplained IVF Failure. RIF is an important and problematic condition for couples in IVF cycles. RIF defined by more than 2 or 3 times unsuccessful IVF cycles [1] or more than 10 embryo transfers (ET) failure [2-5]. The causes of RIF are various including; reduced endometrial receptivity, embryonic defects or multifactorial causes. Overall, implantation failure (especially the unexplained form) could be attributed to immunologic abnormalities, for example the numbers of T cells subpopulations in the blood have been shown to correlate with outcomes [6, 7]. Local or systemic immunologic factors play an important role in reduction of the allo- nic blastocytes immune response so, implantation facilitated and pregnancy occurs. Immunotherapy procedures like Lymphocyte Immunization Therapy (LIT), recommended managing this condition [8-11] although it is controversial. LIT is a procedure whereby white blood cells from the prospective father are injected intradermal to the prospective mother to prepare the maternal immune system in the development of immunologic tolerance to the genetically foreign pregnancy tissues. In this study, repeated unexplained IVF failure patients are candidate for LIT and followed for the pregnancy outcomes to estimate the crude effect of this procedure.

**MATERIAL AND METHODS**

This non-experimental study was performed on the 61 cases that have history of multiple (>2) prior IVF failures, over the past 2 years (March 2010- March 2012) with unexplained infertility and have a negative WBC cross match for Anti-Paternal of Cytotoxic Antibody (APCA) as inclusion criteria. LIT procedu-
re initiated by taking 20 ml of blood from the fathers and immunization was done by the prepared lymphocytes in at least 2 times of 0.5 cc intradermal injection with 3 weeks intervals. Immunization condition was checked by WBC cross matching 2 weeks later for detecting the APCA. APCA rate above the 30% was positive (see Fig.1) and then cases had been candidate for the subsequent IVF cycle. Patients followed for their pregnancy by serum β-HCG testing and the booster injection was done for β-HCG+ ones in 1st trimester. Take home baby rate was the final outcome of our study. SPSS software was used for data analysis.

Fig. 1. Non-concentrate maternal serum cross-match with paternal lymphocytes.

RESULTS

A total of sixty-one studied infertile patients have mean age of 34.4 ± 4.6 years and mean infertility duration of 8.07 ± 4.5 years. Frequencies of the infertility are 46.2% and 53.8% for primary and secondary infertility, respectively 35.7% of the patients has male infertility. Mean of failures in past IVF processes in patients’ history was 2.95 ± 0.85 times. The frequencies of the other infertility causes are 19.6% and 4.9% for female infertility and PCO, respectively. Treated endometriosis seen in 20% (12/61) of cases that 31.25% (5/16) of them became pregnant. The pregnancy rate per LIT cycle was 31.1% (19/61), and among these pregnant patients; the «take home baby rate» was 84.2% (16/19). The live birth rate per LIT cycle or overall success rate by «take home baby rate» was 26.23% (16/61). The highest success rate based on beta-hCG; belong to group of 30-39 years old patients. There were no significant relationship between positive beta-hCG test and infertility duration, follicles numbers, previous FET and ET numbers. Out of 19 pregnant women in our study, 7 cases are 2nd day embryo, 6 cases are 3rd day embryo and 6 cases are
FET. Mean embryo number for transfer after LIT procedure are 2.17 (1-4 range) for fresh embryos and 1.5 (1-4 range) for frozen embryos.

CONCLUSIONS

This study examined the effectiveness of immunization and presents of anti-parental antibody in success pregnancy among women with history of recurrent implantation failure. In Kling et al. study, pregnancy rate is 39.9% per 1.5 transferred follicles in under 30 years old women and 16.9% for over 39 years old group [1]. The pregnancy rate after LIT in another study by Check et al. is 38.3% compare with 28.7% in non-interventional group [12]. According to our study results, the previous studies confirmed more IVF cycles, more therapy failure. So that, in the third cycle the success rate is to 22-50 percent reduced [2-5]. Based on this study it seems that, LIT procedure can be helpful optional treatment choices for the unexplained IVF failure women with negative WBC cross-match. These results could be confirmed by the RCT study for efficacy analysis of this treatment option in future.

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REFERENCES


Low-Level 45,X/46,XX Mosaicism up to 10% of Aneuploidy in Women Undergoing IVF Procedure Seems Not To Be Associated with a Higher Prevalence of Congenital Cardiovascular Disease and Thoracic Aorta Dilatation: a Prospective Cardiovascular Magnetic Resonance Study

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SUMMARY

We present results of cardiovascular magnetic resonance study (MRI) focused on prevalence of congenital cardiovascular disease and thoracic aorta dilatation in women with low-level sex chromosome mosaicism undergoing IVF procedure. There is a considerably higher prevalence of the congenital heart
defects in patients with Turner syndrome (TS), i.e. with the 45,X/46,XX karyotype, as being observed in a number of recent studies. The estimated maternal risk of rupture or dissection of the aorta in pregnancy in such individuals is 2% or higher and the risk of death during pregnancy is increased as much as 100-fold. Nevertheless, there is a lack of knowledge concerning the 45,X/46,XX mosaic forms, in particular the mosaicism below 10% of aneuploidy. The study group was comprised of 25 women with the low-level 45,X/46,XX mosaicism (ranging from 3.3% to 10%) who were from 2009 to 2013 referred to two reproductive medicine units because of infertility and were karyotyped. In accordance with the recommendation of the Practice Committee of the American Society for Reproductive Medicine for patients with Turner syndrome, all participants underwent, prior to the IVF procedure, a careful cardiovascular screening for the congenital cardiovascular disease, particularly for a bicuspid aortic valve (BAV) and coarctation of the aorta (CoA); and for the thoracic aorta dilatation (AoDil), including the non-contrast cardiovascular magnetic resonance study. No higher prevalence of risk factors for aortic dissection in women with the low-level 45,X/46,XX mosaicism without any noticeable features, but the infertility, was detected.

**Keywords:** Turner syndrome, aortic dissection, bicuspid aortic valve, aortic coarctation, pregnancy, thoracic aorta dilatation.

**INTRODUCTION**

Turner syndrome is caused by a complete or partial monosomy (i.e. mosaicism) for the X chromosome, by a major deletion of the X chromosome short arm or by the abnormalities of the X chromosome such as ring X chromosome and Xq isochromosome during the embryonic development.

The most characteristic features are short stature, premature ovarian failure and congenital cardiovascular defects [1]. Patients with TS are usually identified after birth or in their childhood by phenotypical features suggestive of this syndrome. Nevertheless, there is a large proportion of less severe phenotypes with mosaic karyotypes. Although the mosaic forms of TS have been recently more often properly diagnosed, the highly varied phenotype is liable for the fact that up to 30% of females with TS never get a correct diagnosis [2].

Since one possible reason for infertility is caused by a number of chromosomal abnormalities, it is now a common practice in most in-vitro fertilisation centres to perform the cytogenetic screening in patients seeking infertility treatment. Cytogenetic studies of female partners of couples enrolled in an ICSI programme have shown an unexpectedly increased incidence of abnormal
karyotypes ranging from 1.1 to 9.8% when cases with the low-level sex chromosome mosaicism were also included [3].

The cardiovascular disease seems to be one of the most common and clinically significant phenotypical symptoms in live born individuals with TS. Dissection or rupture of the aorta have been recognised as the major causes of premature mortality in adults. A known risk factor for dissection has been present in most cases, either a systemic hypertension or a predisposing cardiac malformation such as BAV, CoA or AoDil; or both. On the contrary, cases with no risk factors identified have occurred only rarely [4].

Congenital cardiovascular abnormalities affect approximately 50% of individuals with TS. Dilatation of the ascending aorta represents another clinically important cardiovascular risk factor affecting 25-30% of girls and women with TS.

Pregnancy in all probability represents an additive risk factor that has emerged only recently with the increasing number of TS women becoming pregnant through oocyte donation. The risk of death from aortic dissection or rupture, preeclampsia and its complications during the perinatal period in women with TS is approximately 2% [5].

There is no sufficient information available whether women with the low-level 45,X/46,XX mosaicism are at an increased risk of maternal cardiovascular mortality or not, and whether they should undergo a careful cardiovascular screening before getting pregnant. The frequency of congenital heart disease in these individuals has not been established yet.

**MATERIAL AND METHODS**

Our study group was recruited from women who had been karyotyped from 2009 to 2013 prior to having the assisted reproductive technologies performed at both FERTIMED, Infertility Centre in Olomouc and the Infertility Centre of Department of Gynecology and Obstetrics, University Hospital Olomouc.

Once having the low-level 45,X/46,XX mosaicism diagnosed, clinical and biological data were collected. The mosaicism was defined as low-level when the aberrant cell line constituted 10 % or less of all analysed metaphases. As the 45,X cell line is often associated with a single cell finding for other sex chromosomal abnormalities (e.g. 47,XXX), these latter were reported as well.

A routine cardiac evaluation including physical examination, blood pressure measurement, ECG and transthoracic echocardiography was completed at the Department of Cardiology, Department of Paediatrics, University Hospital Olomouc. Subsequently, all patients underwent the non-contrast cardiovascular magnetic resonance study before the IVF procedure. The goal of the magnetic
resonance study was to identify those individuals with risk factors for aortic dissection such as CoA, BAV and AoDil.

The non-contrast cardiovascular magnetic resonance study was done in all individuals. All examinations were performed on a 1.5 Tesla whole-body MR scanner (Magnetom Avanto, Siemens, Erlagen, Germany). Magnetic resonance imaging included breath-hold ECG-gated Cine sequences of the heart in standard cardiac planes, breath-hold ECG-gated Gradient Recalled Echo sequences and black-blood Turbo Spin Echo sequences of the thoracic aorta both in axial plane and «candy cane» view.

Aortic diameter measurements were systematically acquired by MRI on the axial slice at the level of the right pulmonary artery origin. Values considered to be normal were those ranging up to the 95th percentile indexed to body surface area. The upper normal limit of the proximal ascending aorta was defined as 2.0 cm/m² [6].

Aortic valve morphology was performed in all women by MRI study using the in-plane cine sequence. The BAV was determined as a partial or complete fusion of two cusps, with or without a central raphe, resulting in either a partial or complete absence of a functional commissure between the fused leaflets.

RESULTS

Our study group consisted of 25 women with the low-level 45,X/46,XX. The percentage of the 45,X/46,XX mosaicism in peripheral blood lymphocytes ranged between 3.3 and 10% (median 5%).

The age of the women involved was between 27.3 and 39.4 years (median 35.3 years). No participant expressed any characteristic phenotypical feature of TS such as short stature, low posterior hairline, low-set ears, high arched plate, webbing of the neck, multiple nevi or cubitus valgus. None of them underwent any growth hormone treatment in their childhood either. Further, all females had shown a complete spontaneous pubertal development with menarche. Out of these 25 patients, 21 women (84%) suffered from a primary infertility, 4 females (16%) had a secondary one.

Participants’ body height ranged from the 4th to 91st percentile (median 55th percentile), e.g. from -1,15 to +1,7 standard deviation (median +0,12 SD). There were none patients with BMI < 18.5 kg/m², i.e. underweight; 17 patients with BMI 18.5-24.9 kg/m² (74%), i.e. normal weight; 4 patients with BMI 25.0-29.9 kg/m² (17%), i.e. overweight; 2 patients with BMI 30.0 to 39.9 kg/m² (9%), i.e. obese; and none patients with BMI ≥ 40 kg/m², i.e. extreme obese. There was a median BMI 23,7 kg/m².
**Cardiovascular Magnetic Resonance Study Results**

Morphology of the aortic valve during MRI study was visualized in 24 out of 25 cases. One woman could not complete the MRI study due to claustrophobia, and she, therefore, underwent the thoracic aorta MRI study only. The morphology of her aortic valve seemed, nevertheless, to be tricuspid on echocardiography. In the remaining 24 patients, a normal tricuspid aortic valve was identified in 23 women by the magnetic resonance aortic valve study. A BAV without any stenosis or regurgitation was found in one woman.

The ascending aortic diameter normalised to body surface area (i.e. aortic size index – ASI) was below the 95th percentile in all 25 cases. A coarctation of the aorta was not detected in any individual.

**CONCLUSION**

In summary, this study reports the first prospective measure of the prevalence of both the congenital heart defects and dilatation of the thoracic aorta in women with the low-level 45,X/46,XX mosaicism up to 10% of aneuploidy evaluated before the IVF procedure. With the aim to guarantee the sensitivity of the cardiovascular screening, the non-contrast cardiovascular magnetic resonance study was used in accordance with the recommendation of the Practice Committee of the American Society for Reproductive Medicine [7]. Nevertheless, no statistically significant higher prevalence of the above-mentioned risk factors for aortic dissection during pregnancy was proved in our study group comprised of women with the low-level 45,X/46,XX mosaicism.

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**REFERENCES**

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SUMMARY

A direct influence of FSH on bone metabolism is discussed [1]. This observational study sought to characterize the effects of therapeutic doses of GnRH on female bone metabolism. Furthermore ovulatory and anovulatory cycles were compared.

Keywords: FSH, bone resorption, GnRH, bone markers, ovulation, IVF, bone turnover.

INTRODUCTION

Osteoporosis is one of the ten most important illnesses worldwide. In 1946 E. C. Reifenstein jr. and F. Albright were the first to show the positive effects of estrogen on bone [2]. Today we know that one of the strongest risk factors for osteoporosis is the lack of estrogen, which occurs in postmenopausal women. But estrogen does not seem to be the only sex hormone influencing bone metabolism. A direct effect of FSH on osteoclasts is under discussion since 2006, as it could be observed that ovarectomised rats experienced more bone loss than ovarectomised and hypophysectomised rats [1]. FSH with or without
LH is used in therapeutic dosages for IVF. This lead to the question whether FSH stimulates bone resorption in women undergoing gonadotropin-therapy for assisted reproduction.

**MATERIAL AND METHODS**

The participants of this prospective observational study were recruited in a university-affiliated IVF-center. After informed consent, information on the patients’ personal and family history, cycle data and lifestyle was documented. Inclusion criteria were initial FSH under 20 mIU/ml and age between 30 and 45 years. Serum samples for bone markers and hormonal values of women undergoing controlled oocyte stimulation were collected on up to four pre-specified visits per patient: T1 = second half of the preceding cycle, T2 = beginning of the stimulation cycle, T3 = oocyte retrieval day, T4 = luteal phase of the stimulation cycle. Serum bone markers collected to describe bone metabolism were bone alkaline phosphatase (BAP) and osteocalcin to indicate bone formation, as well as c-terminal peptide (CTX) and tartrate-resistant acid phosphatase to indicate bone resorption. Samples were taken in the morning to avoid differences caused by circadian rhythms, cooled at 4 degrees Cesius and processed within 12 hours. The analysis subsequently described is based on long protocol cycles. GnRH analogues were used for down-regulation before stimulation start and during stimulation. A combination of FSH and HMG or FSH alone was given for stimulation, starting around day three in the menstrual cycle.

**RESULTS**

Out of 100 participants 59 received long protocol treatment and completed all necessary visits per cycle. The presented analysis is based on these 59 cycles. Average age was 38 years, average vitamin D3 was 20,5 ng/ml and average serum calcium 2,4 mmol/L. At T1 27 participants were ovulatory and 32 participants anovulatory which was defined by T1 progesterone ≤ 6 ng/ml. Mean age of participants with an anovulatory cycle was comparable to those with an ovulatory cycle, average vitamine D 3 levels were slightly higher in anovulatory cycles (22,4 ng/ml) than in ovulatory cycles (18,1 ng/ml). Down regulation between T1 and T2 took 13 days on average in both groups.

We compared initial values of bone markers and sex hormones between ovulatory and anovulatory cycles at visit T1. All bone markers were slightly lower in ovulatory cycles than in anovulatory cycles, CTX showed nearly significant lower levels in ovulatory cycles (p = 0,055). While estrogen was compa-
Fig. 1. Boxplots for FSH (left) and the bone resorption marker c-terminal telopeptide/CTX (right) at baseline (T1, during the second half of the natural cycle) in ovulatory (n = 26) vs. non-ovulatory cycles (n = 32). The cut-off for proof of ovulation was progesterone > 6ng/ml. Average patient age was 38 years, average serum vitamin D3 was 20.5 ng/ml. Both CTX (p = 0.055) and FSH (p = 0.002) were lower in ovulatory cycles.
Fig. 2. Change in FSH (left) and CTX (right) under GnRH-analogue down-regulation for an average of 13 days in initially ovulatory cycles (both upper diagrams) and initially non-ovulatory cycles (both lower diagrams). The cut-off for proof of ovulation was progesterone > 6ng/ml. Average patient age was 38 years, average serum vitamin D3 was 20,5 ng/ml.
FSH decreased between T1 (left bar in all diagrams) and T2 (right bar in all diagrams) in the anovulatory group (p = 0.027 for change), but remained higher (p = 0.144 for change) in previously ovulatory patients. No significant changes were seen for bone resorption during this time period as reflected by CTX (p = 0.9 for change in the group with formerly anovulatory cycles, p = 0.08 for change in the group with formerly ovulatory cycles).
rable in both groups (p = 0.867), initial FSH levels were significantly lower in ovulatory cycles (p = 0.002). The boxplots show the differences of initial FSH and CTX levels between the two groups [see Figure 1].

Furthermore the period of down-regulation with GnRH analogues from T1 to T2 was compared between the ovulatory and the anovulatory group. Estrogen values in T1 and T2 were comparable in both groups and fell significantly during the observed period due to GnRH influence (p = 0.000). FSH levels however showed significant differences both at T1 (p = 0.002) and during the following period (p = 0.007).

While the ovulatory group started with lower average FSH levels in T1 (median FSH 4.4 mIU/ml), FSH remained at median values of 5.05 mIU/ml in T2 (p = 0.144). The anovulatory group started with median FSH values of 6.3 mIU/ml which decreased to median T2 values of 4.85 mIU/ml (p = 0.027).

No significant differences were observed in the course of BAP (p = 0.962) and osteocalcin (p = 0.140) in the ovulatory group. In this group TRACP decreased from T1 to T2 (p = 0.004) and CTX tended to rise (p = 0.084).

In the anovulatory group osteocalcin (p = 0.680) and CTX (p = 0.904) remained stable from T1 to T2 while TRACP (p = 0.054) and BAP (p = 0.049) decreased.

Figure 2 shows the development of FSH and CTX between T1 and T2 for the ovulatory and the anovulatory group [see Figure 2].

No significant correlation could be found between CTX and FSH in either group. The course of osteocalcin correlated positively with the course of FSH in the ovulatory group (p = 0.021), while the courses of TRACP (p = 0.015) and CTX (p = 0.037) correlated positively with the one of estrogen in the anovulatory group.

CONCLUSIONS

The courses of FSH and CTX seemed to be associated but no positive correlation between both could be found. As osteocalcin is interpreted as a marker for bone formation in particular but as a marker for bone turnover in general [3], the positive correlation with FSH can be interpreted as an association between FSH and bone turnover. This significant correlation however is only seen in the ovulatory group.

The positive correlation between estrogen and bone resorption markers does not correspond to the common comprehension of estrogen effects. This leads to the suggestion that confounding variables have to be recognized and their impact has to be considered. Further analyses are under way.
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REFERENCES

Comparative Study Between the Differentiation Abilities of Placental Derived Stem Cells and Wharton Jelly Derived Stem Cells

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SUMMARY

Objective: Compare the differentiation abilities of placental mesenchymal stem cell (MSC) and Wharton Jelly-MSC (WJ-MSC) under different culture conditions using endometrial conditioned medium (ECM), follicular fluid (FF) medium and medium containing both.

Background: The differentiation of Placental and WJ-MSC to glandular cells could help treatment of endometrial atrophy and improve implantation rates in IVF as it might improve endometrial receptivity when stem cells are deposited intra-uterine through an IUI catheter if differentiation of Placental and WJ-MSC succeeded to form glandular cells that express progesterone receptors.

Materials and Methods: Placental and umbilical cord samples were collected during elective cesarean section provided there is no chorioamnionitis and no meconium staining. MSC were isolated and incubated in three culture conditions; ECM, FF medium and medium containing both.

Main outcome measures: The differentiation ability of placental derived MSC and WJ-MSC under different culture conditions was examined through morphological assessment and appearance of glandular pattern and progesterone receptors expression and the proliferative ability of MSC was examined by glandular cell count.

Results: This study shows that Plac-MSC and WJ-MSC can differentiate to
cells showing glandular pattern that express progesterone receptors. The results showed that Plac-MSC had higher proliferative potential than WJ-MSC, the appearance of glandular pattern in Plac-MSC was highly significant 100% compared to only 53.3% in WJ-MSC, Pg receptor expression in Plac-MSC was highly significant 90% compared to only 33.3% in WJ-MSC. The best culture medium was FF+ECM.

**Conclusion:** Placental-MSC is superior to WJ-MSC as regards its differentiation ability to glandular cells that express progesterone receptors. The culture conditions are best obtained with a combination of FF and ECM.

**Keywords:** placenta, Wharton Jelly, stem cells, glandular cells and progesterone receptors.

**INTRODUCTION**

Recently, there is enormous interest in stem cells (SC) as a new treatment modality for regenerative medicine. Embryonal stem cells is superior to fetal and adult stem cells regarding their proliferation and differentiation potential however obtaining them is rather controversial (1). Therefore stem cells obtained from available postnatal retrieved fetal adnexae (placenta, cord or membranes) is a good alternative as they are less immunogenic and their expansion potential is superior to that of adult stem cells which are usually obtained through bone marrow aspiration which is a rather invasive procedure (2).

The differentiation properties of fetal mesenchymal stem cells (fMSC) have been very well documented. They have adipogenic, osteogenic and chondrogenic abilities but differentiation into myocytes, neural-like cell and endothelial tissue have also been described (3).

Several recent findings in stem cell biology have resulted in new opportunities for the treatment of reproductive disease. Endometrial regeneration can be derived by bone marrow derived stem cells. This finding has potential implication for the treatment of uterine disorders as Asherman’s syndrome and diminished endometrial receptivity leading to impaired embryo implantation (4).

The aim of our study is to compare the differentiation abilities of placental-MSC and WJ-MSC under different culture conditions using: supernatant fluid from endometrial cell culture, follicular fluid (FF) and medium containing both.

FF was added to the culture medium because several studies revealed a pivotal role played by this fluid in the process of fertilization and implantation. At the time of ovulation, FF normally enters the fallopian tubes during ovum pick-up and it reaches the uterine cavity as well and many studies suggested a crucial role for this fluid in modifying uterine receptivity (5, 6, 7). Actually
Mitsunari study was the first to succeed in illustrating the expression of stem cell factor (normally present in FF) and its receptor c-kit in mouse embryos and in the stromal and epithelial cells of the uterine endometrium by reverse transcription-polymerase chain reaction (RT-PCR), he revealed that it exerts paracrine and autocrine action on the process of implantation by stimulating trophoblast outgrowth through its receptor c-kit (8). Therefore, FF was added to the culture medium in our study to promote the differentiation of MSC.

MATERIALS AND METHODS

A. Human Tissues Sampling

1. Placental & umbilical cord tissue samples
   10 placental tissues samples and 10 umbilical cord segments of 5-10 cm were collected during elective cesarean sections from normal full-term deliveries provided there is no chorioamnionitis and no meconium staining.

2. Endometrial samples
   Endometrial tissues were collected by curettage of fresh hysterectomy specimens from ovulating women undergoing hysterectomy for multiple fibroids with no endometrial pathology.
   All the tissues were collected under complete aseptic conditions in sterile cups in 0.9% normal saline and sent directly to the lab to be processed within 24 hours.

3. Follicular fluid sample
   Follicular fluid left over after ovum retrieval following ovum pick-up during IVF cycles was collected in sterile tube instead of being discarded (2 samples each about 5cc were used).
   Only follicular fluid aspirate that was transparent without blood contamination was used.
   Informed consents were obtained from the patients before tissue sampling and follicular fluid collection and the protocol has been approved by the research approval committee of the department of obstetrics and gynecology at Cairo university.

B. Methodology

1. Isolation of MSCs
   Isolation of MSCs from placental tissue and Wharton Jelly was done using the method of Chen et al (2009). Under complete aseptic conditions, placental
tissue and WJ were cut into small pieces and trypsinized by the addition of 0.05% trypsin-EDTA for 30 minutes at 37°C. The action of trypsin was stopped by the addition of 0.5 ml fetal bovine serum (FBS). The tissue was filtered using 9 mm cell strainer. The resultant cell suspension was washed twice with phosphate buffered saline. Mononuclear cells were separated using density gradient centrifugation. The resultant mononuclear cells were seeded in T25 flasks with the addition of DMEM (Dulbecco’s modified essential medium), 10% FBS, 1% penicillin-streptomycin and 1% amphotericin. Cultures were incubated in CO2 incubators at 37°C for 5 days. Non-adherent cells were removed and medium replenished twice weekly. Cultures were observed under inverted microscope until 80% confluence was reached. MSCs were harvested by trypsinization using 0.05% trypsin-EDTA for 30 min. The resultant cells were washed and characterized using immunophenotyping and trilineage differentiation potential.

2. Preparation of conditioning media

2.1 Preparation of Follicular Fluid (FF) Conditioned Medium

Follicular fluid was centrifuged and the pellet containing cell aggregate was separated and the follicular fluid was left over with the growth factors, cytokines and hormones only.

2.2 Preparation of Endometrial Conditioned Medium

Endometrial Tissue was dispersed by pipetting and subjected to collagenase digestion for 30 minutes at 37°C. The tissue was washed twice using phosphate buffered saline then inoculated in liquid culture system using DMEM, 20% L-Glutamine, 20% Fetal calf serum, 10% penicillin-streptomycin, 5% amphotericin. Cultures were incubated in CO2 incubator at 37°C for 7 days. Culture supernatant was collected, aliquoted and kept at -4°C.

2.3 Preparation of Endometrial and FF Conditioned Medium

Follicular fluid and the supernatant fluid of endometrial cell culture were collected and used for transdifferentiation.

3. Differentiation Protocols

MSCs isolated from both placenta and Wharton Jelly were plated at a density of 500,000 cells/plate. MSCs were subjected to culture in T25 flasks using complete medium as above in addition to one of the following differentiation media:

- Follicular fluid medium or endometrial conditioned medium (ECM) or both (FF+ECM).

C. Evaluation

Morphological: Cultures were examined for the appearance of glandular structures, clusters of cells or tubules.
Progesterone receptor evaluation: Immunohistochemical detection of progesterone receptor was done using peroxidase labeled anti-human progesterone receptor antibody. This was done twice after MSC isolation and then repeated after differentiation of MSC.

Proliferative response: Cells were counted and fold increase in cell count was calculated.

**D. Statistical method**

Using Microsoft Excel 2003 and SPSS v18.0 for Microsoft Windows 7, the clinical and laboratory data were statistically analyzed to obtain:

1. Descriptive statistics: a. \( \bar{x} \) = mean; b. SD = Standard deviation.
2. Analytical studies. Comparisons between means using: Anova test for comparison between quantitative data. Chi-square test for comparison between qualitative data. P value indicates significance when \( P < 0.05 \) and highly significant when \( P < 0.01 \) (Bland, 2000 and Kirkwood, 2003).

**RESULTS**

After isolation of MSC, progesterone receptor expression was assessed and no receptors were found. Then, the obtained mesenchymal cells were cultured in 3 different media; FF medium, ECM and both. The MSC obtained from each of the 10 placental tissue samples and the 10 umbilical cord samples were divided into 3 to be cultured under 3 different culture conditions. So we analyzed 30 samples of Placental derived MSC and 30 samples of Wharton Jelly derived MSC. After differentiation; progesterone receptors were assessed and were found in 90% of differentiated Placental derived MSC and in 33.3% of differentiated Wharton Jelly derived MSC.

**Comparison between WJ-MSC and Placental-MSC regarding proliferative potential, appearance of glandular pattern and Pg receptor expression in all 3 media:**

- Placental-MSC showed highly significant proliferative potential (2 folds increase in cell count) compared to WJ-MSC (1.3 folds) as shown in table 1.
- Appearance of glandular pattern in Placental derived MSC was highly significant (100%) compared to only (53.3%) in WJ-MSC as shown in table 1.
- Progesterone receptor expression in Placental -MSC was highly significant (90%) compared to only (33.3%) in WJ-MSC as shown in table 1.
Comparison between different media regarding proliferative patterns in MSC:

WJ-MSC showed highly significant proliferative patterns when cultured in FF + ECM media and FF media rather than endometrium conditioned media (ECM), while Placental-MSC proliferative pattern in all three media was insignificantly different as shown in table 2.

Comparison between different media regarding appearance of glandular pattern in MSC:

- Appearance of glandular pattern was more significant in WJ-MSC cultured in FF + ECM (80%) and FF (70%) compared to endometrium conditioned media (10%) as shown in table 3.

- 100% of Placental-MSC showed glandular differentiation in all 3 culture media. However, the glandular pattern was more prominent in FF+ECM than FF media and ECM as shown in table 3 and figure 1.
Comparison between different media regarding Pg receptor expression in each type of MSC:

- Pg receptor expression was more significant in WJ-MSC cultured in FF + ECM (50%) and FF conditioned medium (40%) than those cultured in ECM (10%) as shown in table 4.
- Pg receptor expression in Pl-MSC was 100% in FF + ECM and FF conditioned media compared to 70% in ECM as shown in table 4.
Fig. 1.

Appearance of glandular pattern in differentiated Wharton Jelly - MSC

Appearance of glandular pattern in differentiated Placental - MSC
This study showed that Placental-derived MSC and Wharton Jelly-MSCs can differentiate into mesenchymal lineages and be induced to form glandular cells positive for progesterone receptors. This was expected as fibroblasts from term human decidua closely resemble endometrial stromal cells by induction of prolactin and IGFBP1 expression. Decidual fibroblast may be considered to have properties similar to those of human endometrial stromal fibroblast because of their endometrial niche (9).

Our study showed that the best culture media was FF+ECM. This was expected as the follicular fluid contains growth factors that enriched the media, increased the proliferation of the glandular cells as evident by the increase in cell count and increased the receptivity as evidenced by the increase in Pg receptor expression. This agrees with Somigliana study who reported the ability of follicular fluid to stimulate endometrial cell proliferation (10).

Our results appear to be consistent with the observation by Xiaoqing Yang et al who reported that MSCs derived from WJ of the human umbilical cord can ameliorate damage to human endometrial stromal cells (ESCs). They cultured ESCs with mifepristone, a potent progestational antagonist used for medical termination of pregnancy and emergency contraception and known to cause
endometrial damage. Then the damaged ESCs were co-cultured with WJ-MSCs. They noticed that the proliferation of these damaged cells was significantly increased and apoptosis percentage decreased (11).

Another case report study made by Gargett and Healy of a patient with severe Asherman’s Syndrome where intrauterine administration of bone marrow stem cells regenerated her endometrium sufficiently to support a pregnancy. Ultrasound monitoring of the endometrial thickness and Doppler measurements indicated intraendometrial vascularity. This resulted in a successful IVF pregnancy but questions arise on the mechanism involved in regenerating the endometrium. Did the bone marrow cells incorporate into endometrium and trans-differentiate into endometrial epithelium or did the bone marrow cells provide trophic factors that promoted angiogenesis, prevented apoptosis of remaining endometrial cells? (12, 13). Our study could answer these questions as it proved for the first time that MSC could be isolated from placental tissue and WJ and these MSC can be made to differentiate to glandular tissue that express progesterone receptors abundantly and could proliferate in sufficient amounts for therapeutic purposes.

Our current study provided the proof that MSC can differentiate to glandular tissue positive for progesterone receptors which may be of benefit in endometrial regeneration. These data open the field of research for the potential of placental and WJ stem cells to be used in the treatment of uterine disorders as regeneration of the endometrium in asherman’s syndrome and also treatment of refractory cases of thin endometrium not responding to hormonal therapy. This finding might have potential implications in improving implantation rates in recurrent ICSI failure (14). All of this needs further studies which we recommend to assess the role of stem cells in treating such uterine disorders by injecting these differentiated stem cell in the endometrial cavity through an IUI catheter, these placental or Wharton jelly-MSC could be provided from the same patient if she had her placenta and umbilical cord cryopreserved at her birth for future use.

REFERENCES


First Trimester Aneuploidies Screening in Singleton Pregnancies Achieved by Intrauterine Insemination: a Case-Control Study

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SUMMARY

Problem statement: To evaluate whether achieving a singleton pregnancy by intrauterine insemination (IUI) affects the results of first-trimester screening compared to naturally conceived pregnancy.

The effect of Artificial Reproductive Techniques (ART) on first combined screening has been examined in several studies that have yielded contradictory and inconclusive results. All the most are referred to IVF or IVF/ICSI but there are only a few referred to IUI. The positive rate for Down syndrome would be higher in pregnancies achieved by IUI because nuchal translucency (NT) thickness is increased (only in some studies) and PAPP-A levels are lower. Other studies didn’t show any differences.

Methods: Retrospective case-control study. Pregnancies achieved by intrauterine insemination (partner or donor semen) and naturally conceived singleton pregnancies are compared between 2009 and 2012.
The study was performed at University-level hospital in Valladolid, Spain. Controls were selected according next criterias: same age, same gestation week at first ultrasound diagnosis and same parity than cases. Variables evaluated were crown-to rump length (CRL), NT, PAPP-A and free β-hCG maternal serum levels.

**Results:** Pregnancies achieved by IUI treatment have not showed differences in CRL, NT and PAPP-A or free β-hCG maternal serum levels. Positive biochemical risk (greater than 1/100) was higher in pregnancies achieved by IUI (13,5% vs 3,8%; OR 3,88 IC 95%: 0,76-19,69; p = 0,16) but not significantly. Positive combined risk (greater than 1/270) was similar in both groups (5,8% vs 3,8% OR 1,53; IC95%: 0,2-9,5; p = 0,5). Number of invasive procedures was similar in both groups (11,5%). No one Down syndrome was diagnosed after amniocentesis.

**Conclusion:** Our results indicate that the first trimester combined screening positive rate for Down syndrome is similar in pregnancies achieved by IUI than that for natural conceptions.

**Keywords:** prenatal screening, sterility, intrauterine insemination, Down Syndrome.

**INTRODUCTION**

Over the last three decades, prenatal screening has become an integrated part of antenatal care in developed countries. A 90% detection rate with a 5% false positive rate may be achieved by combining maternal age, two first-trimester

### Tab. 1. Comparison of first-trimester screening markers in IUI vs spontaneous pregnancies.

<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>N</th>
<th>Media</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CRL (mm)</strong></td>
<td>IUI</td>
<td>52</td>
<td>60,6731</td>
<td>6,82369</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>52</td>
<td>59,9308</td>
<td>7,76537</td>
</tr>
<tr>
<td><strong>NT (MoM)</strong></td>
<td>IUI</td>
<td>52</td>
<td>5,7535</td>
<td>2,7864</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>52</td>
<td>5,8000</td>
<td>2,8869</td>
</tr>
<tr>
<td><strong>Free βHCG (MoM)</strong></td>
<td>IUI</td>
<td>52</td>
<td>1,3588</td>
<td>1,03825</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>52</td>
<td>1,2433</td>
<td>1,01077</td>
</tr>
<tr>
<td><strong>PAPP-A (MoM)</strong></td>
<td>IUI</td>
<td>52</td>
<td>1,0342</td>
<td>0,50582</td>
</tr>
<tr>
<td></td>
<td>control</td>
<td>52</td>
<td>1,1220</td>
<td>0,55591</td>
</tr>
</tbody>
</table>
serum markers (pregnancy associated plasma protein-A (PAPP-A) and free β-human chorionic gonadotropin (β-hCG), and nuchal translucency thickness (NT) measurement [1].

The aim of prenatal screening program is to identify women at high risk of carrying a fetus with a chromosomal abnormality. Amniocentesis or chorionic villus sampling are used to diagnose fetal chromosomal aberrations; however these procedures carry the risk of miscarriage. Whether first trimester screening is influenced by mode of conception is a controversial issue. Several studies have found that serum markers levels, especially PAPP-A, seem to be altered in IVF pregnancies [2]. But there are very few publications about these items in IUI pregnancies. The purpose of our study is to assess the influence of ovarian stimulation and intra-uterine insemination (IUI) on the results of maternal serum Down syndrome (DS) screening.

MATERIALS AND METHODS

Retrospective paired case-control study

The study was performed at University-level hospital in Valladolid, Spain. Cases were 52 patients with pregnancies achieved by IUI (partner or donor semen) between June 2009 to June 2012. Controls were 52 patients with naturally conceived singleton pregnancies selected in the same period of time.

Fig. 1. Box plots show the MoM values of NT measurements in both groups.
Controls were selected according next criterias: same age, same gestation week at first ultrasound diagnosis and same parity than cases. Variables evaluated were crown-to rump length (CRL), NT, PAPP-A (MoM) and free β-hCG maternal serum levels (MoM).

Demographic data, ultrasound findings and the results of biochemical testing and combined risks were retrieved from our computerized obstetrical and laboratory records.

DS risk was calculated using PRISCA (Software of the Risk Calculation of Trisomy 21, 18 and Neural Tube Defects, Siemens Medical). Values of 1 in 100 or greater were considered to indicate high biochemical risk for DS and values os 1 in 270 or greater, high combined risk.

Statistical analysis of data was performed with SPSS v. 15.0 (SPSS Inc. 1989-2006). Values were compared between two groups using Student’s t-test categorical data were compared using $X^2$ and Fisher test. A $p$-value of $< 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The ultrasound and serum screening results of both groups are shown in Table 1. There were no significant differences between control and IUI group in fetal CRL, NT, PAPP-A and free β-hCG maternal serum levels (Figures 1-4).
Positive biochemical risk (greater than 1/100) was higher in pregnancies achieved by IUI (13.5% vs 3.8%; OR 3.88 IC 95%: 0.76-19.69; p = 0.16) but not significantly. Positive combined risk (greater than 1/270) was similar in both groups (5.8% vs 3.8% OR 1.53; IC95%: 0.2-9.5; p = 0.5). Number of invasive
procedures was similar in both groups (11.5%). No one Down syndrome was diagnosed after amniocentesis.

Previous studies have published differences in serum maternal markers between IUI pregnancies and natural conceptions on first and second trimester screening [3-5]. Most of them showed reduced PAPP-A levels and NT measurements greater in the IUI group. They suggested that controlled ovarian stimulation before conception, IUI and progesterone support may play an important factor influencing positive DS screening results [2, 6]. However, our results indicate the value of free ß-hCG and PAPP-A were not significantly different between the two groups. False positive rate using combined risk in DS first-trimester screening was lower than false positive rate using only biochemical markers.

CONCLUSIONS

Our results indicate that first-trimester combined screening for DS is similar in pregnancies achieved by IUI than that for natural conceptions. Previous publications showed different results, with PAPP-A levels lower in IUI group than in control group.

According to this study, pregnancies achieved by ovarian stimulation and IUI can be managed in the same way than spontaneous pregnancies, so we can use DS screening protocols without correction factors.

REFERENCES

Intrauterine Insemination: Predictive Factors for Pregnancy

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SUMMARY

Introduction: Intrauterine insemination (IUI) is still a valid procedure in couples with idiopathic sterility and anovulation. Male factor is debated. Woman’s age, etiology and sterility length, drugs for ovarian stimulation, number of motile sperm and type of catheter used have been widely discussed as predictive factors for this technique.

The aim is to determine the predictive factors for pregnancy after controlled ovarian hyperstimulation and intrauterine insemination. This study reports the experience on three years in a new reproduction unit.

This study reports the experience on three years in a new reproduction unit.

Methods: It’s a retrospective observational study. 544 IUI cycles in 203 couples were studied between 2009 and 2012. The study was performed at University-level Hospital in Valladolid, Spain.

IUI indications were: at least one permeable fallopian tube, an FSH level under 10 UI/L or antimullerian hormone more than 1 ng/mL, age below 38 years and motile sperm more than 5 million.

Predictive factors evaluated were female age, duration of sterility, cause, drugs used in ovarian stimulation, motile sperm and type of catheter.
**Results:** Woman’s mean age was 34 ± 2.91 years. Sterility length was 2.38 ± 1.13 years. More than 30% were unexplained sterility, 22% anovulation and 13% male factor.

Primary outcome measures were clinical pregnancy (14.34%) and live birth rates (11.58%). These results were compared with Spanish Society of Fertility dates (Registro SEF) and there were no significative differences. Among the predictive factors evaluated separately, woman’s age (< 31 vs ≥ 32 years, p = 0.002) and duration of sterility (< 2.5 vs > 2.5 years, p = 0.045) significantly influenced the clinical pregnancy rate. Multivariate logistic regression analysis was also used, but in this case, only woman’s age (< 32 years) was significative (OR: 2.30 IC95%: 1.30-4.07).

**Conclusion:** IUI with ovarian stimulation is an effective option in young women and short time sterility, and can be offered in patients with anovulation or unexplained sterility as first line treatment. Other situations will decrease success and should be considered when planning treatment.

**Keywords:** intrauterine insemination, predictive factors, pregnancy rate, ovarian stimulation, sterility.

**INTRODUCTION**

Intrauterine insemination (IUI) is an effective treatment for idiopatic sterility and anovulación [1]. In case of male factor infertility, IUI is a debated treatment [2]. Only in Spain 28,204 IUI (22,087 partner y 6,117 donor) had been performed in 2010 according to Spanish Fertility Society (SEF) register [3]. IUI is commonly accompanied by ovulation stimulation, which increase the pregnancy but markedly elevate the risk of multiple birth.

At present, it is generally admitted that IUI should be limited to four or six cycles and that IVF should be performed in the event of failure. The choice between IUI and IVF as the first-line treatment for couples, when the woman’s age and the duration of infertility were appropriate, is a major theme of debate between authors [4].

The aim of this retrospective study was to report on 3 years of IUI practice at Spanish public hospital and determine the predictive factors for successful pregnancy.

**MATERIAL AND METHODS**

Observational retrospective study between 2009-2012. We studied a total of
544 cycles in 203 couples. All IUIs were performed with partner semen. IUIs with donor semen were excluded from the present study. Clinical pregnancy rates were analyzed according to the woman’s age, the length of infertility, cause, the total motile spermatozoa count, drugs for ovarian stimulation (FSH or hMG) and type of catheter. Results were expressed as mean ± SD. Categorical variables were compared using a $X^2$-test, and continuous variables were analyzed using Student’s $t$-test. A $p$-value of $< 0.05$ was considered statistically significant.

**RESULTS AND DISCUSSION**

The clinical pregnancy rate per cycle was 14.34% (IC95%: 11.3-17.37). Accumulated pregnancy rate per couple was 35.96% (IC95%: 29.11-42.89). Ongoing pregnancy rate per cycle with «baby at home» was 80.5% (11.58% per cycle). Multiple pregnancy rate were 16.47% of total pregnancies. These results don’t show differences compared with Spanish Fertility Society (SEF) year 2010 register.

**Woman’s age**: the woman’s mean age was 34.1 ± 2.8 years. Woman’s age significantly influenced the clinical pregnancy rate at 23.9% for the under 32 versus 12.4% for the over 32 ($p = 0.004$). In multivariate logistic regression analysis woman’s age under 32 is the only variable statistically associated with occurrence of pregnancy (OR 2.31; IC95%: 1.3-4.0). Younger age is the strongest predictor of success in all indications and techniques (IUI or IFV) [5-8].

**Length of sterility**: another success factor for post-IUI pregnancy is the length of sterility. Nuojoa-Huttunen et al. [6] reported significantly differences when the length was below or above 6 years. Our results confirmed statistically differences on pregnancy rate per couple was the length of sterility was below or above 2.5 years: 40.3% vs 25.4% ($p = 0.04$). Other recent studies (Merviel at al. [8]) didn’t show differences. Most of protocols indicate to perform IVF if the length is over 4 years.

**Cause**: table 1 shows ethiological distribution in couples with and without clinical pregnancy. Most of success were anovulatory indications and unexplained sterility. A Cochrane Library review about IUI for unexplained subfertility [1] concluded that there is evidence that IUI improves the odds of becoming pregnant for couples with unexplained subfertility when combined with fertility drugs to induce ovulation.

In case of male factor sterility, we considered only the number of spermatozoa, and the results didn’t show any signifivative difference according to these parameters. The data outlined in a Cochrane Library review demonstrated that
for couples with male subfertility, the evidence is insufficient. No firm conclusions can be drawn about the efficacy of IUI with or without ovarian stimulation [2]. According to *ESHRE Capri Workshop Group* review in 2009 [9] stimulated IUI is ineffective in male infertility and the effect on other diagnoses is small.

**Drugs:** several investigators have demonstrated the superiority of FSH or hMG over clomiphene citrate alone [10]. Our study there were no differences on pregnancy rate between FSH or hMG. (14.6% vs 14%). GnRH antagonist were used for avoid weekends and were not considered in this study. Some reviews didn’t find advantages of using urinary or recombinants products [11]. In other Cochrane Library review in 2008, it is still not clear which stimulation protocol and which dose is the most cost-effective [12].

**Catheter:** when soft catheter is used, pregnancy rate was greater than using firm catheter (14.7% vs 11.9%) but without significative difference. Firstly, we use soft catheter in order to minimize risk of endometrial lesion, and in case of difficulty firm catheter is used. Van der Poel *et al.* revised this item and concluded that there was no evidence of a significant effect difference regarding the choice of catheter type for any of the outcomes [13].

---

**Tab. 1.** Ethiology of sterility in 203 couples.

<table>
<thead>
<tr>
<th>CAUSE</th>
<th>CLINICAL PREGNANCY AFTER IUI</th>
<th>NO CLINICAL PREGNANCY AFTER IUI</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNEXPLAINED</td>
<td>34 (46.6%)</td>
<td>52 (40.0%)</td>
<td>86</td>
</tr>
<tr>
<td>ANOVULATION</td>
<td>19 (26%)</td>
<td>23 (17.7%)</td>
<td>42</td>
</tr>
<tr>
<td>MALE FACTOR</td>
<td>8 (11%)</td>
<td>17 (13.1%)</td>
<td>25</td>
</tr>
<tr>
<td>FALLOPIAN TUBE FACTOR</td>
<td>4 (5.5%)</td>
<td>17 (13.1%)</td>
<td>21</td>
</tr>
<tr>
<td>MORE THAN ONE FACTOR</td>
<td>3 (4.1%)</td>
<td>9 (6.9%)</td>
<td>12</td>
</tr>
<tr>
<td>ENDOMETRIOSIS</td>
<td>2 (2.7%)</td>
<td>6 (4.6%)</td>
<td>8</td>
</tr>
<tr>
<td>ANATOMICAL FACTOR</td>
<td>2 (2.7%)</td>
<td>4 (3.1%)</td>
<td>6</td>
</tr>
<tr>
<td>LOW OVARIAN RESERVE</td>
<td>1 (1.4%)</td>
<td>2 (1.5%)</td>
<td>3</td>
</tr>
<tr>
<td>TOTAL</td>
<td>73 (100%)</td>
<td>130 (100%)</td>
<td>203</td>
</tr>
</tbody>
</table>
Cicle: figure 1 show pregnancies distribution per cycle. Major percentage was found in first and second cycles (42.3% and 34.62% of total of pregnancies). Differences between first-second cycles and third-fourth cycles on pregnancy rate were statistically significative (16.4% vs 10.1%, \( p = 0.04 \)). The best balance between cost and efficacy is found in the first three IUI cycles [8]. At present, it is generally admitted that IUI should be limited to four or six cycles and that IVF should be performed in the event of failure.

![Fig. 1. Pregnancies (number and percentage of total) distribution per cycle.](image)

**CONCLUSIONS**

In summary, we can say that couples with the best probability of pregnancy are those in which the woman is under 32, with a length of sterility under 2.5
years, suffers from anovulation or unexplained sterility and in the first or second cycle of IUI. In multivariate analysis, only woman’s age is a significant predictor factor.

REFERENCES


11. Bayram N vWM, van der Veen F. Recombinant FSH versus urinary gonadotrophins or recombinant FSH for ovulation induction in subfertility associated with polycystic ovary syndrome. *Cochrane Database of Systematic Reviews* 2008 Issue 3. UK.


Influence of Teratozoospermia on Intrauterine Insemination Outcomes


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SUMMARY

Although sperm morphology criteria are clearly defined, there are still slight differences among laboratories; moreover, the influence of teratozoospermia on the results of intrauterine insemination is not still clear. We have examined the influence of abnormal semen morphology on clinical pregnancy rate (CPR) in intrauterine insemination cycles with conjugal fresh semen, in 626 cycles between January-2011 and October-2012. We conducted two study groups, according to the severity of the teratozoospermia (cut off 4 %). Differences were statistically significant between the two groups. We have analyzed the results by diagnostic groups: male factor, unexplained infertility, ovulatory, and other factors. When the results were analyzed by diagnostic groups, only the «male factor» group proved similar results. According to our results, insemination with semen with severe teratozoospermia is not efficient and other options, such as ICSI, should be considered.

Keywords: Intrauterine insemination, teratozoospermia, male factor, male infertility, sperm morphology.

INTRODUCTION

Morphology is the most subjective variable within a semen analysis. In spite of clearly defined criteria that have been assessed in several guidelines pu-
blished by World Health Organization (WHO), the latest in 2010 [1], significant interpersonal and intra-laboratory variations can be still detected among different laboratories. [2] In our Assisted Reproduction Unit there are different treatment options available for couples with male infertility or subfertility, varying from natural methods to highly sophisticated ICSI techniques. Some authors have found that teratozoospermia can reduce pregnancy rates when using intrauterine insemination (IUI) techniques [3], but this point is still quite controversial as recent studies have not found significant differences in pregnancy rates between normozoospermic and teratozoospermic patients. [4] Albeit we usually don’t take into account morphology as criteria to determine what kind of therapy is offered to our patients, we have examined the influence of abnormal semen morphology on clinical pregnancy rate (CPR) in intrauterine insemination cycles with conjugal fresh semen.

**MATERIAL AND METHODS**

This study is a retrospective analysis of 626 stimulated cycles, performed on 190 couples between January-2011 and October-2012. Because it was a retrospective analysis of data available in our database, no specific approval of the institutional review Committee was required. Inseminations with donor sperm were excluded from the study. The final outcome was clinical pregnancy defined as visualization of gestational sac on ultrasound at 6-7 weeks. The abnormal morphology was evaluated using the strict Kruger/Tygerberg criteria in at least two semen samples per patient. We conducted two study groups, according to the recent recommendations made by WHO 2010 [1] for semen analysis:

**Group A:** insemination cycles performed with sperm from males whose semen sample showed less than 4% of normal forms.

**Group B:** insemination cycles performed with sperm from males whose semen analysis showed 4% or more of normal forms. Subsequently, we analyzed the results by diagnostic groups: male factor, unexplained infertility, ovulatory, and other factors.

Ovarian stimulation was conducted with clomiphene citrate 50 or 100 mg/day from day 5 to 10 or with hMG alone or in combination with FSH, (75 to 150 UI/day) from the second day of the cycle. Follicular growth was monitored by ultrasound and hCG (10000 UI) was administered when one or two follicles greater than 17 mm were seen. IUI was performed 36 hours after hCG injection. Sperm samples were analyzed, centrifuged in standard medium and selected with standard swim-up technique. Samples were subsequently incubated in IVF medium at 37°C and 5% CO₂. The insemination was performed using a Wallace catheter.
Data were collected using a database designed for the study with the licensed software Microsoft Access® 2007 (Microsoft, Redmond WA, USA) and statistical analysis was performed using free software EpiInfo 7, provided by CDC. Differences in pregnancy rate between groups were statistically evaluated by the Kruskall-Wallis test and $\chi^2$ test.

RESULTS

Both groups were homogeneous considering women age, number of follicles on the day of hCG injection, infertility diagnosis and difficulty of catheter insertion. We have analyzed both results per cycle and per couple. The CPR per cycle in A group was 4.5% vs. 8.9% in B group with an OR = 0.48 for a confidence interval (CI) of 95% (0.21-1.06), $p = 0.05036$. The CPR per couple in A group was 13.9% vs. 30.4% in B group. This represents an OR = 0.37 for a 95% CI (0.15-0.87). This result is statistically significant with a «$p$» value of 0.01211.

When the results were analyzed separating the distribution by diagnostic groups, only the «male factor» group proved similar results showing a CPR per cycle of 2.8% vs. 8.4%, $p=0.05878$. CPR per couple was 8.8% vs. 30.0% in B group, with a significant «$p$» value of 0.02024). The other diagnostics groups (unexplained infertility, ovulatory, and other factors) did not showed statistical differences between both group A and B.

Results are summarized in table 1.

Tab. 1. Teratozoospermia in conjugal insemination.

<table>
<thead>
<tr>
<th>Diagnostics</th>
<th>Cycles (n)</th>
<th>Couples (n)</th>
<th>Pregnancy (n)</th>
<th>CPR x cycle (%)</th>
<th>CPR x couple (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td></td>
<td>B</td>
</tr>
<tr>
<td>All</td>
<td>200</td>
<td>426</td>
<td>9</td>
<td>4.5*</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>125</td>
<td>38</td>
<td>8.9*</td>
<td>8.9</td>
</tr>
<tr>
<td>Male factor</td>
<td>107</td>
<td>178</td>
<td>3</td>
<td>2.8†</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>34</td>
<td>50</td>
<td>15</td>
<td>8.4†</td>
<td>8.4†</td>
</tr>
<tr>
<td>Unexplained</td>
<td>32</td>
<td>164</td>
<td>3</td>
<td>9.4</td>
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</tr>
<tr>
<td></td>
<td>9</td>
<td>50</td>
<td>14</td>
<td>33.3</td>
<td>28.0</td>
</tr>
<tr>
<td>Ovulatory</td>
<td>30</td>
<td>67</td>
<td>2</td>
<td>6.7</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>23</td>
<td>11</td>
<td>16.4</td>
<td>22.2</td>
</tr>
<tr>
<td>Others</td>
<td>18</td>
<td>30</td>
<td>1</td>
<td>5.6</td>
<td>11.1</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>6</td>
<td>1</td>
<td>3.3</td>
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</tr>
</tbody>
</table>

A: Normal morphology <4%; B: Normal morphology >4%. CPR: Clinical Pregnancy Rate. A vs. B: ' $p = 0.05036$; ' $p = 0.01211$; ' $p = 0.05878$; ' $p = 0.02024$. 

59
CONCLUSIONS

Intrauterine insemination (IUI) is frequently used as a first line strategy in the treatment of infertile couples because of its relatively low cost and simplicity. It has been used with variable success for the treatment of numerous indications in the infertile couples [5]. Several semen parameters have been evaluated as predictors of a successful outcome with IUI, such as total sperm count or total motile sperm count. [6]

When evaluating the influence of teratozoospermia on IUI outcomes, results are confusing and sometimes contradictory. There are studies that have not found differences between normal sperm and teratozoospermia not only on IUI outcomes [7] but also on «in vivo» fertilization rates [8]. But other studies concluded that intracitoplasmic sperm injection should be recommended in patients with teratozoospermia because teratozoospermia worsens significantly IUI outcomes [4, 9]

In our study we have not found significant differences when analyzing data without taking into account what was the infertility diagnosis. However, when we have considered only couples with «male factor» diagnosis, we found significant worse results in the teratozoospermia group.

Although we have not separated teratozoospermia from another sperm abnormalities such a low sperm count or low sperm motility, our results confirms that teratozoospermia is an important factor that must be considered when counseling about the most adequate type of reproductive treatment for a couple, especially when other alterations coexist in the semen. According to our results, we believe that insemination with semen samples that exhibit persistent teratozoospermia is not efficient and we should consider other options with more chance of success, such as ICSI.

REFERENCES


Ovarian Stimulation with Urofollitropin (uFSH) Results in a Lower Number of Oocytes Compared to Recombinant FSH (rFSH), Nevertheless, uFSH is at Least as Effective as rFSH: Preliminary Results of a Retrospective Study with Antagonist Cycles in an IVF/ICSI Program

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SUMMARY

Differences in the mode of action between recombinant FSH (rFSH) preparations and urinary derived FSH (uFSH) or hMG preparations have been reported in cycles down-regulated by GnRH-agonists. The aim of our study was to determine, if these differences also exist in cycles down-regulated by GnRH-antagonists.

GnRH-antagonist cycles performed between 2009-2012 were divided into two groups:

1. Cycles stimulated with rFSH preparations (n = 646);
2. Cycles stimulated with Urofollitropin (uFSH, n = 405).

Cetrorelix or Ganirelix were used as GnRH-antagonists. All patients received 75 IU hMG additionally from day 6 of stimulation onwards up to the day of hCG administration.
Taking into consideration factors evaluated by logistic regression analysis which made positive contributions (numbers of oocytes retrieved and embryos transferred) and negative contributions (age and IVF cycles-ever experienced) to predict pregnancy, it was possible to create comparable rFSH and uFSH groups for younger and older patients separately.

There were no differences in basic personal data and gonadotropin consumption between the groups. Stimulation with rFSH resulted in a significantly higher yield of oocytes compared to uFSH, however, the results of the following reproductive outcome parameters were all in favour of uFSH in both age-groups: oocyte maturation rate, fertilization rate, hCG-positive pregnancy rate, clinical pregnancy rate, implantation rate, embryo-cryopreservation rate and abortion rate.

These results support the concept that uFSH produces fewer oocytes than rFSH, but the oocytes produced by uFSH are of better quality than those produced by rFSH. Basic studies have shown that different FSH isoforms with different elimination kinetics in the two gonadotropin preparations could be responsible for this different effects.

Our preliminary results, based on a retrospective study, have to be confirmed, however, by well designed prospective randomized studies.

Keywords: Urofollitropin, uFSH, rFSH, FSH glycosylation, IVF.

INTRODUCTION

There is a highly-purified uFSH preparation (Fostimon; IBSA, Switzerland) that belongs to a new generation of urinary FSH preparations that have especially acidic glycosylation content. A number of advantages of this product over rFSH preparations have been reported so far, which we have summarized recently in the introduction of a publication including most of the now presented results [1]. Since these studies were all conducted using agonist cycles, and we predominantly use antagonist cycles, we were interested in determining whether the reported advantages of Urofollitropin could be achieved in antagonist cycles as well. Accordingly, this study reports a retrospective comparison of both rFSH preparations (Puregon®, MSD and Gonal-F®, Serono) with Fostimon®.

MATERIALS AND METHODS

Patients were selected for the study if they had undergone IVF/ICSI treatments in our clinic between January 2009 and December 2012. They were allocated to one of three groups by the physicians:
Group I = Follitropin-alfa (Gonal-F®) + Cetrorelix (Cetrotide®);
Group II = Follitropin-beta (Puregon®) + Ganirelix (Orgalutran®);
Group III = uFSH (Fostimon®) + Cetrorelix (Cetrotide®).

In addition to the different FSH preparations, all the patients received an injection containing 75 IU FSH and 75 IU LH (Merional®) daily from stimulation day 6 until the day of hCG administration. There was no randomization of patients, with doctors free to decide which treatment to prescribe.

Group I and Group II were aggregated to the rFSH-group which was compared with Group III, the uFSH-group.

A logistic regression analysis demonstrated that the predictors age of patients (p<0.001) and number of cycles ever performed (p=0.003) made negative contributions and number of oocytes retrieved (p=0.076) and number of embryos transferred (p<0.001) made positive contributions to prediction of hCG-positive pregnancy.

In a first step only first cycles-ever with 2 embryos transferred were analysed (figure 1). It is evidently shown that there were fewer oocytes in the uFSH-

![Fig. 1. First-ever IVF cycles, 2 embryos transferred. Comparison of the number of oocytes retrieved after rFSH or uFSH stimulation. In the group as a whole, significantly fewer oocytes were retrieved in the uFSH group compared to the rFSH group (p=0.009), however, the difference was not significant in younger patients between 25 and 35 years (p=0.272).](image-url)
group compared to the rFSH- group and the difference was – based on the whole sample – highly significant (p=0.009). However, considering only the younger patients up to 35 years, the difference did not reach significance (p=0.272).

Taking this into consideration, comparable groups could be created by including only first-ever cycles with more than 10 oocytes retrieved and 2 embryos transferred for patients < 36 years and cycles with > 6 oocytes retrieved and 2 embryos transferred for the age-group 36-42 years.

RESULTS

The comparison of rFSH with uFSH in both age groups didn’t show significant differences between the groups in terms of BMI, cause of infertility, duration of infertility, cigarette smoking and consumption of FSH and HMG dosages. And there were no significant differences in the results either, however, it was remarkable, that even though in the uFSH-group age was somewhat

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>rFSH group</th>
<th>uFSH group</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (X ± SD)</td>
<td>33.1 ± 4.9</td>
<td>33.7 ± 5.1</td>
<td>.559</td>
</tr>
<tr>
<td>Oocytes (X ± SD)</td>
<td>15.6 ± 3.9</td>
<td>14.4 ± 2.9</td>
<td>.171</td>
</tr>
<tr>
<td>Mature (MII) Oocytes (%)</td>
<td>76.5 ± 16.3</td>
<td>79.0 ± 13.6</td>
<td>.485</td>
</tr>
<tr>
<td>Fertil. 2 PN (%)</td>
<td>53.6 ± 17.4</td>
<td>58.6 ± 21.1</td>
<td>.211</td>
</tr>
<tr>
<td>Fertil. 2 PN ICSI (%)</td>
<td>68.9 ± 16.1</td>
<td>72.4 ± 21.9</td>
<td>.394</td>
</tr>
<tr>
<td>Embryo Score 4 (%)</td>
<td>25.7 ± 22.4</td>
<td>31.1 ± 28.3</td>
<td>.299</td>
</tr>
<tr>
<td>Endometrial thickness (X ± SD)</td>
<td>11.0 ± 2.3</td>
<td>11.0 ± 1.3</td>
<td>1.00</td>
</tr>
<tr>
<td>Pregnancy rate (%)</td>
<td>43.9 ± 49.9</td>
<td>59.3 ± 50.1</td>
<td>.159</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>19.9 ± 30.2</td>
<td>29.6 ± 39.9</td>
<td>.171</td>
</tr>
<tr>
<td>Clinical pregnancy rate (%)</td>
<td>33.7 ± 47.5</td>
<td>44.4 ± 50.6</td>
<td>.306</td>
</tr>
<tr>
<td>Abortion rate (%)</td>
<td>14.0 ± 35.1</td>
<td>12.5 ± 34.2</td>
<td>.887</td>
</tr>
<tr>
<td>Cryopreserved embryos (%)</td>
<td>19.3 ± 24.7</td>
<td>30.5 ± 42.8</td>
<td>.085</td>
</tr>
</tbody>
</table>

Tab. 1. Results of antagonist protocols for first-ever IVF cycles with > 10 oocytes retrieved and 2 embryos transferred, stimulated with either rFSH or uFSH.
higher and the number of oocytes somewhat lower compared to the rFSH-group, the rate of mature oocytes, the fertilization rate, the rate of embryos score 4, the hCG-positive pregnancy rate, the clinical pregnancy rate, implantation rate and the rate of cryopreserved embryos were all higher in the uFSH-group, and the abortion rate was lower compared to the rFSH-group (tables 1 and 2).

| Tab. 2. Results of antagonist protocols for IVF cycles in patients aged 36-42 years with > 6 oocytes retrieved and 2 embryos transferred, stimulated with either rFSH or uFSH. |
|--------------------------------------------------|------------------|------------------|------------------|
| Patients (n)                                      | rFSH group       | uFSH group       | P               |
| Age (X ± SD)                                      | 38.4 ± 1.6       | 38.8 ± 1.9       | .159            |
| IVF cycles-ever                                   | 2.0 ± 1.4        | 2.4 ± 1.6        | .115            |
| Oocytes (X ± SD)                                  | 11.9 ± 4.6       | 11.0 ± 3.5       | .148            |
| Mature (MII) Oocytes (%)                          | 78.0 ± 15.6      | 79.3 ± 16.4      | .628            |
| Fertil. 2 PN (%)                                  | 57.7 ± 16.3      | 58.6 ± 21.1      | .211            |
| Fertil. 2 PN ICSI (%)                             | 73.6 ± 17.8      | 75.8 ± 19.8      | .453            |
| Embryo Score 4 (%)                                | 23.7 ± 26.0      | 26.4 ± 27.0      | .504            |
| Endometrial thickness (X ± SD)                    | 10.7 ± 2.5       | 10.0 ± 1.9       | .054            |
| Pregnancy rate (%)                               | 35.2 ± 48.0      | 37.5 ± 48.8      | .762            |
| Implantation rate (%)                            | 13.7 ± 24.2      | 17.5 ± 29.1      | .357            |
| Clinical pregnancy rate (%)                       | 24.6 ± 43.2      | 26.6 ± 44.5      | .770            |
| Abortion rate (%)                                 | 23.6 ± 42.7      | 12.5 ± 33.8      | .293            |
| Cryopreserved embryos (%)                         | 12.8 ± 19.8      | 14.5 ± 32.3      | .670            |

**CONCLUSION**

We are well aware of the fact that a retrospective study is always distorted by confounding factors. In our previous publication we found good arguments against possible confounding influences of the facts that two different antagonists and two different rFSH preparations have been used and additional HMG was given from day 6 onwards [1].
In contrast to the mentioned factors, however, there are indeed powerful confounding parameters, as age, number of previous cycles, number of embryos transferred and number of oocytes retrieved. By taking these into consideration we finally succeeded in creating comparable groups (tables 1 and 2). Although no significant differences were found between the rFSH and uFSH group, it is worth noting, that the mean values of all the reproductive parameters were in favour of the uFSH group, thus supporting our conclusion that uFSH produces fewer oocytes but oocytes with a better quality compared to rFSH. These findings are consistent with the results of a study where highly purified menotropin (hphMG), was compared with rFSH in antagonist cycles with compulsory single blastocyst transfer [2].

According to ample basic research, this mode of action is now mainly attributed to the fact that uFSH is more glycosylated and more acidic than rFSH [3-6]. However, the clinical results should be confirmed by well designed prospective randomized studies.

ACKNOWLEDGMENTS

The authors thank Prof. Dr. Anselm Eder, Department of Statistics, Institute of Sociology, University of Vienna, Austria, for supervising the statistical analysis. The authors also cordially thank Dr. Sharon Mortimer, Oozoa Biomedical Inc, Canada, for her critical contributions and her linguistic support in preparing the manuscript.

REFERENCES

1. Kemeter P, Stroh-Weigert M, Feichtinger W. Ovarian Stimulation with Urofollitropin (uFSH) Results in a Lower Yield of Oocytes Compared to Recombinant FSH (rFSH), Nevertheless, uFSH is at Least as Effective as rFSH in Younger Patients: Preliminary Results of a Retrospective Study with Antagonist Protocols in an IVF/ICSI Program. The Open Reproductive Science Journal, 2013; 5, 1-16.
Massive Vulvar Edema in a Patient with Hyperstimulation Ovarian Syndrome

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ABSTRACT

Vulvar edema is not uncommon in pregnancy, but massive isolated vulvar edema, accompanying a hyperstimulation ovarian syndrome (HOS), is a rare situation and could be due to lymphatic stasis from compression or hypoproteinemia. We are reporting a patient with massive vulvar edema, due to lymphatic stasis from compression, which was resolved with correction of clinical parameters of HOS.

Keywords: edema, vulva, HOS, lymphatic stasis, adnexectomy.

INTRODUCTION

Clinically detectable edema may be observed in majority (80%) of pregnant women, mainly at the third trimester of pregnancy (1). Although isolated vulvar edema is an extremely rare entity, it may be associated with edema at other places in women with an underlying systemic disease (2). Due to presence of abundant loose of areoler tissue in this region, vulvar edema at times may be out of proportion to the edema at other sites and may become the presenting feature. We are reporting a patient, after IVF, with massive vulvar edema, due to lymphatic stasis from compression, which was resolved with correction of clinical parameters of HOS.
CASE REPORT

A 25 year old woman, came at the Department of Gynecology of our University Hospital for Obstetrics and Gynecology «Koço Gliozeni» Tirana, Albania on 08.11.2011. She refers an extreme distension of abdomen, accompanied by abdominal pain, massive vulvar edema and difficulty in walking due to vulvar edema. The patient had performed an IVF procedure and had done the transfer of 2 embryos on 29.10.2011. On her history she had performed some years ago a right adnexectomy for ovarian cyst and later on a laparoscopy for adherentiolisis. Four days after transfer of embryos displayed a rapid, progressive edema of vulva, more developed on the left side, corresponding with the left hyperstimulated ovary side. The patient was hospitalized with diagnosis of HOS, moderate forme, and underwent relevant examinations.

**Abdominal Ultrasound.** Uterus with normal size. Great multifollicular left ovary, measured 152 x 121mm. There is abundant free liquid in abdomen.

**Vaginal examination.** Examination of vulva revealed a non-inflammatory pitting, edematous swelling of labia majora and minora extending to mons pubis and reaching to the level of upper one third and lower two third of thighs. Examination of vagina and cervix did not reveal any abnormality. The left ovary was as big as a grapefruit, painful and with limited movement. There was no regional lymphadenopathy, vein varicosis or signs of deep vein thrombosis. There was not any edema of lower extremities.

Complete blood count showed hemoglobin of 10.7 gm%. Renal and hepatic function was normal. Total serum proteins (7 gm/dl). The urine analysis, blood sugar, serum calcium and hemoglobin electrophoresis were normal. Potassium hydroxide and a wet mount preparation of vaginal smears were also normal. \( \beta \)-hCG = 727 mUI/ml and after two days was 4274 mUI/ml, so we confirmed that the patient was pregnant. Patient was treated with Human Albumin for 3 days, Utrogestan 3x2 tab/oral and Calciparine 1250 UI 2x1/2 amp s/c for 7 days. Patient was recommended to follow a hydric diet about 3 liter liquids per day as well as bed rest from right side. Diuresis was monitored daily (see tab. 1). Topical cream with hialuronic cream and lukewarm water was applied against dermal irritation. The patient was kept under observation for 10 days. Edema resolved gradually together with clinical parameters (see fig. 1, 2 and 3).

**DISCUSSION**

Massive vulvar edema in pregnancy is unusual and a cause for concern. Total body water increases to the tune of 6-8 liters in normal pregnancy, two-third of which is extracellular (1). Any change in factors controlling renal sodium and
Tab. 1. Diuresis of 24 hours.

<table>
<thead>
<tr>
<th>Date</th>
<th>Liquids intake in ml</th>
<th>Urine in ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>08.11.2011</td>
<td>2400</td>
<td>2200</td>
</tr>
<tr>
<td>09.11.2011</td>
<td>1800</td>
<td>1600</td>
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<tr>
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<tr>
<td>11.11.2011</td>
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<tr>
<td>12.11.2011</td>
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<tr>
<td>13.11.2011</td>
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</tr>
<tr>
<td>14.11.2011</td>
<td>2200</td>
<td>2000</td>
</tr>
</tbody>
</table>

Fig. 1. Edema of vulva on day 1.

water and interstitial colloid osmotic pressure, can precipitate edema during pregnancy (1). Underlying systemic disease is a common cause of vulvar edema, associated with some degree of pedal edema (1, 2, 3). Massive vulvar edema has been reported to occur following tocolysis, vulvovaginitis, Crohn’s disease,
Fig. 2. A small reduction of edema on day 4.

Fig. 3. An evident reduction of edema on day 7.

artificial ascites for adesiolyis with dextran, pre-eclampsia and hypoproteinem-ia (4, 5, 6, 7, 8, 9, 11), but all this cases are reported on the third trimester
of pregnancy and never in the first trimester. Differential diagnosis of vulvar edema includes infections, neoplasm, congenital lymphatic anomalies, trauma, inflammatory and metabolic disorders. Interestingly, vulval edema occurring in immediate post-partum period has been reported to cause maternal death, due to vascular collapse in six patients with an 80% mortality rate (10).

At our case, we thought that probably, vulvar edema in a patient with HOS, in the first days of pregnancy was due to hypoproteinemia, but it was not confirmed from relevant examinations. The second version was the compressive phenomenon of the enlarged ovary and ascites on the lymphatic canals. This version is more probable because even the edema of vulva (labia minor and major of the left side) were more evident and corresponding with the left enlarged ovary (see photo 1). Our Hospital, is a tertiary level hospital in which are referred the most severe diagnoses from all over the country, but this type of isolated massive vulvar edema has not been observed before. To the best of our knowledge vulvar edema due to compressive phenomenon of enlarged ovary and ascites on the lymphatic ways in a 4-5 weeks pregnant woman, with HOS, has not been described before. Edema resolved fortunately without complications, gradually together with clinical parameters of HOS.

REFERENCES

Does the Use of Highly Purified Human Menopausal Gonadotrophin (HP-HMG) Avoid Ovarian Hyperstimulation Syndrome (OHSS) in Polycystic Ovary (PCO) Patients in Assisted Reproduction (IVF/ICSI)?

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ABSTRACT

Problem/statement: In reproductive age the polycystic ovary (PCOS) patients have quite common endocrinopathies, affecting 5-10% of women, patients with (PCOS) are presented with low ovulation and cycle irregularity which lead the women to seek treatment for infertility. It is a heterogeneous syndrome both in its clinical presentation and in its laboratory manifestations. Although a significant improvement has been seen in the frequently out comes with induction of many stimulation protocols, the incidence of ovarian hyperstimulation syndrome (OHSS) is still high. Ovarian hyperstimulation syndrome can be potentially very risky to ladies lives. Our objective is to evaluate the role of Highly Purified Human Menopausal Gonadotrophin (HP-HMG) in preventing and reducing OHSS in Polycystic ovary (PCO) patients. This study was carried out in Lamis IVF Center Misurata, Libya.

Methods: This study is a prospective study from 1st Aug. 2012 until end of Jan. 2013 over 6 months, during this period 500 patients were treated for infertility due to different causes. All these patients were treated by ICSI procedure. 20% of patients were proved by investigations to be polycystic ovary patients (PCO). So, one hundred (100) patients were observed for the symptoms and signs of ovarian hyperstimulation syndrome, (OHSS).
The main outcome measure(s) are diagnosis of Polycystic ovary, age distribution, dose of highly purified human menopausal gonadotrophin (HP-HMG) 300-450IU from BBT/day, protocols used (short agonist protocol and flexible antagonist protocol), pregnancy outcome, any OHSS and hospital admission due to any complication were all observed and recorded.

**Results:** Age distribution counted from 20 years to 44 years old. The total number of polycystic ovary patients (PCO) were 100 patients. They were having successful ovarian induction with highly purified human menopausal gonadotrophin from BBT. The type of eggs retrieved were considered with very good quality. These eggs were fertilized and produced high quality embryos. The pregnancy outcome was about 50%. The good news that there was no single case of moderate and severe ovarian hyperstimulation syndrome (OHSS). This can lead to the fact that using Highly Purified Human Menopausal Gonadotrophin (HP-HMG) reduces the problem with ovarian hyperstimulation syndrome (OHSS) and protects patients from moderate and severe ovarian hyperstimulation syndrome (OHSS).

**Conclusion:** Using Highly purified Human Menopausal Gonadotrophin is safe in any ovulation induction protocol in cases of polycystic ovarian syndrome and can prevent or reduce the ovarian hyperstimulation syndrome with good pregnancy outcome.

**INTRODUCTION**

In reproductive age the Polycystic ovary syndrome (PCOS) is quite common in endocrinopathies, affecting 5-10% of women (1). Women with PCOS is present with low ovulation, hyperandrogenism and polycystic ovary syndrome (2, 3) women seek treatment for infertility due to unovulation (4). It is a heterogeneous syndrome both in its clinical presentation and in its laboratory manifestation (5, 6).

Although there is significant improvement has been seen in the pregnancy outcomes with the introduction of many stimulation protocols, the incidence of ovarian hyperstimulation syndrome (OHSS) still high. OHSS is a potentially life threatening complication associated with controlled ovarian hyperstimulation and IVF, ovarian hyperstimulation syndrome can potentially rupture, hemorrhage or undergo torsion (7, 8).

The pathogenesis of OHSS is unclear. The variables closely related to OHSS are beta-sub-unit of human chorionic gonadotrophin (B-hCG), S. estradiol, number of follicles, vascular endothelial growth factor (VEGF), interleukin-6, the ovarian renin angiotensin system and prostaglandins (7, 9, 10). High levels of serum estrogen is associated with increased risk of OHSS (6). The syndrome of
OHSS has been reported in extremely low S. estrogen levels (9, 10). Cancellation of the cycle to avoid the risk of OHSS but at the expenses of losing the cycle (11) with holding the human chorionic gonadotrophin (hCG) injection, coasting and elective cryopreservation of the embryos. These are all tried to avoid the flare up of OHSS. This study is randomized for patients with all ages who request management of their infertility by ICSI. This ICSI management includes short protocol and antagonist protocol for ovulation induction. The total number of patients are 500. One hundred patients were having antagonist protocol. We were using 450 IU/day intramuscular of highly purified human menopausal gonadotrophin (HP-HMG) «Diclair of BBT» contain FSH and LH in 50% of each to find out how effective in preventing OHSS. Diclair HP HMG 150 starting from day three of cycle for 7 days but in some cases we added 2-3 days. In short protocol decapeptyl 0.05 mg (half amp) was start on day two of the cycle by injecting subcutaneously until the day of giving Human chorionic gonadotropin. In cases of antagonist protocol we were using flexible type and we give Organolutran 0.25 mg S.C. on day of the loading follicles measured 15-16 mm.

PATIENTS AND METHODS

Five hundred infertile women were included in this study. These patients attending the Lamis IVF Center Misurata. One hundred of them were diagnosed as Polycystic ovary syndrome based on European Society for Human Reproduction and Embryology (ESHRE)/American Society for Reproductive Medicine guideline (Rotterdam criteria 2003), as including at least two of the following three criteria: 1.) Chronic anovulation, 2.) Clinical or biochemical signs of hyperandrogenism; and 3.) Polycystic ovary morphology shown on ultrasound scan, defined as the presence of ≥12 follicles (with one ovary being sufficient for diagnosis) measuring 2-9 mm in diameter. Our inclusion Criteria were 1.) No patient showed hyperprolactinemia, thyroid problems, liver or kidney dysfunction, 2.) All patients were for ICSI procedures, 3.) Age between 20 and 44 years. The study started from 1st August until end of January 2013 (6 months). All patients were given 450 IU of highly purified human menopausal gonadotrophin (HP-HMG) contains LH and FSH in equal form (three ampules contains 150 Iu FSH and 150 Iu LH in each ampule).

This dose was given daily intramuscular from day three of cycle until the leading follicles are measured in diameter 17-22 mm (the time of human chorionic gonadotrophin 10,000 Iu to be given IM, in the same time 1/2 ampule of decapeptyl 0.1 mg was given S.C. daily, this is named short protocol. The other protocol was used in a Flexible Antagonist. Protocol (same dose of HP-HMG
450IU/IM daily from day three of cycle until follicles are measured in diameter 15-16 mm. The Human chorionic gonadotrophin 10,000 IU/IM after 24 hours.

The number of eggs and Quality were observed. Quality of embryo and pregnancy were studied. Side effects and any symptoms of hyperstimulation syndrome were followed up.

RESULTS

Tab. I. Age Distribution.

<table>
<thead>
<tr>
<th>AGES</th>
<th>20-24</th>
<th>25-29</th>
<th>30-34</th>
<th>35-39</th>
<th>40-44</th>
<th>&gt;44</th>
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<td>84</td>
<td>106</td>
<td>136</td>
<td>108</td>
<td>48</td>
</tr>
<tr>
<td>Short protocol</td>
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<td>84</td>
<td>96</td>
<td>126</td>
<td>80</td>
<td>36</td>
</tr>
<tr>
<td>Antagonist protocol</td>
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<td>0</td>
<td>10</td>
<td>10</td>
<td>28</td>
<td>12</td>
</tr>
</tbody>
</table>

Tab. II. Number of Eggs were collected.

<table>
<thead>
<tr>
<th>One egg</th>
<th>2-5 eggs</th>
<th>6-10 eggs</th>
<th>11-15 eggs</th>
<th>16-20 eggs</th>
<th>21-25 eggs</th>
<th>&gt;25 eggs</th>
<th>Bad and no eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>40 patients</td>
<td>150 patients</td>
<td>120 patients</td>
<td>74 patients</td>
<td>32 patients</td>
<td>28 patients</td>
<td>26 patients</td>
<td>30 patients</td>
</tr>
<tr>
<td>8%</td>
<td>30%</td>
<td>24%</td>
<td>14.8%</td>
<td>6.4%</td>
<td>5.6%</td>
<td>5.2%</td>
<td>6%</td>
</tr>
</tbody>
</table>

There were 20 patients have no eggs.

Tab. III.

<table>
<thead>
<tr>
<th>Total number of eggs</th>
<th>Good Quality eggs which were injected by sperms</th>
<th>18% of Eggs collected were very poor and were not injected by sperm</th>
</tr>
</thead>
<tbody>
<tr>
<td>3748</td>
<td>3128</td>
<td>620</td>
</tr>
</tbody>
</table>

Tab. IV. Embryo Quality.

<table>
<thead>
<tr>
<th>Poor Quality Embryo G3 and G4</th>
<th>Excellent Embryo G1 and G2</th>
</tr>
</thead>
<tbody>
<tr>
<td>286</td>
<td>1764</td>
</tr>
</tbody>
</table>
We had 15 patients had no eggs and 15 patients had poor eggs and there was no fertilization. The total number of patients who had no embryo transfer were 50 patients equal to 10%. In the other hand 90% of our patients in this study were having embryo transfer. Our pregnancy rate was 55% and the early abortion was 6%. These results were almost the same in both protocol. In these five hundred patients there was no single admissions due to hyperstimulation syndrome and there was no patients got any treatment against hyperstimulation syndrome.

The total number of patient who have polycystic ovarian syndrome in Lamis IVF Center during this six (6) months study were one hundred patients this is equal to 20% of total patient were treated in this time by ICSI and ET procedures.

<table>
<thead>
<tr>
<th>Tab. V. Age Distribution of Polycystic.</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-24 years</td>
</tr>
<tr>
<td>5 patients</td>
</tr>
</tbody>
</table>

Most of patients with polycystic ovary were at age 25-34 73%.

<table>
<thead>
<tr>
<th>Tab. VI. Number of Eggs/Patients of Polycystic.</th>
</tr>
</thead>
<tbody>
<tr>
<td>6-10 eggs</td>
</tr>
<tr>
<td>10 patients</td>
</tr>
</tbody>
</table>

63% of patients produce more than eleven (11) eggs. 13% of patients gave eggs more than 25. The good thing here to say is that all patients gave more than 6 eggs to any patients in any age and have good quality eggs.

In this study we found that eggs number per patient were from 6- >25 eggs. In the same time there were no evidence of any moderate or severe symptoms or signs of hyperstimulation syndrome.

The results from this study showed no single patient needs admission for any management of hyperstimulation syndrome. The clinical pregnancy rate were >45%.

The patients who had Antagonist Protocol only 20% of total patient and 20%
of polycystic patient. The result in the eggs and Embryos Quality were same as in the Short Protocol using Highly Purified HMG 150Iu FSH and 150 LH via Intramuscular showed.

Almost the same result in Quality and Pregnancy were recorded.

**DISCUSSION**

The patients with ovarian hyperstimulation syndrome (OHSS) remain a challenge to manage. The ideal and successful management of high responders especially in polycystic ovary syndrome, would use as a treatment that minimizes the patient’s risk while achieving optimal cycle outcome. The main advantage of using highly purified human gonadotrophin (diclair from BBT) 150 Iu is the prevention of OHSS without the need to cancel the cycle. In this study more advantage were seem in continuation of all and High pregnancy rate >50%.

Coasting 4 or more days reduces the implantation and pregnancy rate. Chen et al. (12) demonstrated increased in cancellation rates when coasting was longer more than 4 days. In our study using diclair highly purified HMG has no cycle cancellation and has good embryo implantation and pregnancy rate.

The incidence of OHSS is 20-30% for mild, 3-6% for moderate and 1-2% for severe OHSS (13, 14). According to our results OHSS has 0%. To manage severe OHSS requires ICU admission and intensive treatment. Newer management suggest treatment with cabergoline to decrease hemoconcentration and ascites by blocking the vascular endothelial growth factor (VEGF-2) receptor (15, 16). Cycle cancellation can eliminate the risk for developing OHSS completely (8, 11), but the coast is not eliminated. Patient psychology will be affected from cancellation of cycle.

In this study there was no cancellation needed and no ICU management was required to a single patient, so using highly purified HMG can be the alternative and ideal drug for ovulation induction in high risk patient.

In this study the number and quality of eggs were good and not affected in fertilization, while in coasted cycles the number and quality of eggs retrieved were reduced (17).

**CONCLUSION**

In conclusion, this study revealed that using Highly Purified HMG 450 Iu/Im (diclair from BBT) can save patients with polycystic ovaries from severe OHSS and in the same time gives good pregnancy outcome with no cycle cancellation or holding of giving HCG.
REFERENCES

Using Multi-Micronutrients Supplement in One Capsule Twice a Day Improves the Sperm Quality and Increases the Pregnancy Outcome

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ABSTRACT

Objective: to evaluate the effect of Multi-micronutrients in one capsule twice a day in sperm parameter and pregnancy outcome in idiopathic sub-fertile males.

Design: 300 sub-fertile males were treated in a period of 12 months (1st May 2012 to 30th of April 2013) all cases were idiopathic and have sperm count < 20 million/ml, progressive motility < 20% and normal sperm morphology < 20%.

Setting: Lamis IVF center, Misurata, Libya.

Patient(s): during one year of study, 400 patients were included. One hundred patients were taken as control no treatment was given for 3 months. Three hundred patients were received the micronutrients for at least 3 months. All patients were sub-fertile according to semen analysis results twice. In one week in between. All patients had semen analysis after 3 months and 6 months. Follow up included any pregnancy during this period.

Main outcome measure(s): all patient included in the study and diagnosed sub-fertile had semen analysis after 3 months and 6 months. During the study of one year, all semen parameters changes were recorded and any spontaneous pregnancy in both group were noticed (control and study group).
**Result(s):** age distribution counted from 25 yrs to 55 yrs. 55% patients improved to normozoospermie, 11% pregnancies in follow up, sperm count (significantly improved and to normal density 64%). Progressive sperm motility (significantly improved and to normal motility) in 63%. Sperm Morphology (significantly improved to normal morphology in 63%). In the control group an overall improvement was seen in 10%, spontaneous pregnancy in 3%.

**Conclusion:** using Multi-micronutrients for male sub-fertility for 3 months or more has a good prognosis to treat male infertility spontaneously or by assisted reproduction. Significant increase of pregnancy rate could be shown.

**INTRODUCTION**

Male subfertility related primary and secondary factors exists in 25-30% of all infertility causes (1, 2) in Libya according to our statistics. Male factor is counted more than 50% of all infertility cases. Several causes have been identified: hypogonadism, gonadotrophin deficiency, genital tract infection and obstruction or sperm auto immunity. More than 50% of causes are not known, male patients are seeking for treatment. Abnormal sperm parameters are strongly associated with pregnancy outcome (3, 4). Low sperm quality increases the therapeutic activity (1, 5). In the last 25 years there was no evidence to support the androgen and gonadotrophin treatment to enhance male fertility. The uses of anti-estrogen, aromatase found to be insufficient. There is no therapeutic drug found to treat idiopathic male infertility in term of natural conception (6).

The etiology of idiopathic sperm parameters is very poorly understood. May be due to multi factorial disorders, in which many genetic, environmental, nutritional and lifestyle related factors act together. Any of these factors can lead to abnormal spermatogenesis and sub fertility. Nutrition may be of major therapeutic interest, as DNA and spermatogenesis might be affected by unknown diet. Therefore, concentration of required nutrient and other relevant factors may have substantial effects on sperm quality and reproduction (7,8). Compensation of underlying nutritional imbalance in order to support optional sperm production and function is aim of multiple past studies.

A number of nutrients such as trace elements, amino-acids, vitamins and many other agents involved in spermatogenesis have been examined and advocated as a way of optimizing sperm production and quality.

Many single nutrient or combination of two showed improvement of the

In this study we use eight nutrients combined in one capsule. Each capsule contains L-carnitine, L-arginine, zinc, vitamin E, glutathione, selenium, coenzyme Q10 and folic acid. The purpose of this study was to investigate whether the use of multi nutrients together in one capsule will provide better results in sperm parameters and pregnancy outcome.

**MATERIAL AND METHODS**

A total of three hundred sub-fertility males were included in the study group. These are all males who have sperm count < 15 millions/mL, forward sperm progress movement < 20% and sperm morphology < 20% normal sperm morphology. These three parameters can be present in one patient or one parameter or two together in a patient. In this study any patient who showed these three parameters or one of them was selected in the treatment group. Patients in this study group where randomized for treatment with one capsule of the active compound (PROfertil® provided by Lenus Pharma Gesmbh, Vienna, Austria), which consist of a combination of eigth micronutrients L-carnitine 440 mg, L-arginine 250 mg, Vitamin E 120 mg, Folic acid 800 mcg, Zinc 40 mg, Selenium 60mcg, Glutathione 80 mg and Coenzyme Q10 15 mg in the morning and the second one in the night, with 12 hours apart, for three months or alternatively in the control group. Two baseline semen analysis were taken a week between the two samples. These semen analysis were done by one laboratory technician and repeat again for the second semen analysis after three months from starting the therapy. Revision to all cases who receive the micronutrients therapy for three months or more. The notice was observed on semen parameter, pregnancy and any side effects.

The control group was where one hundred patients with the similar inclusion criteria (semen parameters) as in therapeutic group. They were taken randomly between patients. This group has no treatment but observed for later treatment after 3 months. Some of them tried ICSI Management.
RESULTS

Age Distribution

<table>
<thead>
<tr>
<th>Age Group</th>
<th>Number of Patients</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;25 years old</td>
<td>3</td>
<td>9%</td>
</tr>
<tr>
<td>25-29 years old</td>
<td>24</td>
<td>34%</td>
</tr>
<tr>
<td>30-34 years old</td>
<td>102</td>
<td>25%</td>
</tr>
<tr>
<td>35-39 years old</td>
<td>75</td>
<td>19%</td>
</tr>
<tr>
<td>40-44 years old</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>45-49 years old</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>50-54 years old</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>&gt;55 years old</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>234</td>
<td>78%</td>
</tr>
</tbody>
</table>

- 78% of our patients in age group between 30-44 years old. Only 1% in age group < 25 years old and 1% in age group > 55 years old.

3-6 months of treatment

- 15% become normal semen parameters according to WHO classification. 11% of therapeutic group their wives became pregnant.
  The semen parameters in these patients before treatment:
  - Sperm count were 10-50 million/mL;
  - Forward progressive motility between 5-20%;
  - The sperms morphology were between 10-20%.

Changes in sperm count

<table>
<thead>
<tr>
<th></th>
<th>The sperm count improved significantly and some become normal</th>
<th>No changes in the count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>193</td>
<td>107</td>
</tr>
<tr>
<td>Percentage</td>
<td>64.33%</td>
<td>35.67%</td>
</tr>
</tbody>
</table>

The changes in the forward Progressive Motility

<table>
<thead>
<tr>
<th></th>
<th>Increasing significantly and become normal 30% or more</th>
<th>Stay the same/ no changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>191</td>
<td>109</td>
</tr>
<tr>
<td>Percentage</td>
<td>63.67%</td>
<td>36.33%</td>
</tr>
</tbody>
</table>

The changes in the Sperm Morphology

Increased sperm morphology 62.67% in the treatment group. Significant less changed from pathological to normal due to the low normal range in WHO IV. The improvement in all semen parameters are > 60% which is significantly to concede.
In this study we use an active compound contained L-carnitine (440 mg), L-arginine (250 mg), zinc (40 mg), vitamin E (120 mg), coenzyme Q10 (15 mg), glutathione (80 mg), selenium (60 mcg) and folic acid (800 mcg). The patients in the treatment group took one capsule in the morning and the other one in the evening. In another study of Imhof M. (2011) the same combination was administered as two capsules together at once (9). His results can approximately similar excluding morphology. Other studies using single micronutrient had some improvement on sperm parameter such as in L-carnitine the effect was on sperm count, motility and motile sperm density (10) (11) (12). In normal sperm count, L-arginine improved sperm motility (13), Vitamin E improved sperm motility (14), vitamin E in combination with selenium increased sperm motility and normal morphology rate (15) (16). Despite this about selenium, the supplementation data is conflictive increased (17), unchanged (18) or decreased (19). Zinc supplementation has shown positive effect on sperm counts and other parameters (20) (21). Folic acid is required for DNA synthesis and impotent for spermatogenesis (22) (13). Supplementation of folic acid alone failed to show beneficial effects on sperm concentration (23). Lower glutathione levels related to abnormal sperm motility and morphology (24). Glutathione is important for DNA synthesis and its supplementation has positive effect on sperm motility and morphology (25) (26). Coenzyme Q10 supplementation has been confirmed by increased sperm motility on asthenospermic Mon (27) (28). As described above the use of micronutrient alone or in combination has a positive effect on semen parameters.

Semen analysis repeated after three months without any treatment; 6 patients 6% became normal semen parameters according to WHO classification and only two pregnancy occurs to their wives.

The control group showed changes of sperm parameters from male fertile pathology to normozoospermia in 8%. 92 patients had no significant improvement.

**DISCUSSION**

In this study we use an active compound contained L-carnitine (440 mg), L-arginine (250 mg), zinc (40 mg), vitamin E (120 mg), coenzyme Q10 (15 mg), glutathione (80 mg), selenium (60 mcg) and folic acid (800 mcg). The patients in the treatment group took one capsule in the morning and the other one in the evening. In another study of Imhof M. (2011) the same combination was administered as two capsules together at once (9). His results can approximately similar excluding morphology. Other studies using single micronutrient had some improvement on sperm parameter such as in L-carnitine the effect was on sperm count, motility and motile sperm density (10) (11) (12). In normal sperm count, L-arginine improved sperm motility (13), Vitamin E improved sperm motility (14), vitamin E in combination with selenium increased sperm motility and normal morphology rate (15) (16). Despite this about selenium, the supplementation data is conflictive increased (17), unchanged (18) or decreased (19). Zinc supplementation has shown positive effect on sperm counts and other parameters (20) (21). Folic acid is required for DNA synthesis and impotent for spermatogenesis (22) (13). Supplementation of folic acid alone failed to show beneficial effects on sperm concentration (23). Lower glutathione levels related to abnormal sperm motility and morphology (24). Glutathione is important for DNA synthesis and its supplementation has positive effect on sperm motility and morphology (25) (26). Coenzyme Q10 supplementation has been confirmed by increased sperm motility on asthenospermic Mon (27) (28). As described above the use of micronutrient alone or in combination has a positive effect on semen parameters.
In this study we used eight micronutrients in one capsule twice a day, which gives very positive result in all semen parameters up to 60% improvement in count, motility and even morphology. These improvements are significant when compared to the results in the control group. Many idiopathic infertile men became normal in semen parameters and significant pregnancy rate were reported. As extensively described and discussed above, using micronutrient in combination gives very good results in improving semen parameters in idiopathic male infertility and increases the pregnancy outcome.

CONCLUSION

Treatment with a standardized combination of eight micronutrients for a period of three to six months improves the semen parameters in > 60% of patients significantly and even up to normozoospermia. Especially for idiopathic sub-fertile men, the administration with the micronutrient compound can be an initial treatment of choice. Treatment showed a significant increase of pregnancy rate in sub-fertile men without appearance of adverse effects.

REFERENCES


Meaning Attached to Infertility in Turkish Society/Stigmatization

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SUMMARY

Infertility is defined as not being able to get pregnant despite having regular and unprotected sex for one year. Infertility affecting 10-15% of population at reproductive age is an abrupt and unexpected life crisis for many couples. It is estimated that there are 1.1 million infertile couples in Turkey. Infertility lowers life quality of couples through causing physical, psychological, socio-cultural, economic and marriage or sexual life-related problems. Inability of reproduction frequently creates a social stigma. Stigmatization for infertility happens when predefined social norms cannot be met. Thus, infertility causes individuals to feel incompetent, inferior and different from others and causes a significant life stress that depreciates individual identity. In Turkish society, having a child is accepted to have economic, psychological and social dimensions. Therefore, marriage brings along having children and certain problems could occur in childless marriages. Not having a child emotionally affects both genders; however, women are seen to feel more stress and pressure. In this review, it is aimed to investigate life experiences of infertile women and meaning attached by society to infertility in light with the results of some other studies.

Keywords: infertile women, life experiences, Turkish society, meaning of infertility, stigma.
INTRODUCTION

In many cultures, pregnancy and parenthood are regarded as an important developmental milestone and infertility generally creates a social stigma [1, 2]. Infertility could result in an important life stress that depreciates individual identity and makes individual feel incompetent, inferior and different from others [1, 2, 3]. Stigmatization for infertility happens when predefined social norms cannot be met [4]. Stigmatization might ruin social interactions of individuals and restrict social networks, lower quality of life and cause low self-esteem and depressive symptoms [5].

For many people, being a parent is a natural part of life. Seeing the next generation growing up is defined as a pleasing and supportive power; therefore, any limitation of the natural condition might negatively affect the couples [6]. Ability to have a child could be a determinant factor of sexual power for women and men in communities, but women are generally held responsible for deterioration of this ability. Motherhood is still regarded as the primary role of women in society. Nearly in all communities, infertile women are held responsible for fertility problems; however, women living in traditional culture are seen to bear a higher burden of infertility [1]. In Africa, women with fertility problems could be despised, disregarded or even blamed by their spouses and families. In Nigeria, women must have a child for proving their womanhood. Childless women are isolated from other members of the community, excluded from social activities and ceremonies and forbidden from touching newborn babies, while childless men are forced to make another marriage [7, 8].

Child delivery is given more importance in Islamic and Eastern counties. Having a child gives balance to family and increases the marital satisfaction. In the absence of a child, making another marriage or divorcement could take place. For infertile women, interference of husband’s family and negative attitude of family, friends and neighbors might cause psychological problems. In general, women could be exposed to such problems as disrupting family order and cruel criticisms [9, 10].

In Turkish society, child is an important factor that has economic, sociological and psychological dimensions. Though these values are generally same for women and men, the priority level can be different. For women, such psychological values as strengthening marital bonds, intimacy with spouse and maternal instinct come first, while economic and traditional values like sustaining family surname and old age security are more important for men. Child is regarded as an important labor force in agricultural communities. In addition, having a child could also be regarded as a factor that brings individuals certain privileges and prestige. On account of all these reasons, couples unable to have a child suffer more psychological pressure due to social effects [11, 12].
In Turkish society, marriage brings along having a child and certain problems can occur in childless marriages. As in many other countries, the words «woman and mother» are used synonymously in Turkey and inability to have a child is regarded as a condition that lowers status of woman. In rural parts of Turkey, childless couples could be alienated by the community, women can be blamed, and they might even be obliged to settle for her spouse to have a second wife in order to have a child [13].

In this review, it is aimed to investigate the life experience of infertile women and meanings attached by community to infertility in Turkish society in light of the results of the previous studies.

MATERIAL AND METHODS

This study was a review of the literature.

RESULTS

According to the results of some anthropological studies carried out in Turkey; For Turkish Women, being a mother is equal to being able to give birth to a baby. Giving birth to a baby is the only way to get rid of the «infertile woman» stigma and to find acceptance in society [14]. Children are regarded as necessary in order to protect social status and secure the future [15]. Women are directly blamed for childlessness. Sex of the child depends on women [14]. Childless women are looked down on in the family and among neighbors [15, 16]. Childless women are seen as «bad luck», «waste», «fruitless tree», «dry wood», or «unproductive land» [15, 16]. Childless women are not allowed to put henna on the bride’s hands during the henna night (a Turkish tradition, ceremony made one day before wedding) [16]. Childless women are deemed to deserve to be the second wife [16]. For Turkish Men; Having children is necessary to maintain social status, increase personal reputation within the family and among friends, ensure the continuity of their surnames and secure the future [14].

Van Rooij et al. (2007) reported that infertile women in Turkey and infertile Turkish women living in Holland experience higher emotional stress than infertile Dutch women. This study demonstrates that Emotional stress experienced in infertility is not related to living place but directly related to cultural norms and values. Cultural norms and values in Turkish society encourage couples for marriage and reproduction, which is considered to cause women to be more affected by infertility [17].
Teskereci and Oncel (2013) determined that life quality of infertile women is lower than infertile men. In literature about infertility in Turkey, it is seen that the studies are generally conducted on life experience of infertile women [18]. In the study of Kocyigit (2012), one of each two women receiving infertility treatment is exposed to social pressure, which is initially shown with the question «When will you make a child?». In questions about children, women stated that they get exhausted, feel guilty, uncomfortable with their peers having children, despised, alienated, and threatened for divorce or a second wife. On the other hand, some women emphasized that their spouses become unfaithful. Women experiencing intensive social pressure said that they are seen as «dead tree» or «infertile soil» [19]. In the study of Ilerisoy (2012), most of the women receiving infertility treatment defined inability to have a child as «incompetence» or «deficiency» [4]. They find the question of social environment «Why do you still not have a child?» as annoying and stigmatizing. In the study of Sen (2011), infertile women said that they do not want to communicate with society due to negative reactions of the community [20]. In the study of Bayram Onat (2009), infertile women stated that their social life is adversely affected and demonstrate such behaviors as locking themselves at home, avoiding environments with children, not wanting to speak or see friends, stigmatization, hiding their treatment, lying about having a child, making up excuses, fulfill their longing with others’ children, and communicating with other childless families [21]. In the study of Kirca (2013), infertile women stated that having a child is the most important thing in their life; they feel uncomfortable with questions about having a child, are adversely affected by conversations about children and feel uncomfortable with environments with children [22]. Yildizhan et al. (2009) reported that some infertile women are exposed to violence by their husbands or husbands’ family after diagnosed with infertility [23].

CONCLUSIONS

When having children is seen as a typical norm in the society, childlessness is considered as a form of «abnormal behavior». This situation reduces self-esteem and body image of women exposed to stigmatization more in particular, it weakens their social support networks and has negative effects on their interpersonal relationships [3, 24]. Although it is not possible to make generalization for the entire Turkish society, some study results indicate that infertile couples are exposed to a great pressure. Couples can feel incompetence or guiltiness and be subject to pressure of both family and friends. In order to decrease the negative experiences of couples who cannot have children, individual coping resources and family dynamics should be strengthened and stigmatizing attitu-
des should be reduced by increasing public awareness about infertility [4]. In this subject, nurses should take more responsibility to raising awareness of the community and provide guidance.

REFERENCES


Intra-Uterine Insemination with Donor Semen in Non-Stimulated Cycles: a Large Retrospective Cohort Study

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SUMMARY

Intrauterine insemination using donor semen (D-IUI) is a widely accepted and successful treatment for patients with partners suffering from severe male infertility (from severe OAT to azoospermia) or severe genetic problems. Single or lesbian women can also be treated with D-IUI. To date, few studies have focused on the success and efficacy of D-IUI especially when used in non-stimulated cycles.

We report here a large retrospective cohort study of 2242 D-IUI in non-stimulated natural cycles and demonstrate that high OPR’s (8.7%) and high COPR’s (42.1% after 6 cycles) with a very low risk for a multiple pregnancy (<0.5%). Can be obtained. Our data confirm that outcomes in D-IUI are related to donor sperm quality at the time of insemination and also to age of the female patient.

INTRODUCTION

Intrauterine insemination using donor semen (D-IUI) is a widely accepted and successful treatment for patients with partners suffering from severe male infertility (from severe OAT to azoospermia) or severe genetic problems. Single or lesbian women can also be treated with D-IUI. To date, few studies have focused on the success and efficacy of D-IUI especially when used in non-stimulated cycles.

The aim of this study was to analyse the outcomes of intra-uterine insemi-
nation with donor semen in a large cohort of non-stimulated natural cycles. Primary outcome (1) was the ongoing pregnancy rate (OPR). Secondary outcome was the cumulative ongoing pregnancy rate (COPR) after 6 D-IUI in natural cycles. Influence of female age and donor sperm quality on D-IUI's outcomes were also analysed.

**MATERIALS AND METHODS**

*Study design.* Retrospective cohort study evaluating outcomes of all D-IUI's in non-stimulated cycles during a twelve month period (January till December 2011). The setting is Stg Geertgen, a private fertility treatment centre in Esendorp, the Netherlands.

*Patients.* Patients underwent counseling for use of donor gametes as well as an extensive clinical interview and a gynecological examination. Patients signed an informed consent before undergoing D-IUI.

*Patient's protocol.* Urine LH peak detection as well as ultrasound of both ovaries was used for ovulation timing in the natural cycles.

*D-IUI procedure.* The procedure for D-IUI was performed according to hospital specific protocols. Frozen donor semen was thawed from the Stg Geertgen Donor Sperm bank. The sample was washed by centrifugation, supernatants was removed and the pellet resuspended in 0.4ml of HTF A sample was taken for sperm analysis to define concentration and motility (WHO, 2010). Sperm suspension was kept in a test tube at 36°C until the time of insemination. Patients were inseminated 24-36 hours post hCG injection or post LH peak. The 0.4 ml prepared donor semen was aspirated in an PM (Gynotec) or Wallace (Laprolan) catheter and immediately used for insemination. D-IUI was performed irrespective of sperm quality obtained. No luteal phase support was given.

*Sperm quality.* Sperm quality was defined after sperm preparation as total motile count (TMC) available for insemination and is the total sum of grade A and grade B motile spermatozoa in the prepared sample (WHO, 2010 (2)).

*Ongoing pregnancy.* Ongoing pregnancy was defined as the presence of gestational sac with fetal cardiac activity heartbeat during transvaginal ultrasound at 10 to 12 weeks post IUI. Data: Ongoing pregnancy rates (OPR) and multiple pregnancy rate were noted. Cumulative ongoing pregnancy rates after 6 cycles of D-IUI were also calculated.

RESULTS

In 2011, a total of 2242 Donor-IUI’s using natural cycles were performed at Stg Geertgen. Patient’s age had a mean of 35,9 years (range 21-45 years; SD: 4,4 years; median: 36,3 years). A mean TMC of 1,6 million spermatozoa was noted (range: 0,1-32,3; SD 2,1; median: 0,9). Twelve weeks post D-IUI, 195 healthy ongoing pregnancies were recorded, resulting in an OPR of 8.7%. After 6 cycles an overall COPR of 42,1% was obtained. Only one monozygotic twin pregnancy was noted (0,5% multiple pregnancy rate).

A TMC $\geq$ of 1 106 resulted in an OPR of 11,9%; a TMC of $\geq$ 0.5 106 in 6,5% OPR, whereas with a TMC of $< 0.5$ 106 a 2.5% OPR was noted.

A 22,2% OPR was noted for patients < 25 years; 12,9% between 25 and 30 years; 10,6%, when age was between 30 and 35 years; 9,2% OPR when between 35 and 40 years and 4,2% when > 40 years old.

CPR after 6 Donor-IUI’s was 77,8% for patients younger than 25 years; 56,3% for patients between 25 and 30 years old; 48,9% for patients between 30 and 35 years old, 44% between 35 and 40 years of age and 22,7% for those patients over 40 years of age.

CONCLUSIONS

This retrospective large cohort study demonstrates that D-IUI in non-stimulated cycles can result in high OPR’s (8,7%) and high COPR’s (42,1% after 6 cycles) with a very low risk for a multiple pregnancy (<0,5%). D-IUI in natural cycles is therefore a good treatment alternative to D-IUI in controlled ovarian stimulation regimes (COS). IUI data presented in the 2009 ESHRE consortium paper (3) showed higher delivery rates (13.4%) when D-IUI was performed in COS but also demonstrated a clear increased risk for obtaining multiple pregnancies: twin pregnancy rate >10.3% and triplet pregnancies >0.5 (29235 D-IUI cycles) were observed.

Our data confirm that outcomes in D-IUI are related to donor sperm quality at the time of insemination and also to age of the female patient (4): D-IUI in natural cycles showed to be a poor treatment option for women >40 years of age. Use of D-IUI in natural cycles may also require more cycles of treatment for obtaining a pregnancy. D-IUI in natural cycles is however non-invasive and patient friendly; the treatment regime is practically medication free and costs are limited. D-IUI could be considered as the first line treatment before going to IVF with donor semen.

There is a need for prospective randomised trials comparing the efficacy of IUI with donor semen in both stimulated and non-stimulated cycles.
REFERENCES

FETOMATERNAL MEDICINE
Etiology of Fetal Presentation: One (Still) Personal Approach

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SUMMARY

Problem statement: The hypothesis on the dependence of fetal presentation on the postural development presumes that cephalic-anterior presentation (CP) allows uncompromised posture of the fetus. Changing gravity vector in intrauterine environment for 180° should cause a change of presentation. When the postural development is absent, the fetus assumes a presentation randomly, with equal probability for breech-posterior presentation (BP) and CP.

Methods: Investigating gravity depended behavior of the sheep and guinea pig fetus. Investigating types of movements which the fetus use to change its presentation in human newborns from BP. Investigating in human species as well as in quadrupedal mammals probability of BP.

Results: Righting reflex from supination to pronation has been confirmed in the guinea pig, as well as decreased righting reflexes and locomotor movements in human newborns from BP. The guinea pig is not a proper experimental model for examination of gravity-dependent presentation due to lack of the possibility of presentation changes in the second half of gestation. The short-term exposure of sheep fetuses to an upside-down position has not confirmed or disproved the gravity-dependent fetal presentation. The highest probability of BP is 50%

Conclusion: It is necessary to keep sheep fetuses in the upside-down position for a prolonged period to prove or disapprove the hypothesis. Before experiment the horn of the uterus with the sheep fetus inside should be surgically fixed. BP represents a mere filling of the intrauterine cavity, with an equal probability for CP and BP. CP group is heterogeneous, which creates bias when comparing CP group with BP group.
**Keywords**: breech presentation; mammals; postural development; gestation; probability.

**INTRODUCTION**

Etiology of fetal presentation in mammals has not been fully clarified yet. In a published paper it was postulated that fetal presentation is a consequence of postural development [1]. In human species development of the subcorticospinal system, which maintains postural reflexes, occurs prenatally and has a caudo-cranial direction of development. After the onset of fetal postural development, the cephalic presentation (CP) enables easier performance of movements of the legs. The direction of the gravity force in the human species is reversed compared to quadrupedal mammals. In quadrupedal mammals postural development has craniocaudal direction and occurs during the second half of gestation. The fetus’ anterior presentation (CP), in which the cranial part of the body is above the caudal part of the body, releases the cranial part of the body from the weight of the caudal part. This enables easier performance of movements of the cranial part of the body. When the postural development has affected all segments of body, the hind legs are the source of the main propulsive force and anterior presentation provides an optimal mobility. When postural development is absent the fetus assumes a presentation randomly, with the same probability for a CP and breech (posterior) presentation (BP). To test this hypothesis, investigations have two directions. The aim of one direction is to test gravity-dependent reaction of the fetus in experimental animals. An ultimate goal is to prove that gravity-dependent presentation of the fetus occurs after its postural development takes place. The aim of the second direction is to prove that the probability of BP in any given medical (veterinarian) entity is no more than 50% [1].

**MATERIAL AND METHODS**

To prove or disprove the gravity-dependent behavior and presentation, experiments have been conducted with two precocial species: guinea pig and sheep. In the guinea pig, prenatal onset of the righting reflex and gravity-dependent presentation of the fetus were examined. Ultrasound examinations were performed from gestation day (GD) 26 until term delivery on GD 65. For examination of the righting reflex from a supine to prone position, the fetus was brought into a supine position relative to the gravity vector. During the next two minutes any changes in fetal presentation were observed. In this study 40 pregnant females with one to four fetuses were included. The experimental procedure was descri-
bed in detail in a previous article [2]. For examination of gravity-dependent presentation, eight singleton gestations were included and the fetuses were brought into a head-down position relative to gravity and any changes in fetal presentation were observed for 5 minutes.

Reaction of sheep fetuses to an upside-down position were examined on GDs 83, 86 and 116. The inclusion criteria were singleton gestation, and CP of the fetus. Ultrasound examinations were performed using a Model DP 6600 instrument (Mindray Biomedical Electronics Co Ltd, Manchan Chenzhen, China) with a sector probe of 3.5MHz. Each experimental animal was first examined in an upright quadrupedal position in order to determine fetal presentation. The ewe was then positioned into a sitting position so that their trunks were vertical to the ground and kept in that position for two minutes, during the second ultrasound examination. After returning the animal to an upright quadrupedal position, the third scan was performed. Fourteen fetuses were examined on GD 83 and ten fetuses on GD 86. On GD 116 examination was performed twice: first in 12 fetuses and after three hours it was repeated in 11 fetuses. During the examination on GD 116 all fetuses assumed a transverse presentation in response to the ewes’ vertical position. Examination was therefore repeated after three hours. In order to keep the fetus in a longitudinal presentation, the ewe’s abdominal wall was manually pressed on the opposite side from the fetus. The experimental procedure was described in detail in a previous article [3].

Examination of the types of movements which human BP newborns use from the second to the fourth day of life, which are identical to fetal movements, included: popliteal angle; extension of the hip-joint; ventral flexion and dorsal extension in the axis; spontaneous displacement; crawling reflex; righting reaction in vertical and sitting positions; righting reaction in horizontal suspension, the automatic walking. Subjects were 50 BP and 87 CP term newborns delivered by elective cesarean section following a regular course of pregnancy, without any fetal, neonatal or maternal disease. The experimental procedure has been described elsewhere [4].

To prove that maximum probability of BP in any given medical or veterinarian entity is 50%, investigation included incidence of fetal presentation during gestation in the human general population as well as in quadrupedal mammals, incidence of BP in medical entities that have a higher probability of BP than the general population (>3%), related to single series of medical entities, collection of series of medical entities, data obtained from repeated observation under same conditions, series of two concomitant medical entities.

RESULTS

The righting reflex from a supine to prone position in the guinea pig fetus
may be induced, and the highest percentage of positive responses is in period from GD 61 to GD 66 (16.51%). The most probable type of the righting reflex is contact-righting. Although the percentage of positive responses is small, the righting reflex shows that there is continuity between prenatal and postnatal gravity-dependent behavior [2]. After the occurrence of the first movements on GDs 25 and 26, the guinea pig fetus is capable of changing presentations only for 5-6 days. After that, throughout the second half of gestation, its intrauterine presentation is fixed (unpublished data). Published data show that in humans and herd mammals there is a change in presentation in the second half of gestation. The incidence of presentations and lies during gestations with one fetus in human species, rhesus monkeys, and precocial mammals that live in a herd indicates the existence of three stages. The beginning of the first stage is characterized by equal proportions of longitudinal and transverse lies with equal proportions of CP and BP within the longitudinal lie. During this stage, there is an increasing incidence of longitudinal lie with a proportional decrease in transverse lie. In the second stage, which occurs in the second half of gestation, a transverse lie is almost completely absent, whereas in the longitudinal lie there is an increasing incidence of CP and a proportional decrease in BP. By the end of this stage, around 95% of fetuses are in a longitudinal lie with CP. In the third stage, during the last weeks of gestation, there is a further mild increase in the incidence of CP [3,5]. As expected in the period of equal incidence of CP and BP, turning sheep fetuses upside down did not result in changes of fetal presentation. When fetuses were put in an upside-down position by manually pressing the ewes’ abdominal walls during the gestation period characterized by predominance of CP, only two of five cases changed the position to BP and returned to CP at the end of experiment, when the ewes were again in quadrupedal position. However, one of the three cases that did not change CP for BP in an upside-down position, and changed CP for BP only after returning the pregnant ewe in a quadrupedal position [3].

Compared with human CP newborns, human BP newborns had a significantly lower score at the first and second examination in spontaneous displacement, crawling reflex, righting reaction in vertical and sitting positions, and the automatic walking [4].

Available data related to single series of more than 50 known medical entities that have a higher probability of BP than that which occurs in the general population revealed that the probability of BP is between 4-50%. Review of the available literature data on the incidence of BP in several series of same medical entities shows clustering of the incidences of BP: First twin 17-30%, Second twin 28-39%, Anencephalus 6-18%, Congenital hydrocephalus 24-37%, Spina bifida 20-30%, Leiomyoma uteri 9-20%, Uterus didelphys 30-41%. Data obtained from repeated observations under the same conditions shows that the pro-
bability of BP at first and second birth among multiparas with a congenitaly malformed uterus are the same. Also, in the general population, probability of repeated BP does not exceed 38% even after three previous breech deliveries. Simultaneous presence of two medical entities, twin gestation in a uterus with two bodies, each associated with an increased incidence of BP relative to general population, are not associated with a probability of BP over 50%: Twin A 14.29%, Twin B 18.52% [5].

CONCLUSION

The guinea pig is not appropriate as an experimental model to investigate gravity-dependent presentation after appearance of postural development, because during this period there are no changes of presentations. It is necessary to surgically fix the horn of the uterus with the sheep fetus inside, in the stable position prior to studying fetal reaction to changes of gravity vector in the intrauterine environment [3]. The obtained data confirm the assumption that BP represents a mere filling of the intrauterine cavity, with an equal probability for CP and BP. Group of CP fetuses is heterogeneous: some fetuses assume CP «intentionally» while others assume CP randomly. The number of fetuses that randomly assume CP is identical to the number of fetuses in BP. Prior to comparison, the bias should be eliminated by subtracting from the CP group the number of fetuses/newborns that is identical with the number of BP fetuses/newborns with identical characteristics (excluding those related to delivery mode). This subtracted group of CP fetuses/newborns should be added to the BP group before comparison with the rest of the CP fetuses/newborns [5].

ACKNOWLEDGEMENTS

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REFERENCES


Uterine Rupture Intrapartum – About a Clinical Case

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SUMMARY

It is well documented that the risk of cesarean delivery for women is higher with the increasing number of cesarean sections. The authors describe the case of a 31-year-old pregnant patient with a history of one previous cesarean section 6 years ago. It was a pregnancy followed in a private practice, uneventful, with an elective cesarean section at 38 weeks and 5 days for breech presentation. Upon entry into the abdominal cavity, an haemoperitoneum was found intraoperatively with a rupture in the anterior uterine wall at the isthmic region and a free fetus in the abdominal cavity. A uterine rupture is a serious complication for the mother and for the newborn, which occurs in 0.2 to 0.8 % of labors after cesarean.

Keywords: uterine rupture, previous caesarean section, hemorrhagic shock.

INTRODUCTION

Uterine rupture is a rare peripartum complication associated with severe maternal and perinatal morbidity and mortality and previous caesarean section is the main risk factor for uterine rupture [1]. This risk is decreased in case of previous vaginal delivery and increased in case of classical C-section, previous uterine rupture, inductions of labor, more than one previous caesarean section, fetal macrosomia and interval less than 6 months before the next pregnancy [2].
This includes potentially serious complications such as bleeding, surgical complications and adherent placenta.

The uterine rupture is defined as a solution of continuity of the uterine wall during pregnancy or labor. It corresponds to two entities of different prognostic: the complete and the incomplete uterine rupture. The complete rupture can be accompanied by a solution of the fetus and/or placenta into the abdominal cavity; it is the most symptomatic and needs an urgent surgical repair. The incomplete uterine rupture or sub-serous, often referred to as dehiscence, respects only the visceral peritoneum. It is often asymptomatic and has better maternal-fetal prognosis than the complete rupture.

The uterine rupture can occur suddenly and the antenatal predictive risk markers are often faulted. An early diagnosis is essential to minimize the fetal-maternal mortality and morbidity. The clinical manifestations may vary and the clinical symptomatic triad – continuous and intense suprapubic pain, decreasing intensity of contractions and metrorrhagia – often fails. The most frequent sign is the rough and severe anomaly of the fetal cardiac rhythm (found in 55-87% of all uterine ruptures). In 50 to 70 percent of cases there is an intense secondary abdominopelvic pain that persists between the contractions or is resistant to analgesia. The genital hemorrhage and the macroscopic hematuria are less common (28 and less than 5 percent). The uterine dynamic modifications can be seen (hypertonicity or hyperkinesia). The non-perception of the fetal presentation when previously perceived and the maternal hemodynamic instability are other signs of rupture. At last, the echographical signs of an haemoperitoneum can not delay the surgical intervention.

**MATERIAL AND METHODS**

The authors describe the case of a 31-year-old pregnant patient with a history of one previous cesarean section (low transverse incision) 6 years ago because of a failure to progress. It was a pregnancy followed in a private practice, uneventful, with an elective cesarean section at 38 weeks and 5 days for breech presentation.

**RESULTS**

Upon entry into the abdominal cavity, a complete uterine rupture with an haemoperitoneum was found intraoperatively. She had no contractions detected on cardiotocograph and no maternal or fetal symptoms were detected before the
intervention. The rupture was located in the anterior uterine wall at the isthmic region. The fetus was found free in the abdominal cavity but the placenta was still adherent. The newborn was extracted, weighing 3030gr and with a first and five-minute Apgar score of 8 and 9. After clamping the cord, findings of placenta increta caused excessive blood loss requiring a life-saving partial removal. The postpartum woman evolved with hemorrhagic shock was transferred to the Intensive Care Unit, with hemoglobin of 5.3 g/dL and Simplified Acute Physiology Score (SAPS II) score 26, where she remained for 15 days. While in ICU, she required multiple transfusions – a total of 6 units of red cell concentrates and 2 units of fresh frozen plasma, as well as triple antibiotics coverage and vasopressor support.

CONCLUSIONS

A uterine rupture is a serious complication for the mother (mortality <1 percent, serious morbidity 15 percent) and for the newborn (3 to 6 percent term mortality, perinatal asphyxia 6 to 15 percent), which occurs in 0.2 to 0.8 percent of labors after cesarean [2].

Women with previous caesarean are also at higher risk for abnormal placenta insertion, the strength of the association increasing with the number of previous caesareans: twice higher risk of placenta praevia and greater maternal morbidity associated with placenta praevia; major risk factor for placenta accreta in particular in women combining previous caesarean and placenta praevia [3].

The rupture in a healthy uterus is a rare event, with an estimated incidence of 0.3-0.8 per 10000 deliveries in high socioeconomic countries, increasing for 1 percent in emerging countries.

The available data do not reveal the exact frequency of complete or incomplete uterine rupture. The risk is higher in case of corporal vertical scar (classical or T-incision).

Complete uterine ruptures that occur at prelabor elective caesarean section with low vertical uterine scar and transverse scar in the lower corpus indicate that the type of scar plays a role in prelabor ruptures and elective caesarean section should be scheduled no later than 38 weeks [1].

The individual risk of uterine rupture is multifactorial and so the scores have no predictive value in clinical practice. Women with uterine rupture were significantly more likely to have postpartum complications than women without uterine rupture [4].

The subsequent pregnancy is not formally contraindicated but there is a high risk of new rupture and recurrent cesarean section in new pregnancy is, therefore, recommended.
REFERENCES


Preterm Birth and Teenage Pregnancy: a 2 Years Review

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SUMMARY

Teenage pregnancy has been associated with maternal morbidity, mortality and with poor perinatal outcomes. Pregnancy in this age group is often related to preterm births. The objective of this study was to compare preterm birth rate in adolescence and adult groups, as well as the mode of delivery, birth weight and Apgar score (AS) at 1st minute. There were no statistically significant differences between the two groups for any of the parameters analyzed.

Keywords: Adolescent, pregnancy, preterm, delivery.

INTRODUCTION

Presently the increased incidence of pregnancies at the extremes of reproductive age group is a reality. In Europe, Portugal has one of the highest rates of pregnancies in the 15-19 years age group, although the birth rate among teenagers in our country has fallen in recent years (16,17 per 1000 in 2008 and 12,15 per 1000 in 2012) and remains lower than the general fertility rate (36,29 per 1000 in 2012) [1]. However, teenage pregnancy is an important threat, since it has been associated with a higher rate of maternal and fetal morbidity, mortality, with increased preterm delivery, fetal growth restriction, and newborns with low birth weight for gestational age [2, 3], factors worthy of consideration in relation to perinatal morbidity and mortality. Those associations however have not been consistently demonstrated and different arguments have been proposed to
justify the less favorable outcomes of these pregnancies, such as poor health habits with no adjustments in lifestyle that are required to promote a healthy pregnancy [3].

Although nonconsensual, has yet been proposed higher incidence of instrumented deliveries and lower cesarean rate in this age group [4]. That may be due to the immaturity of the birth canal. However, recent studies contradict this association based on the fact that teenagers have a higher rate of preterm birth and lower weight newborns [3].

MATERIAL AND METHODS

In the study, all the parturients in HESE between 2011 and 2012 were divided into two groups: adolescents (10-19 years old) and adults (20-34 years old). Women over 34 years old were excluded due to the higher incidence of medical complications and poor perinatal outcomes in this population. The variables analyzed included gestational age at the time of delivery, type of birth, birth weight and AS at 1st minute. The data was processed and analyzed using the SPSS Statistics 20. The test used for comparison of results was the $\chi^2$ and significance level of 0.05 was adopted.

RESULTS

We evaluated 2081 births. The mean age in adolescent group was 17.7 years old with a median age of 18 years old. The rate of teen births was 5.6%, with a lower rate of prematurity and cesarean sections compared to adults, but higher incidence of newborns with low birth weight and AS<7 at first minute (Table 1) and instrumented deliveries. The difference was not statistically significant between the two groups in any of the parameters analyzed (Table 1).

CONCLUSIONS

Although there was a lower cesarean rate and prematurity among teens, but a higher incidence of instrumented deliveries, low birth weight and AS <7, the differences were not statistically significant between the two groups. This may be due to the existence of a differentiated monitoring of these young pregnant women in our Hospital, which is in agreement with recent studies showing that if a pregnant teen receives high quality maternity care, the maternal and perinatal outcomes will be identical to or even better than those of the remaining pregnancies [5, 6].
Tab. 1. Comparison between adolescent and adult groups for gestational age at delivery, birth weight, Apgar score at 1st minute and type of birth (very low birth weight [VLWB], low birth weight [LBW], normal weight [NW], Apgar score [AS] at 1st minute, no significant [NS]).

<table>
<thead>
<tr>
<th>Type of birth</th>
<th>Teens</th>
<th>Adults</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLWB (&lt;1500 g)</td>
<td>2 (1.7%)</td>
<td>32 (1.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>LBW (≥1500-&lt;2500 g)</td>
<td>13 (11.1%)</td>
<td>133 (6.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>NW (≥2500-&lt;4000 g)</td>
<td>100 (35.5%)</td>
<td>1727 (87.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Macrosomic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥4000 g</td>
<td>2 (1.7%)</td>
<td>72 (3.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>AS&lt;7</td>
<td>4 (3.42%)</td>
<td>71 (3.21%)</td>
<td>NS</td>
</tr>
<tr>
<td>AS≥7</td>
<td>112 (95.73%)</td>
<td>1873 (95.37%)</td>
<td>NS</td>
</tr>
<tr>
<td>Stillbirth</td>
<td></td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>?</td>
<td>1 (0.81%)</td>
<td>20 (1.02%)</td>
<td>NS</td>
</tr>
<tr>
<td>Eutocic</td>
<td>56 (48%)</td>
<td>925 (47%)</td>
<td>NS</td>
</tr>
<tr>
<td>Distocic</td>
<td>25 (21%)</td>
<td>356 (15%)</td>
<td>NS</td>
</tr>
<tr>
<td>C-section</td>
<td>36 (31%)</td>
<td>743 (38%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

REFERENCES

Investigation of Thermal Effects Caused by Interaction of Drugs with Soft Tissues

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SUMMARY

In conditions of the experiment and clinics in the infrared range of a spectrum of radiation of fabrics we undertook a study of the radiation intensity of the heat and the state of the following parts of the body: the skin, subcutaneous fat, of the subcutaneous blood vessels and installed into the veins of intravascular catheters as well as the cornea and mucous membranes cavity of the conjunctiva, before, during and after the local application of high-quality medicines. The high security, informative and promising applications of infrared thermal imaging and thermography for safe beam diagnostics in medicine with the purpose of evaluation the local safety of medicines.

Keywords: temperature, infrared thermography, injection disease, drugs.

INTRODUCTION

For a long time the process of introduction of drugs in the organism of patients not controlled by the reaction of tissues, interacting with drugs as their absorption and penetration of the blood, because there were no methods of safe and informative visualization processes of local pharmacokinetics [1-5]. In
connection with this remained unknown features of the local drug interactions with various tissues and causes of their injuries caused by the introduction of certain medicines into the organism. Moreover, these complications associated with the violation of the technologies of introduction of drugs, so the perpetrators of complications were medical workers, violated the technology of introduction of drugs [6-10].

Today there is no specific solutions for injection, produced exclusively for safe introduction into the body of newborns. Therefore, to the body of children administered drugs, formulated for administration to the body of adults. However, the post-injection safety of these drugs for liquid and soft tissues of children and adults is not carried out, so any injection of any drug can cause local reversible or irreversible damage of tissues [11-20].

However, in recent years, there is evidence that the cause local damage can be themselves drugs, because modern standards of their quality admit that they have денатурирующего action [21-23]. In these circumstances, the identification of universal indicators of the local pharmaceutical aggressiveness of drugs is an urgent task.

MATERIAL AND METHODS

We conducted a retrospective analysis of the areas of local application of solid and liquid medicines in 1000 patients in the hospital and out-patient treatment during the 2000-2012. The study of the condition of the places of injections in the visual, ultrasonic and infrared study of 100 patients are female and have 100 patients of male sex during their hospital treatment in the various branches of several city hospitals of the city of Izhevsk in 2012. The study was approved by the Ethics committee at Izhevsk State Medical Academy and complied with the Declaration of Helsinki. All patients gave informed consent.

Experiments were carried out on 20 keepers of pigs, which have carried out monitoring of a condition of the tissues of the oral cavity and congestion after local application of tablets and eye drops (respectively) and the condition of the skin and subcutaneous fat after subcutaneous injection of solutions 40 drugs before and after reconstitution with water. Dynamics of a tissue with the introduction of these drugs was estimated in different years on the eye, with the help of the ultrasonic brand Aloka SSD-900 and through determination of the Dynamics of temperature and infrared radiation of bodies by ThermoTracer TH9100XX (NEC, USA). The obtained data were processed using software Thermography Explorer and Image Processor.
RESULTS

Our results indicate that every patient in the hospital and out-patient treatment is being daily from 1 to 12 oral tableted medicines and from 1 to 25 of hypodermic, intramuscular and intravenous injection of solutions of medicines. Therefore, today the absolute majority of the drug is introduced into the organism of patients through intravenous injection.

However, the medical records do not contain any information about the state of tissues on the introduction of the pills, eye drops and solutions for injections. Including the absence of information about the appearance of inflammation, bruises, hematomas and abscesses in the field of local action of eye drops, tablets and solutions for injections. At the same time, the observation of the state of organs of vision in adult patients with conjunctivitis and the babies with the introduction of these solution 25% sodium sulfacyl, conducted by the inspection of the skin of the buttocks and the elbow of the basins in pregnant women after multiple intramuscular and intravenous injections of plasma substitutional solutions and hemostatic products, as well as conducted by the observation of the state of mucous membranes of the mouth, gums and cheeks for women in the resorption of tablets acetylsalicylic acid, showed the presence of local inflammation at all 100% of patients.

Then we conducted an analysis of the legal list of controlled indicators of the quality of solutions for injection and the ranges of their possible values. It turned out that Russian, British and European Pharmacopeas requirements do not regulate the production of solutions for injection with a pH of 7.4, with an indicator of osmotic activity 280 mOs/m/l of water, as well as with the lack of local inflammatory (annoying) and denaturation (caustic) of action on the tissues in the ways the introduction of drugs. Therefore, solutions for injections that are considered today quality, can provide a cauterizing an (denaturation of action) action.

With this in mind we have conducted a study in experiments. The observation of the state of organs of vision in the pigs with the introduction of these solution 25% sodium sulfacyl, conducted by the inspection of the skin showed the presence of local inflammation at all 100% of pigs. We have obtained data testify to the fact, that for the forecast of the local drug interactions is sufficient to determine the dynamics of local tissue temperature for 10 minutes after the start of their interaction with the drugs. It is established that the drugs have annoying and/or cauterizing an action, cause, and medication, deprived of pharmaceutical aggression, not a cause in this period of time the local hyperthermia in places of local interactions.

After this we decided to use the tissue temperature in the role of indicator urgent rapid assessment of their reaction to drugs. As the most secure, accurate,
urgent and documented way of measuring the surface temperature of a thermal imager, to solve this problem we used a monitoring with the help of thermal imager of heat emission. Our results show that urgent monitoring with the help of thermal imager the local temperature of the mucous membranes of the lips when resorption of tablets acetylsalicylic acid, ketorol, or ascorbic acid, as well as the mucous membranes of the conjunctiva in instillation in the eye of the eye drops containing a solution of 25% sodium sulfacyl, or the skin of the buttocks when the intramuscular injection and/or the skin of the antecubital fossa with injections in cubital Vienna 5 ml solution 25% magnesium sulfate really allows you to receive early information on the fate of tissue on the introduction of drugs. Moreover, we have obtained data testify to the fact, that for the forecast of the local drug interactions is sufficient to determine the dynamics of local tissue temperature for 10 minutes after the start of their interaction with the drugs.

As an example, the image of the person of the newborn on the screen thermal imager at the time of and after 2 minutes after the introduction of drops of solution 25% sodium sulfacyl at a temperature of + 24 °C in the cavity of the conjunctiva of the left eye.

It is established that the drugs have annoying and/or cauterizing an action, cause, and medication, deprived of pharmaceutical aggression, not a cause in this period of time the local hyperthermia in places of local interactions.

Therefore, the registration of the dynamics of the local temperature tissue in places of local interactions of medicines, implemented for the first 10 minutes after injection of medications, can claim the role of a universal indicator of Express-diagnostics of the pharmaceutical tissue damage to the introduction of drugs injection site appears bruise pharmaceutical irritation and local non-infectious inflammation of skin and soft tissues, a new disease, which received the name of «Injecting disease of skin and subcutaneous fat» [24, 25].

Established that the cause of the disease is interstitial physical-chemical burn, which cause the medicine with high pharmaceutical aggression. Proposed original methods and tools for rapid diagnosis, prevention and treatment of injection disease.

CONCLUSIONS

Changing the heat radiation of tissue during their interaction with the drugs proposed to consider as a universal criterion of the local drug safety when administered to the mother, her fetus and newborn. It is shown that the temperature of the tissue at the site of safe drug may decrease for a short time, but then normalized. Medicine, with irritant or cauterizing action, cause long-lasting local hyperthermia.
It is shown that many medicine has high pharmaceutical aggression, so their injections cause the disease of skin and soft tissue, which was named injecting disease.

Established that the thermal monitoring the local temperature in the injection site has a high prognostic value for the diagnosis and treatment of injection disease.

REFERENCES

Impact of Advanced Maternal Age and Parity on the Pregnancy Outcome

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SUMMARY

Advanced maternal age has been described as a risk factor for an adverse pregnancy outcome, with a higher incidence of preeclampsia, gestational diabetes, placental abruption and preterm delivery. Older women’s deliveries are also more often induced, instrumented or by caesarean section. There are studies that also support an association between increased incidence of cesarean section in elderly nulliparous women (≥ 35 years-old).

Keywords: Older pregnant women, instrumented delivery, caesarean section, nulliparity.

INTRODUCTION

Nowadays, delayed childbearing and the increased incidence of pregnancy in elderly women is a reality all over the world and Portugal is not exception. The possibility of a late fertility offered by Assisted Reproduction Techniques in last decades and career investment delayed the plan of motherhood in women, currently. The aim of the study was to compare demographic characteristics and clinical outcomes of pregnancy in advanced and normal reproductive age women. The study goal was also to analyse the prevalence and reasons of cesarean section and instrumental deliveries in pregnant women in advanced age (≥ 35 years), as well as the impact of parity on pregnancy outcome in the Espírito Santo Hospital in Évora (Portugal).
MATERIAL AND METHODS

This was a retrospective study in pregnant women in labour at Espírito Santo Hospital in 2012, divided into two groups: young (20-34 years) and advanced maternal age (≥ 35 years). The variables were gestational age at the time of delivery, parity and type of delivery. Pregnant adolescents were excluded because they are also associated with adverse pregnancy outcome in some publications. Data were obtained by consulting the clinical processes and analyzed using SPSS Statistics 20. The tests used for comparison of results were the Student’s t test and chi-square test ($\chi^2$) and significance level adopted was 0.05.

RESULTS

The study included a total of 1218 women. The rate of births in older women group was 24.3% and the average of maternal age was 37.05 years-old in this series. There was a higher incidence of preterm birth and cesarean section in nulliparous pregnant older women. The difference was statistically significant between the two groups in parameters analyzed, except about dystocic delivery in elderly women, that was lower.

<table>
<thead>
<tr>
<th>Type of Birth</th>
<th>young women (20-35)</th>
<th>Elderly women (≥ 35)</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eutopic</td>
<td>429 (46.52 %)</td>
<td>133 (44.93 %)</td>
<td>NS</td>
</tr>
<tr>
<td>Distocic</td>
<td>145 (15.73 %)</td>
<td>24 (8.11 %)</td>
<td>S</td>
</tr>
<tr>
<td>C-section</td>
<td>348 (37.74 %)</td>
<td>139 (46.95 %)</td>
<td>S</td>
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<tr>
<td>sub-total</td>
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<td>296</td>
<td></td>
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</tbody>
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<table>
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<th>Preterm birth</th>
<th>young women (20-35)</th>
<th>Elderly women (≥ 35)</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60 (6.51 %)</td>
<td>33 (11.15 %)</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Parity</th>
<th>young women (20-35)</th>
<th>Elderly women (≥ 35)</th>
<th>Significance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous</td>
<td>50 (58.14 %)</td>
<td>36 (41.86 %)</td>
<td>S</td>
</tr>
<tr>
<td>Multipara</td>
<td>89 (42.88 %)</td>
<td>121 (57.62 %)</td>
<td>S</td>
</tr>
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</table>
CONCLUSIONS

Advanced maternal age appears to influence the pregnancy outcome, with a higher rate of cesarean section, preterm but not instrumental delivery. It was also found that nulliparity is a risk factor for cesarean section in the older group.

The main reasons for this to happen were previous caesarean section, dynamic dystocia, unsuccessful labour induction, fetal distress and complications like pregnancy-induced hypertension and preeclampsia.

REFERENCES

1. Klemetti R, Gissler M. Associations of maternal age with maternity care use and birth ou-


Matrix Metalloproteinases and Its Inhibitors: the Role in the Pathogenesis of Preeclampsia

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SUMMARY

The article reports about matrix metalloproteinases 2 and 9 and inhibitor of metalloproteinase type 2 as a relatively new factors that play a role in the pathogenesis of preeclampsia. Presents data about different concentrations of these factors in the blood of women with severe preeclampsia compared to healthy non-pregnant and pregnant women. The work found significant correlations of concentration of metalloproteinases with the clinical manifestations of preeclampsia, pro-angiogenic and anti-angiogenic factors.

Keywords: angiogenesis, implantation, hypertension, prediction, gelatinase.

INTRODUCTION

Worldwide more than 60,000 women a year die from preeclampsia (PE) [1, 2]. Preeclampsia takes II-III place in the structure of maternal mortality in Russia [3]. Despite the fact that the maternal mortality rate in PE recently reduced, hypertensive complications during pregnancy are one of the most serious and unfortunately the unresolved problems of modern obstetrics.

Was established that in the remodeling of extracellular matrix during implantation leading role played matrix metalloproteinases (MMPs) type 2 and 9, which are crucial effectors of this process. MMPs are unique prote-
olytic enzymes, denaturing the collagen, which is a structural protein of the basal membrane. In experiments in vivo it was shown the participation in the formation of the placenta only gelatinases (enzymes type 2 and 9) [4, 5]. The matrix metalloprotease-2 with MMP-9 is involved in the degradation of type IV collagen which is the main component of the basal membrane and gelatin (denatured collagen) and also destroys other types of collagens (V, VII and X), elastin and fibronectin. There are biological mechanisms limiting tissue proteolysis caused by active MMPs, as stromal cells secretion tissue inhibitors of metalloproteinases (TIMPs). TIMPs are small proteins capable of forming covalent complexes with many of the MMPs [6]. Study of changes in the concentration of MMPs may be a theoretical basis for the development of new methods of diagnosis and prognosis in obstetrics and neonatology, improving perinatal outcomes and reducing the incidence of complications of pregnancy.

MATERIAL AND METHODS

During the work we used a prospective cohort method. The work carried out at the Department of Obstetrics and Gynecology of the Stavropol State Medical University. Pregnant women were examined, they treated in the intensive care unit. They are the main group (n=17). The criteria for inclusion of patients in the main group were: the presence of clinical manifestations of severe PE, singleton pregnancies, lack of somatic diseases, autoimmune diseases and the patient’s informed consent for inclusion in the study. The comparison group consisted of women with full-term physiological pregnancy (n=15) and control group consisted of healthy non-pregnant women (n=15).

Initially we analyzed the following parameters: anamnesis, characteristics of pregnancy and childbirth, delivery and outcomes of newborn. After that we performed enzyme-linked immunosorbent study of angiogenic status. We used for this purpose standard test system Bender MedSystems GmbH (Austria) for determining the concentration of MMP-9 and R&D Systems (USA) for the determination of MMP-2 and TIMP-2. Blood sample was taken from the ante-cubital vein in an amount of 10 ml in vacuum tubes Vacutainer (Becton Dickinson, USA) with a coagulation activator (silica). The material for the study was the blood serum.

Statistical analysis we performed using Statistica 10 (StatSoft, USA). The critical level of statistical significance in testing the null hypothesis was assumed to be 0.05. To analysis of the relationship between the analyzed signs, we used a logistic regression model with stepwise algorithm of inclusion and exclusion of predictors and discriminant analysis.
RESULTS

The age of pregnant women in the main group was between 19 and 43 years (mean age 29.47 years; standard deviation (SD) = 6.9), age of the patients in the control group was 19 to 39 years (mean age 28.46 years; SD = 6.4).

Differences between groups were determined by using the discriminant analysis. From the full data set of clinical and laboratory investigation highlighted a number of features that differ in the maximum degree of the analyzed group.

We also studied the concentrations of other factors which are important in angiogenesis (Vascular endothelial growth factor A, VEGF-A and Soluble fms-like tyrosine kinase-1, sFlt-1). Along with the standard features that explain the differences between women with PE, healthy pregnant and non-pregnant (the values of blood pressure, proteinuria, coagulation parameters) analysis allowed us to identify the main characteristics of the angiogenic status, in which different groups surveyed: VEGF-A (reducing its concentration is reflected in a shift of the distribution of data to the left), sFlt-1 (increase in concentration leads to a shift to the left of observations), and MMP-2, TIMP-2, MMP-9 (Table 1 and Fig. 1).

Mean values with the average error of the mean (m) of considered parameters, including introduced by us in the study of two surrogate markers – TIMP-2/MMP-2 ratio and MMP-9/MMP-2 – considered in the Table 2. Obviously, in the serum of women with severe preeclampsia occurs increase in TIMP-2, a slight decrease in both metalloproteinases, and a characteristic reduction in comparison with healthy pregnant MMP-9/MMP-2 ratio.

Because VEGF-A induces the formation of matrix metalloproteinases involved in angiogenesis, an attempt was made to trace the relationship of the analy-

Tab. 1. Assessment of the contribution of individual indicators of angiogenic status in the formation of the discriminant functions.
We found it necessary the study of the relation of TIMP-2 and its substrate MMP-2. As a result, we had found a significant negative correlation of VEGF and TIMP-2/MMP-2 ratio ($r=-0.52; p \leq 0.0001$).

**Tab. 2.** Concentration of metalloproteinases -2, -9 and tissue inhibitor of metalloproteinase type 2 in the serum of women.

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</thead>
<tbody>
<tr>
<td>Severe preeclampsia</td>
<td>26,27</td>
<td>7,08</td>
<td>319,7</td>
<td>22,08</td>
<td>168,3</td>
</tr>
<tr>
<td>Healthy pregnant</td>
<td>30,01</td>
<td>9,19</td>
<td>283,21</td>
<td>17,18</td>
<td>171,59</td>
</tr>
<tr>
<td>Healthy nonpregnant</td>
<td>27,41</td>
<td>5,83</td>
<td>245,71</td>
<td>11,63</td>
<td>31,26</td>
</tr>
</tbody>
</table>

Fig. 1. The distribution of the analyzed observations in the discriminant functions (the angiogenic status).
Comparison of the concentration of MMP-2 with another protease, which plays an important role in the process of cytotrophoblast invasion (MMP-9), showed a significant positive correlation ($r=0.53; p \leq 0.0001$). The obtained ratio isn’t the same as the majority of studies examining the role of gelatinases in the development of pre-eclampsia. However, given that MMP-2 and MMP-9 facilitates the formation of the vascular network (a process that is disrupted from the beginning of pregnancy complicated by PE in the future), the one-stage-dependent reduction of both proteases is justified.

We didn’t detect significant association between MMP-2 and its inhibitor (TIMP-2) for analyzing the data obtained during the examination of the main group, at the same time in healthy pregnant this pair showed very high and a significant positive correlation ($r=0.575; p \leq 0.0001$). This fact we can explain clearly coordinated process of angiogenesis and implantation in physiological pregnancy. During PE, which is based on a defect of implantation, we observe a violation of the relationship of collagenase and collagenase inhibitor. We also found statistically significant correlations of concentration of MMP-2 with the level of aspartate aminotransferase (negative correlation), height and weight of the newborn (positive correlation), concentration of TIMP-2 with the level of proteinuria, duration of cesarean section (positive correlation), with the level of total protein (negative correlation); MMP-9 with coagulation parameters (platelet count, activated partial thromboplastin time – a positive correlation), features a state-of placenta (estimated by the Apgar score, weight of the placenta – a positive relationship).

**CONCLUSIONS**

Therefore comparing the new markers, while unused for predicting the development or assessment of the severity of manifest PE with clinical and laboratory parameters, long well-established ourselves for this, we can assume that the determination of the content in the peripheral blood of metalloproteinases 2 and 9 types of inhibitor of metalloproteases-2 may be effective for diagnosis already developed PE and for the prediction of its development.

**REFERENCES**

«The Film Study»: a Randomized Controlled Trial of an Informational Film About Prenatal Examinations

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SUMMARY

In Sweden pregnant women are offered ultrasound in the second trimester. Combined Ultrasound and Biochemical screening (CUB) may be offered for risk estimation for chromosomal aberrations but the routines varies over the country. Invasive test, amniocentesis or chorion villus sampling, is offered when the risk for chromosomal aberrations is high due to screening test results or due to the maternal age. It is essential to have access to correct, non-directive information to be able to make decisions, informed choices, about prenatal examinations, and this is a great challenge for the antenatal health care.

An information film was developed to facilitate the information about prenatal examinations. A randomized controlled trial was performed. Women were allocated to either film (n = 184) or not to film (n = 206). All received the usual written and verbal information about prenatal examinations. The data collection was performed by questionnaires in gestational week 27. The primary outcome was informed choice about CUB-test. Further research questions were informed choice about the second trimester ultrasound examination and about worry and anxiety in relation to watching the film.

Conclusions from the study are that the film increased the number of informed choices about CUB but did not affect the number of informed choices about the second trimester ultrasound. An informational film as a complement
did not appear to increase women’s anxiety or worries in a long time. However, the informational film seemed to cause worry at the time of viewing.

The uptake of CUB did not increase in the intervention group.

**Keywords:** Prenatal examinations, Information, Informed choice, Worry, Anxiety.

**INTRODUCTION**

In Sweden all women in early pregnancy are offered a second trimester ultrasound examination and are provided information about it. The vast majority accept this offer [1]. Until 2006 screening for Down syndrome (DS) was not routinely offered in Sweden. From a Swedish review in 2006 [2], it was concluded that CUB (Combined Ultrasound and Biochemistry) [3, 4] was the best method for Down syndrome screening (DSS) in early pregnancy. If the risk is estimated as high an invasive test, amniocentesis or chorion villus sampling is offered. Another conclusion was that the information before screening was inadequate for the women to make informed choices. Information about DSS is complex in many aspects [2, 5]. Information about prenatal diagnosis should enable a woman to make an informed choice [6], that is, a choice based on relevant knowledge, consistent with the decision-makers’ values and behaviorally implemented [7]. Informed choice in the context of prenatal screening means to undergo prenatal examinations when the woman has relevant knowledge about the test, a positive attitude towards it and actually undergoes it.

Relevant knowledge to undergo a screening test include knowledge about the purpose of the test, the likelihood of positive and negative findings, the possibility of false positive and false negative results, the uncertainties and risks attached to the screening process and any significant medical, social or financial implications of screening and follow-up plans [8].

Information about prenatal examinations is a challenge for the antenatal care. A review from 2009 concluded that patient decision aids increase people’s involvement and is more likely to lead to informed, values-based decisions, but the effect varies across studies. The degree of detail that patient decision aids require for positive effect on decision quality should be explored [9].

«The film-study» contented three aims responded to in three different publications (listed below). The first aim was to evaluate the effects of an information film on making an informed choice regarding Down syndrome screening, and women’s knowledge and experiences of information. The second aim was evaluate effects of an informational film on making an informed choice regarding second trimester ultrasound. Finally, the third aim was to explore if an
information film about prenatal examinations affects pregnant women’s’ worry and anxiety.

**MATERIAL AND METHODS**

An information film lasting for 26 minutes, including both medical facts and parents’ experiences, was developed to facilitate the information about prenatal examinations. A randomized controlled trial was performed. Women were allocated to either the intervention group-film (n=184), or to the control group-no film (n=206). All women received the usual written and verbal information about prenatal examinations. The data collection was performed by questionnaires in gestational week 27.

The primary outcome was informed choice about the CUB-test, further research questions were informed choice about the second trimester ultrasound examination and about worry and anxiety in relation to watching the film. Three different measures were combined to measure informed choice; attitudes towards DSS, knowledge about DS and DSS and uptake of CUB.

The same three measures were used about the second trimester ultrasound screening.

Worry and anxiety were measured by the validated instrument STAI (State Trait Anxiety Inventory) and there were complementary questions about worry in the questionnaire.

The research ethics committee at Karolinska Institutet approved the trial (dnr 2009/208-31).

**RESULTS**

More women made an informed choice about the CUB-test - 71.5% (film) vs. 62% (no film). $p<0.05$. More women was satisfied with the information and more perceived the information as enough, comprehensible and correct. The women requested in a less extent further information they showed increased knowledge and an unchanged attitude towards early prenatal examination. The uptake of CUB did not increase in the intervention group.

However, an informational film does not increase women’s knowledge or the number of women making an informed choice about the second trimester ultrasound. Of those in the intervention group, 81.3% made an informed choice regarding second trimester ultrasound examination compared with 76.1% in the control group ($p = 0.21$). Women making an informed choice scored higher in knowledge about the examination ($p < 0.001$), had higher degree of education
(p < 0.001), and spoke more frequently Swedish as mother tongue (89.5% vs. 74.7%, p = 0.01). Women who did not make an informed choice about the second trimester ultrasound had a lower level of education and less knowledge about second trimester ultrasound screening.

There were no statistically significant differences between the groups neither in state nor trait anxiety. Regarding worry about the possibility of something being wrong with the baby and worry about giving birth, there were no statistically significant differences between the groups. The women stated that to see the film increased their worry rather than decreased it.

CONCLUSIONS

The film increased number of informed choices about CUB. Participants were more satisfied with the information received.

An informational film did not increase women’s knowledge or the number of women making an informed choice about the second trimester ultrasound. Women who did not make an informed choice about the second trimester ultrasound had a lower level of education and less knowledge about second trimester ultrasound screening.

An informational film as a complement to written and verbal information about prenatal testing did not appear to increase women’s anxiety or worries in a long time. However, the informational film seemed to cause worry at the time of viewing which should be taken into consideration.

«The film-study» has generated three scientific publications:


REFERENCES


Maternal Pulmonary Arterial Hypertension in Pregnancy: Incidence, Risk Factors, Management and Outcomes

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SUMMARY

Pulmonary arterial hypertension (PAH) of any aetiology during pregnancy is widely considered to pose a high risk of maternal death, due to the inability to tolerate the physiological changes of pregnancy and the stress of delivery. Despite this, there are currently few data on PAH in pregnancy in the UK. This prospective descriptive population-based study used the UK Obstetric Surveillance System (UKOSS) to identify all women in the UK with PAH in pregnancy so as to estimate the incidence and describe its risk factors, management and outcomes. Thirty cases of maternal PAH were reported between March 2006 and February 2012, giving an estimated incidence of 0.6 cases per 100,000 maternities (95% CI 0.4 to 0.9). Twenty-four women (80%) proceeded with their pregnancies and 71% of the neonates were delivered by Caesarean section. Two women died post partum (overall case fatality 6.7%, 95% CI 0.8 to 22; case fatality amongst women with continuing pregnancies was 8.3%, 95% CI 1.0 to 27). There were no neonatal deaths. Maternal New York Heart Association (NYHA)/ World Health Organization (WHO) functional classification was correlated with maternal intensive care admission, major maternal morbidity and preterm delivery (p<0.05). Maternal PAH in pregnancy is extremely rare in the
UK, however neither maternal nor infant mortality appears to be as high as previously reported. Clinicians should be aware of the prognostic value of maternal NYHA/WHO functional classification and thus it should be recorded in all cases to aid risk-stratification.

Keywords: Morbidity, Mortality, UKOSS, Pulmonary vascular disease, Eisenmenger’s syndrome.

INTRODUCTION

Pulmonary arterial hypertension (PAH) refers to a group of diseases of the small pulmonary arteries characterised by a progressive increase in pulmonary vascular resistance, resulting in right ventricular failure [1, 2]. PAH of any aetiology during pregnancy is widely considered to pose a high risk of maternal death, due to the inability to tolerate the physiological changes of pregnancy and the stress of delivery [3-7]. In the UK, between 1994 and 2008, 23 maternal deaths reported to the ‘Confidential Enquiries into Maternal Deaths’ (CEMD) were due to PAH [8]. A systematic review of PAH in pregnancy, which included studies published between 1978 and 1996, showed maternal mortality to be 30-56%, and neonatal mortality to be 11-13% [9]. This review suggested that poor prognosis is linked to late diagnosis, late hospital admission, high pulmonary artery pressures and operative delivery [9]. A more recent review of reports from 1997 to 2007 suggested a reduction in maternal mortality (17-33%), but an unchanged neonatal mortality (7-13%); however, the authors believe a potential publication bias towards cases with favourable outcomes may have led to underreporting of true mortality data [10].

Treatment of PAH has typically involved anticoagulation, diuretics and calcium-channel blockers [4,11]. However, over recent years, specific therapies targeted to the pathophysiological mechanisms of PAH have revolutionised its management; these include prostacyclin analogues, phosphodiesterase inhibitors, endothelin-receptor antagonists and inhaled nitric oxide [4,11]. Studies have demonstrated symptomatic, functional and haemodynamic improvements, and as such, their use has been incorporated into PAH treatment algorithms [2, 4, 5, 12-14]. However, evidence for the use and benefit of advanced PAH therapies in pregnant patients is limited to case reports and data extrapolated from non-pregnant patients [7]. The authors of the systematic reviews have called for more information from prospective studies to differentiate the pregnancy-related risks in PAH and to ascertain the true role of advanced PAH therapies in pregnant women [9, 10]. The aims of this research were to conduct a prospective population-based study using the UK Obstetric Surveillance System (UKOSS)
to estimate the incidence of PAH in pregnancy in the UK, and to describe its risk factors, management and outcomes in order to inform national guidance [15].

**MATERIAL AND METHODS**

This prospective descriptive population-based study used the UKOSS to identify all women in the UK with PAH in pregnancy. Cases were identified through the monthly mailing of the UKOSS between March 2006 and February 2012. The UKOSS methodology has been described in detail elsewhere [15]. A case of maternal PAH was defined as any pregnant woman having either (1) a mean pulmonary artery pressure greater than or equal to 25mmHg at rest, or 30mmHg during exercise, in the absence of a left-to-right shunt, or (2) a pulmonary artery systolic pressure greater than 36mmHg [3]. Cases had either pre-existing or new-onset PAH. Cases of PAH were classified as either idiopathic (iPAH), associated with congenital heart disease (CHD-PAH), or associated with other causes (oPH). CHD-PAH was defined as PAH due to an uncorrected left-to-right shunt from a ventricular-septal defect (VSD), atrial-septal defect (ASD) or patent ductus arteriosus (PDA) [16].

To ensure all cases were identified, we independently contacted all intensive care and coronary care units, who were asked to report any cases of PAH in pregnant women, providing details of their year of birth, date of diagnosis and hospital of origin only. Where a case was identified that had not been reported through the UKOSS, the relevant UKOSS reporting clinician was contacted and asked to complete a data collection form.

Incidence was calculated with 95% confidence intervals using the total number of maternities in the UK over the six year study period as the denominator. Proportions were compared using Pearson’s chi-squared test or Fisher’s exact test as appropriate, and statistical significance was assumed at the $p<0.05$ level. All analyses were conducted using the STATA® 11 (StataCorp LP, Texas) statistical package.

**RESULTS**

All UK consultant-led maternity units participated in the UKOSS throughout the study period. Eighty-eight cases were reported and data were returned for eighty-one (92%). There were four duplicate cases and four cases whose notes were lost. Twenty-six cases were subsequently reported by clinicians as not cases, and seventeen cases for which data collection forms were received did
not meet the case criteria. No additional cases were identified through sources other than the UKOSS. There were thus 30 confirmed cases (34%) of maternal PAH in pregnancy, giving an estimated incidence of 0.6 cases per 100,000 maternities (95% CI 0.4 to 0.9 per 100,000).

Twenty-four women (80%) proceeded with their pregnancies (one of which was a twin pregnancy) and six (20%) underwent terminations. Of the women continuing with their pregnancies, 50% experienced other medical problems in addition to PAH, including thrombocytopenia, pulmonary embolism and gestational diabetes. Mode of delivery was as follows: 17 Caesarean sections (71%), five vaginal deliveries (21%) and two instrumental deliveries (8%). Two women died post-partum (overall case fatality 6.7%, 95% CI 0.8 to 22; case fatality amongst women with continuing pregnancies was 8.3%, 95% CI 1.0 to 27). There were no neonatal deaths; however 16 (64%) infants were premature and 13 (52%) were admitted to the neonatal intensive care unit. Maternal New York Heart Association (NYHA)/ World Health Organization (WHO) functional classification was correlated with maternal intensive care admission, major maternal morbidity and preterm delivery ($p<0.05$). Despite this, the NYHA/ WHO functional classification was only recorded in two-thirds of women.

**CONCLUSIONS**

Maternal PAH in pregnancy is extremely rare in the UK. The current study confirms results from previous studies, such as those by Bedard and Weiss, which suggest that maternal mortality due to PAH is still greatest in the post-partum period, secondary to acute haemodynamic shifts [9, 10]. Consequently both British and European PAH guidelines emphasise the avoidance and prevention of pregnancy in these patients [4, 5]. The results of this study suggest that neither maternal nor infant mortality appears to be as great as previously reported [9, 10]. Similarly, premature delivery occurred less often, at 64% compared with 85-100% [10]. Explanations may include greater use of advanced therapies and better multidisciplinary care. Pre-pregnancy counselling, contraception and access to terminations may also have improved such that higher risk women are not becoming pregnant. However, in light of the apparent low mortality rate that we have described, further checking of case ascertainment of maternal deaths due to PAH is currently underway; until this is complete, we cannot be certain that the true mortality rates are as low as suggested by this study. If shown to be true, guidelines may need to be adapted to support women with PAH who choose to continue their pregnancy.

We have also shown that maternal NYHA/ WHO functional classification appears to be a prognostic feature for both mother and infant. Previous studies
have also shown functional class III/IV to indicate a poor prognosis outside of pregnancy [17, 18]. As such, recording of NYHA/WHO functional classification in all cases may be helpful in stratifying individual women’s risks.

ACKNOWLEDGEMENTS

This study would not have been possible without the contribution of the UKOSS reporting clinicians who notified cases and completed the data collection forms. We would also like to thank the members of the UKOSS Steering Committee who provided advice throughout the study.

REFERENCES


The Influence of Parity in the Outcome of Twin Pregnancies

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SUMMARY

This is the first study done in Albania for multiple pregnancies to analyze the outcome of nulliparous women versus pluriparous women with twin pregnancies. We conducted a retrospective cohort study of 415 twin pregnancies in our department between 2003 and 2009. Population characteristics, complications during pregnancy and delivery, and neonatal outcomes were assessed. Statistical tests were used to examine the relationship between different variables: parity, maternal age, mode of conception, chorionicity, mode of delivery and total twin birth weight (TTBW).

Keywords: nulliparous, pluriparous, assisted reproductive technology, total twin birth weight.

INTRODUCTION

Actually, more than 50% of multiple pregnancies in developed countries are from the use of assisted reproductive technology (ART). This increase is because of the use of ART and with advanced maternal age at the time of remaining
pregnant. In the last decades, there is a trend of increased rate of incidence of multiple pregnancies because of advanced maternal age and assisted reproductive technology (ART). Monochorionic placentation happen often after treatment for infertility with ART than after spontaneous conception of multiples [1]. On this context it is important for the contemporary obstetrician to take advantages from the use of the placenta to determine the zygosity, because today is obvious that monizygotic twins are not fenotipically nor genetically identical [2, 3].

**MATERIAL AND METHODS**

We conducted a retrospective cohort study of 415 twin pregnancies in our department between 2003 and 2009, delivered from 28 weeks of pregnancy on ward. Population characteristics, complications during pregnancy and delivery, and neonatal outcomes were assessed. The Student’s t-test, Fisher exact test, were used to examine the relationship between different variables, parity, maternal age, mode of conception, chorionicity, mode of delivery and total twin birth (TTBW). p < 0.05 was considered significant.

**RESULTS**

During 7 years of study from 2003 until 2009, 415 twin pregnancies were enrolled and gave birth in our department. According to the maternal age the distribution was: < 20 years 6 cases (1.4%), 20-24 years old 72 cases (17.3%), 25-29 years old 124 cases (29.8%), 30-34 years old 117 cases (28.1%), 34-39 years 78 cases (18.75), > 40 years old 18 cases (4.3%) with a mean of 29.9 ± 5.74 years old. In total nulliparous were 281 cases (67.7%) with a mean maternal age 29.48 ± 6.18 years old, divided according to the mode of conception: twin pregnancies conceived spontaneously 121 cases (43.1%), twin pregnancies with ovulation induction 52 cases (18.5%) and twin pregnancies with assisted reproductive technology (ART) 108 cases (39.4%). According to the chorionicity the nulliparous pregnant women were dichorionic 155 cases (55.1%), monochorionic 93 cases (33.0%) and with unknown chorionicity 33 cases (11.9%) (Table 1). The mean gestational age at delivery in nulliparous twin pregnant women was 35.4 ± 2.53 weeks. The mean total twin birth weight (TTBW) was 4666.86 ± 971.8 g. Pluriparous with twin pregnancies were in total 135 cases with a mean maternal age of 30.9 ± 4.57 years old. According to the mode of conception they were divided: twins with spontaneous conception 91 cases (67.4%), with ovulation induction 22 cases (16.2%) and with ART 21 cases
The mean gestational weight at birth was $4978 \pm 975$ g. When we compared both groups nulliparous versus pluriparous we found significance in the maternal age ($p = 0.007$) and in the TTBW ($p = 0.002$). There was no significance for the gestational age ($p = 0.155$) (Table 2).

| Tab. 1. Comparison of different characteristics of nulliparous vs pluriparous. |
|-----------------|-----------------|-----------------|
|                 | Nulliparous ($n = 281$) | Pluriparous ($n = 136$) | p-value |
| Maternal Age (years) | $29.48 \pm 6.18$ | $35.8 \pm 2.48$ | 0.007* |
| Pregnancy age (weeks) | $35.4 \pm 2.53$ | $35.8 \pm 2.48$ | 0.155 |
| TTBW (g) | $4666.86 \pm 971.8$ | $4978 \pm 975.0$ | 0.002* |

*significant

In the comparison we made in the relationship to the mode of conception nulliparous versus pluriparous we found that in the spontaneous conception the OR 0.4 (95% CI 0.2-0.6), in the ovulation induction the OR 2.1 (95% CI 1-1.39) and in the ART the OR 3.4 (95% CI 1.9-5.9).

Tab. 2. Comparison of nulliparous vs pluriparous related to the mode of conception.

| Nulliparous ($n = 281$) | Pluriparous ($n = 136$) | OR 95% CI |
|-----------------|-----------------|-----------------|-----------------|
| N              | %              | N              | %              |              |
| Spontaneous Conception | 121 43.1 | 91 67.4 | 0.5 (0.2-0.6) |
| Ovulation Induction | 52 18.5 | 22 16.2 | 2.1 (1-1.39) |
| ART            | 108 39.4 | 21 15.5 | 3.4 (1.9-5.9) |

In the comparison we made in the relationship to the mode of conception nulliparous versus pluriparous we found that in the spontaneous conception the OR 0.4 (95% CI 0.2-0.6), in the ovulation induction the OR 2.1 (95% CI 1-1.39) and in the ART the OR 3.4 (95% CI 1.9-5.9).

CONCLUSION

In our study we found that pluriparous pregnant women with twin pregnancies conceive more spontaneously and the weight of their twins is increased when compared with nulliparous pregnant women with twin pregnancies. Nul-
liparous women are younger than pluriparous at the time of conception. Nulli-parous women with twin pregnancies have more chances to conceive artificially because also of advanced maternal age at the time of conception.

ACKNOWLEDGMENTS

I would like to express my special thanks of gratitude to my teacher Isaac Blickstein as well as our principal director Rubena Moisiu who gave me the golden opportunity to do this wonderful project on the topic of Twin Pregnancies, which also helped me in doing a lot of Research and I came to know about so many new things. I am really thankful to them. Secondly, I would like to thank my special wife who helped me a lot in finishing this project within the limited time.

REFERENCES

Malaria in Pregnancy and Postpartum – About a Clinical Case

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SUMMARY

We report the case of a 35-year-old nulliparous African female who had been diagnosed with malaria at 30 weeks of gestation. At 33 weeks, she was transferred to our hospital because of the possible need for Intensive Care. With fever, myalgia and respiratory distress and positive smear for *P. falciparum*, an urgent cesarean section was performed, giving birth to a live female newborn. Postpartum immediate transference to an Intensive Care Unit was needed, with Simplified Acute Physiology Score 16. While in ICU, she developed a moderate Acute Respiratory Distress Syndrome, requiring mechanical ventilation for 13 days. Treated with quinine, there was an increase in hemoglobin, resolution of thrombocytopenia and negative smear for *P. falciparum*. Severe forms of malaria should be carefully treated in an Intensive Care Unit.

Keywords: malaria in pregnancy, ICU, *Plasmodium falciparum*, maternal and perinatal mortality.

INTRODUCTION

Human malaria is nowadays one of the main health care problems worldwide, responsible for the death of over one million people every year. It is
estimated that 10000 women and 200000 infants die as a result of malaria infection during pregnancy; more than half of these are due to severe maternal anemia, prematurity and low birthweight (LBW) [1].

The close relationship of Portugal with African countries allows the diagnosis of some African endemic diseases in Portuguese Units. Malaria is one of those diseases and requires a high degree of suspicion for the diagnosis to be made.

Despite the troubling symptoms, when infection is carried out by *Plasmodium falciparum*, it may only lead to more serious clinical cases when it is not timely diagnosed and a proper drug treatment is not performed. The majority of malaria-related deaths are due to this species of Plasmodium, it is the predominant species giving rise to considerable morbidity and mortality in pregnancy. *P. vivax* infection can give rise to some of the same complications as *P. falciparum*; however, the complications are less frequent and less severe.

Maternal malaria in holoendemic areas, such as Sub-Saharan Africa has a 28 percent prevalence [3]. The prevalence of malaria in pregnant women in low-transmission areas outside of Africa ranges from 1.8 to 17.4 percent.

**MATERIAL AND METHODS**

The authors describe the case of a 35 year-old-nulliparous African woman who had been diagnosed malaria at 30 weeks of gestation. She lived in Angola and came to Portugal 3 weeks before she was admitted to our Hospital.

**RESULTS**

At 33 weeks of gestational age, the patient was transferred from another hospital to our department due to the possible need for intensive care. She presented fever, myalgia and respiratory distress. Analytically she was anemic (Hb 8.8g/dL), with thrombocytopenia (platelets 30 x 10^9 / L) and positive smear for *P. falciparum* with 1.5% parasitized erythrocytes.

There were no ecographic signs of infection and no fetal growth restriction detected during pregnancy. The maternal clinical changes required an urgent cesarean section, from which resulted a live female newborn with 1899gr and first and five-minute Apgar score of 6 and 9. No infant’s peripheral smear parasitaemia was detected.

Postpartum immediate transference to an Intensive Care Unit (ICU) was needed, with Simplified Acute Physiology Score (SAPS II) score 16, where she remained for 14 days. While in the ICU, the patient developed a moderate Acute
Respiratory Distress Syndrome (ARDS, ratio \( \text{paO}_2/\text{FiO}_2 \) between 100-200), requiring mechanical ventilation for 13 days. There was progressive increase in hemoglobin and resolution of thrombocytopenia from the 6th day on, without transfusion. She was treated with intravenous quinine for 7 days because of a marked gastric stasis, followed by oral administration until the 11th day, with negative parasitaemia in the 9th day of admission.

The patient recovered well and she was discharged home on postoperative day 15.

**CONCLUSIONS**

Compared to nonpregnant women, pregnant and postpartum women are at increased risk of both acquiring malaria and developing more severe diseases, especially nonimmune nulliparous. They also experience more hypoglycemia and more respiratory complications (pulmonary edema, acute respiratory distress syndrome) [1]. Anemia is a common complication of malaria in pregnancy; approximately 60 percent of pregnant women presenting with malarial infection are anemic and anemia may be one of the few signs of the disease.

The clinical presentation varies according to the underlying endemicity of the region [2]. In holoendemic regions, where partial immunity is common, most malarial infections in pregnant women are asymptomatic. In areas of low or unstable malaria transmission where pregnant women have acquired little immunity, symptomatic malarial disease is the rule and serious complications may occur.

Prompt and accurate diagnosis of malaria is critical for implementation of appropriate treatment to reduce associated morbidity and mortality. The diagnosis should be considered in any febrile pregnant woman who comes from or lives in a malarious region [2]. It is well known that the higher the parasitaemia the greater the systemic inflammatory response. There is a semi quantitative relationship between the degree of parasitaemia and the severity of disease.

The WHO revised in 2010 the criteria for severe malaria, as well as the treatment [1]. Pregnant women with severe P. falciparum malaria must be treated promptly with an effective antimalarial agent to clear parasites rapidly and should receive parenteral therapy (the intravenous route is preferred over the intramuscular). Patients with severe forms of malaria should be carefully treated in an Intensive Care Unit, as well as the ones in special conditions such as pregnant and postpartum women [4].

One special feature of malaria in pregnancy is the capability of P. falciparum parasitized erythrocytes to sequester within the intervillous space of the placenta. Malarial infection of the placenta induce placental thickening and fibrin
deposition, thereby decreasing placental transport of oxygen and nutrients. The mean birthweight of infants born to mothers with no evidence of malarial placental infection is higher than for those newborns from infected placentas. In addition, second trimester infection increased the risk of LBW more than third trimester infection.

REFERENCES

Changes in Body Mass, Size and Composition Following 12 Weeks of Exergaming in Postpartum Females

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SUMMARY

The retention of gestational weight gain (GWG) contributes to the prevalence of obesity in adult females. Most new mothers don’t achieve the recommendations for physical activity (PA), often quoting factors such as a lack of time and access to childcare. Exergaming may tackle some of the barriers to PA and offer an alternative to traditional exercise. Eight females who had given birth within 1 year completed a 12 week exergaming intervention, which required them to exercise at home for 45 minutes on alternate days, using the Wii Fit. Participants self-reported their pre-pregnancy body weight, and visited the laboratory prior to and following the intervention for evaluation of body weight, size (height, regional circumferences, body mass index [BMI]) and composition (fat mass, lean mass and bone mineral content [BMC]). Body composition was evaluated via full-body dual-energy x-ray absorptiometry scan. Participants also completed a three-day weighed food intake at three time-points (0, 6 and 12 weeks). Baseline body mass was 8.2 kg greater than self-reported pre-pregnancy values. Following the intervention, body mass was significantly lower (7%) than baseline and similar to pre-pregnancy. Reductions in BMI (2 kg·m²), waist,
hip and bust circumference (3-6%) accompanied the loss of body mass. Decreases in body mass and size were almost entirely attributable to a loss of fat (15-28%), since there were no changes in lean mass and bone mineral content. Food diaries confirmed participants hadn’t altered their energy intake. Exergaming may aid in preventing the retention of GWG and reducing associated health risks.

**Keywords:** active gaming, pregnancy, weight loss/management.

**INTRODUCTION**

Maternal obesity, specifically the retention of GWG, contributes to the global prevalence of overweight adult females [1]. The average body mass gain from pre-pregnancy to one year postpartum is 0.5-4 kg [2]; 25% of women retain more than 4.5 kg of body mass following child-birth [3].

Regular PA is important for weight management, reducing the risk of developing body mass-related health problems and lowering the mortality rate [4]. Postpartum females are generally less physically active than their childless counterparts [5].

Previous research has shown beneficial effects of regular exercise on body mass loss in the year following parturition [6], however starting exercise in this period is difficult; with new mothers reporting perceived barriers to PA such as tiredness and a lack of time, social support, childcare and confidence [7].

Exergaming requires individuals to use body movements in order to play. We suggest that exergaming has the potential to overcome some of the perceived barriers to PA reported by postpartum females as it offers an opportunity to undertake PA at home, at a time when it is convenient, and does not necessitate childcare.

The aim of this study was to examine changes in the body mass, size and composition of postpartum females in response to a 12 week exergaming intervention. We hypothesised that the intervention would reduce body mass, improve body composition and preserve BMC relative to baseline.

**MATERIALS AND METHODS**

**Participants**

Eight healthy postpartum (range 12-34 weeks) females volunteered for this study. Participants self-reported pre-pregnancy weight was 56.8 ± 5.1 kg. All participants met the inclusion criteria: 18-40 years, viable pregnancy within the
last 12 months, no postpartum complications, eumenorrheic with a menstrual bleed within the last 30 days, no large single dose of radiation exposure within the last 2 years and no musculoskeletal injuries. All participants gave their written informed consent. The study was approved by the Nottingham Trent University’s human ethics committee and conformed to the Declaration of Helsinki.

**Protocol**

At baseline (0 weeks) and following completion of the training programme (12 weeks) participants’ height, weight, upper arm, thigh, bust, waist and hip circumference were recorded. In addition, participants underwent a full-body dual-energy x-ray absorptiometry (iDXA; Lunar iDXA, GE Healthcare, General Electric Company, Little Chalfont, UK) axial scan, performed by a qualified radiographer. Participants were required to exercise at home using a Wii Balance Board (Nintendo Wii, Nintendo Co Ltd, Kyoto, Japan) every other day, for 12 weeks. Participants were provided with three possible Wii Fit training programmes, each containing an aerobic, training plus, muscle and yoga component. The duration of the programmes was 45 minutes. Participants were asked to not to alter their habitual PA levels or engage in any exercise outside of the prescribed programme throughout the duration of the study, and retrospectively confirmed this at the completion of the programme. Participants completed a three-day weighed food intake at three time-points (0, 6 and 12 weeks) and kept a food diary for the entire 12 week period. They were instructed not to alter their dietary habits in any way during the study. Energy expenditure was estimated from food diaries and weighed food intake. Data were analysed using Microdiet version 2 (Downlee Systems Ltd., High Peak, UK).

**Statistical analysis**

Descriptive and outcome statistics are presented as means ± 1SD. Data were analysed using SPSS version 18 (IBM SPSS statistics, New York, USA). All data met the assumptions for parametric tests. Differences between baseline and post-intervention training values were analysed using a paired-samples t-test. Repeated measures ANOVA was used to detect differences between time points in body mass (pre-pregnancy, baseline and post-intervention) and energy intake (weeks 1, 6 and 12), with least squares difference post hoc analysis used to identify specific differences between time points. Statistical significance was accepted at \( P < 0.05 \). Data are also presented as the mean intra-individual percentage change from baseline to post-intervention for each outcome measure, which was calculated using the formula: \( \frac{(post-intervention - baseline)}{baseline} \times 100 \).
RESULTS

Body mass

Body mass was on average 8.2 ± 8.8 kg (14 %) greater at baseline compared to self-reported pre-pregnancy body mass, and five of the eight participants had retained > 4.5 kg. Following the intervention, body mass decreased by 5.1 ± 3.9 kg (7%), such that post-intervention body mass was similar to self-reported pre-pregnancy values, and only 2 participants had still retained > 4.5 kg. The average rate of weight loss from baseline to post-intervention was 0.40 ± 0.03 kg per week.

Body size

BMI at baseline was 24.4 ± 4.9 kg·m², with 6 participants categorised as normal (range 20.2-24.2 kg·m²), one as overweight (27.7 kg·m²) and one as obese (35.2 kg·m²). Following the intervention, BMI was significantly lower (22.5 ± 3.9 kg·m²; \( P = 0.01 \)) compared to baseline. Two individuals classed as overweight and obese were close to moving down a BMI category, with post-intervention BMI values of 25.5 and 30.4 kg·m², whilst the other participants remained within the healthy range (19.4-23.4 kg·m²). Waist, hip and bust circumference decreased from baseline to post-intervention (3-6%, Table 1), but there were no differences in thigh or arm circumference.

Body composition

Total body fat mass (FM) decreased from baseline to post-intervention by 4.0 ± 3.0 kg (Table 2). When evaluated by body region, FM of the legs, trunk, android and gynoid regions decreased (15-28%), whilst there was also a trend for arm FM to decrease (Table 2). Total body relative FM decreased from baseline to post-intervention by 4.0 ± 3.4 % (Table 2). Relative FM of the legs, trunk, android and gynoid regions also decreased (5-18%), but there was no change in relative FM of the arms (Table 2).

Tab. 1. Circumference measurements (cm) by body region.

<table>
<thead>
<tr>
<th>Bodyregion</th>
<th>Waist</th>
<th>Hip</th>
<th>Bust</th>
<th>Thigh</th>
<th>Arm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>83.4 ± 10.6</td>
<td>102.9 ± 6.1</td>
<td>95.4 ± 6.9</td>
<td>49.3 ± 7.8</td>
<td>27.4 ± 2.7</td>
</tr>
<tr>
<td>Post</td>
<td>78.4 ± 6.7*</td>
<td>97.3 ± 5.6**</td>
<td>92.1 ± 9.0**</td>
<td>47.1 ± 4.7</td>
<td>26.3 ± 1.4</td>
</tr>
</tbody>
</table>

*Data are group mean ± SD (N=8). *P < 0.05, **P < 0.01
There was no difference in baseline and post-intervention total body lean mass (LM) (Table 2). Leg LM decreased by 0.4 ± 0.5 kg, but there were no differences in arm, trunk, android or gynoid LM (Table 2).

No differences in baseline and post-intervention BMC were shown for the total body or any of the individual body regions (Table 2).

Dietary analysis

Energy intake at the start of the intervention (2156 ± 97 kcal’d⁻¹) was not significantly different from energy intake during (2090 ± 187 kcal’d⁻¹) or at the end of the intervention period (2025 ± 159 kcal’d⁻¹) (all \( P > 0.05 \)), which were at 6 and 12 weeks respectively. Also the contribution of carbohydrate, fat and protein in the diet did not differ between the 3 time-points (\( P > 0.05 \)). Carbohydrate, fat and protein provided 51%, 34% and 15% of total energy consumed at baseline compared to 49, 42 and 9% at the cessation of the exercise programme.

CONCLUSION

Postpartum females who participated in a novel 12 week exergaming intervention (consisting of aerobic, body-weight resistance exercise, flexibility and...
postural control exercises) showed reductions in total body mass and region-specific circumferences. These declines were largely attributable to reduced body fat since LM and BMC were maintained. Exergaming may offer an alternative to traditional exercise for preventing the retention of gestational weight gain and reducing associated health risks, whilst also maintaining bone mass.

REFERENCES

Diagnosis of Autosomal Dominant Polycystic Kidney Disease During Pregnancy: a Clinical Case

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SUMMARY

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic renal disorder, occurring in approximately 1 in every 400 to 1000 live births. Hypertension is a common early finding in ADPKD, occurring in 50 to 70 percent of cases before any significant reduction in glomerular filtration rate within an average age onset of 30 years of age. We present a case of a twenty-eight-year-old woman with chronic hypertension since she was eighteen years old. During the chronic hypertension study, she did an ultrasound that demonstrated multiple bilateral cysts in kidneys. After observation by a nephrologist, the most likely diagnosis is an autosomal dominant polycystic kidney disease. At 38 weeks the secondary hypertension to a renal disease led to the termination of the gestation. The male newborn weighted 2785 g, Apgar score of 9/10.

Keywords: Autosomal dominant polycystic kidney disease, Pregnancy, Hypertension.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is the most common genetic renal disorder, occurring in approximately 1 in every 400 to 1000
live births. Pregnant women with ADPKD have an increased incidence of hypertension late in pregnancy and a higher rate of perinatal mortality. The diagnosis is easy to establish in patients with symptomatic disease who have a family history of ADPKD. In such patients, the diagnosis is assured by the finding of large kidneys with multiple bilateral cysts on ultrasonography or CT scanning. Although the typical age of clinical onset is in the third to fifth decade of life, rarely ADPKD can occur in utero or in the neonatal period with massive enlargement of the kidneys with preservation of the reniform shape with diffuse hyperechogenicity of both cortex and medulla, and loss of corticomedullary differentiation.

Hypertension is a common early finding in ADPKD, occurring in 50 to 70 percent of cases before any significant reduction in glomerular filtration rate within an average age onset of 30 years of age. Chronic hypertension is defined as systolic pressure ≥ 140 mmHg and/or diastolic pressure ≥ 90 mmHg that precedes pregnancy, is present before the twentieth week of pregnancy, or persists longer than twelve weeks postpartum. Hypertension with or without antihypertensive drug treatment is associated with adverse pregnancy outcome like poor fetal growth, stillbirth and iatrogenic preterm birth and accounts for a substantial fraction of maternal morbidity (pulmonary edema and stroke).

**CLINICAL CASE**

A twenty-eight-year-old woman with chronic hypertension since she was eighteen years old. In her previous pregnancy, five years ago, she developed preeclampsia at 36th week with cesarean section due to non-reassuring foetal status. After this, the study was conducted without finding cause of secondary hypertension.

She was referred to our institution in her 8th week of gestation with non-controlled hypertension and normal renal function. During the chronic hypertension study, she did an ultrasound that demonstrated absence of normal corticomedullary differentiation and multiple bilateral cysts in kidneys. After observation by a nephrologist, the most likely diagnosis is an autosomal dominant polycystic kidney disease.

During the pregnancy she was hospitalized twice with non-controlled hypertension and needed two drugs to control the hypertension (methyldopa 500 mg 3 id and amlodipine 5 mg). The renal function was getting worse with serum creatinine of 1.09 mg/dl and creatinine clearance of 67.97 ml/min. At 38 weeks the secondary hypertension to a renal disease led to the termination of the gestation.

The male newborn weighted 2785 g, Apgar score of 9/10. After delivery she
reinitiated methyldopa 500 mg 3 id and was discharged after four days with normal blood pressure. She keeps on being followed by Nephrology with controlled blood pressure and normal renal function. The exact diagnosis is more difficult because the family do not have a well-diagnosed ADPKD.

CONCLUSIONS

The hypertension and ADPKD represent a high risk factor during pregnancy because they are implicated in adverse pregnancy outcomes. In the management of these patients, it is important the contribution of a nephrologist. We have to increase the frequency of prenatal consultations to ensure the right control of blood pressure, renal function and fetal wellbeing.

REFERENCES

2. UptoDate, Management of hypertension in pregnant and postpartum women.
3. UptoDate, Diagnosis of and screening for autosomal dominant polycystic kidney disease.
4. UptoDate, Course and treatment of autosomal dominant polycystic kidney disease.
5. UptoDate, Prenatal sonographic diagnosis of cystic renal disease.
Ultrasonic Monitoring of the Motor Activity of the Fetus During the Breath of a Pregnant Woman – A New Functional Test for the Stability of the Fetus to Hypoxia

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SUMMARY

We have developed a method diagnostics of the cerebral cortex hypoxia of the fetus with ultrasonic monitoring of the motor activity of the fetus during the breath of a pregnant woman. To improve the accuracy of prediction of perinatal birth outcomes is proposed to estimate the stability of the fetus to intrauterine hypoxia using the developed functional test, called the test Gauskneht. It is shown that the values of the test is more or less than 15 seconds showing of sufficient or insufficient stability of the fetus to hypoxia, as well as a high or low probability of having a healthy baby in the physiological delivery.

Keywords: pregnancy, birth, fetal hypoxia, test Gauskneht, newborn child.

INTRODUCTION

Currently preparing pregnant women for childbirth is carried out without determine the sustainability of their fetuses to intrauterine hypoxia, therefore,
the forecast perinatal outcomes are not accurate enough [1]. Before we developed a method for assessing the sustainability of fetal intrauterine hypoxia on M.Ju. Gauskhneht [2, 3]. Way represents the modified sample rod and allows with the help of ultrasound to determine the reserves adaptation of the fetus to hypoxia duration withstand them a period of breathing in pregnant women [4, 5]. However, the sensitivity, specificity, and predictive value of the proposed method is not sufficiently studied [8]. The aim is to study the relationship between the values of the functional test of the stability of fetal intrauterine hypoxia and indicators of the health of newborn children.

In the absence of finished technologies intrauterine ventilation of the lungs of the fetus breathing gas hypoxia remains invincible factor damage to his brain in childbirth. Save the brain of the fetus allows timely use of the test Gauskhneht [3, 7, 9], which in many country does not apply.

**MATERIAL AND METHODS**

Took part in the survey 200 pregnant women, 80 parturient, 20 of obstetricians and 10 of anesthesiologists. Held transabdominal ultrasound study of the motor activity of the fetuses of a few groups of pregnant women 20-24 and 30 to 34 weeks of pregnancy in conditions of women consultations of the city of Izhevsk. Two groups (40 and 20 women) were pregnant women with normal pregnancy. The third group consisted of 5 pregnant women with signs of Fetoplacental insufficiency degree 1B. Moreover, one of the pregnant women of the third group had a cord entanglement umbilical cord around the neck of the fetus.

In addition, conducted a prospective cohort surveillance of the 61 patients received for urgent deliveries in terms of pregnancy 37-41 week in Maternity hospital № 5 and Perinatal center in Izhevsk in 2011-2012. Ultrasound examination of pregnant womens and fetuses were conducted with use of devices of the expert class ALOKA SSD – ALPHA 10, Medison SonoAce-600-C and standard sensors with a frequency of 3 to 7 MHz on a previously published methods [5, 6]. All pregnant women were tested according to the existing standards of rendering of medical aid [1]. In addition to ultrasound after obtaining the informed voluntary consent of applied functional sample Gauskhneht. For this woman asked to hold your breath and held ultrasonic monitoring of the motor activity of the fetus in the sagittal projection, determined length of time from the moment of breath pregnant women prior to the registration of respiratory excursion fetus’s chest. All newborns were examined by specialists and received the necessary laboratory and clinical diagnostic research.
RESULTS

All respondents noted the low labor intensity and high speed testing (when the result is 1-2 minutes of the study). The results of the analysis of the answers showed that all the respondents midwives and midwifery anesthesiologists recognize the high ease of implementation and the predictive value of the test Gausknecht. In addition, all the doctors recognize the high security test, the possibility of its re-use right up to the final stage of delivery, as well as the high information value and predictive value. All the experts note that the results of test Gausknecht allow timely, more accurately and reasonably select the desired option of delivery, providing safety of a cortex of a brain of a newborn.

Previously we have conducted surveillance of the fetuses in the 20-24 and 30 to 34 weeks of pregnancy at 40 pregnant women whose pregnancy was without any signs of pathology. We managed to install the following:

1. With breath pregnant women do not feel the change of status of the fruit;
2. Ultrasonic monitoring of the motor activity of fruits allows to evaluate their reserves adaptation to hypoxia;
3. In terms of 20-24 and 30-34 weeks in normal pregnancy fruits react to the delay of breath pregnant women in the following way: take a motionless condition and are in it up to exhaustion of reserves of adaptation to hypoxia. Symptom of exhaustion of reserves is the appearance of the «respiratory» excursions ribs;
4. Identical to the values of the sample Rod in adults values samples in fetuses different and reflect the resistance to hypoxia.

The results obtained in the first group of pregnant women showed that almost simultaneously with the termination of the respiratory system of a pregnant woman take the fetuses of stationary for at least 20 seconds, after which each of them sooner or later appear respiratory movements ribs.

In the next group, consisting of 20 healthy pregnant women, found that the values of the samples Gauskhneht 20-24\(^{th}\) week of pregnancy amounted to 22.1 ± 6.0 seconds (P ≤ 0.05, n = 20), and in the 30 to 34 weeks of pregnancy 22.05 ± 7.0 seconds (P ≤ 0.05, n = 20). In other words, with normal pregnancy the values of the functional test of the stability of the fetus to hypoxia in terms of 20-24 and 30-34 weeks of the pregnancy do not change. In addition it is found that in this group of women, all the values of the samples exceeded 20 seconds.

In parallel, the group consisting of 5 pregnant women with pathologies of pregnancy (with signs of Fetoplacental insufficiency IB degree), it is shown that the values of a functional of the sample in the 30 to 34 weeks of pregnancy were in average of 8.1 ± 1.8 seconds (P ≤ 0.05, n = 5). Moreover, the fetus with a
twofold umbilical cord entanglement around the neck value of the functional test was 5 seconds.

Therefore, at a pathology of pregnancy the values of the samples Gauskhneht almost 3 times lower than in the normal. In addition, each individual value of the functional test at a pathology of pregnancy were less than 15 seconds.

The third series of observations was devoted to the evaluation of sensitivity, specificity and predictive significance of values of the sample Gauskhneht 61 pregnant women admitted for urgent deliveries. The women were divided into 2 groups by the values of the samples Gauskhneht. «Cut-off» value sample was defined indicator to 15 seconds. In this regard, the first group consisted of 34 patients, whose fetus antenatal have value of samples for more than 15 seconds, and the second group consisted of 27 pregnant women, whose fetuses antenatal have value of samples less than 15 seconds.

As follows from the above results, the indicators of the functional test for resistance to hypoxia fetus in pregnant women of the first group were in the range of 17 to 40 seconds. The average value of the samples in this group amounted to 26.41 ± 5.69 seconds (P < 0,05, n = 34). It is shown that the pregnancy in the first group, the complicated swelling of 17.64%, mild pre-eclampsia – 23.53%, anemia – 17.64% of women. Chronic fetoplacental insufficiency and chronic fetal hypoxia in this group were diagnosed respectively the 11.76% and 11.76% of pregnant women.

In the second group of women, the results of the sample were in the range of 5 to 15 seconds, the average value by 10,.37 ± 2,53 seconds (P < 0,02, n = 27), that is less than the fetus of the first group of 2.54 times.

In this group of women pregnancy was complicated by the presence of edema of 18,51% of women, anaemia – 22.22% of women, chronic fetoplacental insufficiency and chronic intra-uterine hypoxia of a fetus – at 22,22% and 37,04% of women (respectively).

We then divided the women of the second group, 2-subgroups. The first subgroup was formed from 14 women, the fetus of which had the values of samples less than 10 seconds. The mean value of a functional samples in this group amounted to 7.93 ± 1,81seconds (P ≤ 0,05, n = 14). The second group was composed of 13 pregnant women, the fetuses of which had the values of the sample from 11 to 15 seconds. The mean value of the sample amounted they 13.00 ± 0.92 seconds (P ≤ 0,02, n = 13).

It is shown that at high values of the functional test for resistance to hypoxia fetus newborn significantly more often born more healthy, than at low values. Thus, in the group of women with high values of samples Gauskhneht newborn babies at the at the first and at the fifth minute of life had scores respectively one 7.91 ± 0,22 (P < 0,01, n = 34) and 8,47 ± 0,49 points (P < 0,01, n = 34) on Apgar scale. The health indicators in individual children were in the range...
from 7 to 9 points. In the group of women with the values of samples less than 15 seconds newborn babies at the first and fifth minute of life had scores respectively 7.25 ± 0.88 and 7.85 ± of 0.56 points (P < 0.05, n = 27) on Apgar scale.

More significant differences in the health condition of newborn children were identified in the subgroup of women with the values of the functional test of less than 10 seconds. This group of newborns had evaluation on Apgar scale in the first minute of life in the range from 2 to 8 points with an average value 6.86 ± 1.22 points (P < 0.05, n = 14), in the fifth minute of life – in the range from 3 up to 9 points with an average value of 7.57 ± 0.96 points (P < 0.05, n = 27). In the subgroup women with the values of the samples of fetus from 11 to 15 seconds newborn children to have the evaluation on Apgar scale in the first minute of life in the range of 7 to 8 points with the average values of 7.69 ± 0.43 points (P < 0.05, n =13), in the fifth minute of life – in the range from 8 to 9 points with average values 8.15 ± 0.26 points (P < 0.05, n = 13).

Thus, the indicators of the health of newborn babies for the first and fifth minutes after birth in the group of women with low values of the samples Gauskhneht were 8.69% and 7.31% lower than in the group of women with high values of the sample.

Following this, we analyzed the state of health of all newborns during the first week of life. In the end, in the group of infants with prenatal values of the sample 15 seconds cerebral ischemia of I degree was diagnosed in 5 out of the 34 children (14.7%), and in the group of infants with prenatal values of samples less than 15 seconds in 23 of the 27 children (85%).

In addition to the group of children with high values of samples Gauskhneht (in the first group) jaundice was detected in 4 of 34 children (11.76%). The level of indirect bilirubin blood of children with jaundice in the early neonatal period averaged 80.67 ± 22 mmol/l water (P ≤ 0.05, n = 4). In the group of children with low values of the samples Gauskneht (the second group) jaundice was revealed that every third child. The level of indirect bilirubin blood, they were determined in the range from 20 mmol/l to 254 umol/l water the Average values of this indicator constituted 134.75 ± 77 mmol/l water (P ≤ 0.05, n = 8).

In addition, in the subgroup of infants in history where the values of the samples did not exceed 10 seconds, noted 4 cases of intra-partum asphyxia newborns. Moreover, the average value of the sample Gauskneht these newborns amounted to 8.0 ± 2.0 seconds (P ≤ 0.05, n = 4), and in 3 of them in history there was an indication of Fetoplacental insufficiency during pregnancy. In other words, children with low values of the samples indirect bilirubin level and frequency of the conjugation of jaundice were 1.67 and to 3.15 times higher than that in children with high performance.

Therefore, use of antenatal sample Gauskneht is a way of predicting perinatal
outcomes. The values of samples less than 10 seconds, you can predict asphyxia and cerebral ischemia in newborn infants with sensitivity 85.19% and specificity of 88.24%. Low resistance of the fetus to hypoxia, suggests the possibility of a newborn asphyxia and hypoxic damage to the cortex in physiological delivery with a probability of exceeding an average population value in 4.34 times.

The prognostic value of the values of the samples exceeding 15 seconds, is 98.43%. High values of the samples demonstrate a very high probability of birth of alive and healthy baby during the term physiological childbirth.

CONCLUSIONS

Our research shows that some pregnant women and nearly half of obstetricians and obstetric anesthesiologists in the Republic of Udmurtia aware of the functional test for resistance to intrauterine fetal hypoxia. The information they receive about the readiness of the fetus of the experience one or another hypoxia increases the accuracy and security of applied obstetric and intensive care benefits. The other half of the experts still don’t explores before birth adaptive reserves of the fetus to hypoxia, so the need for caesarean section is for them and unexpected emergency, which increases the risk and the extent of hypoxic damage of a cortex of a brain of the infant. International guidelines on obstetric practice do not use the new function test Gauskneht, which does not contribute to the improvement of the test and reduce cerebral pathology in newborns.

REFERENCES

The Effectiveness of Preinduction of Preterm Labor in Case of PROM at 34-36 Weeks of the Gestation

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SUMMARY

At 35.6 ± 1.12 weeks of gestation was performed the preinduction of labor with Preterm Prelabor Rupture of Membranes (PPROM). Were used prostaglandin (PGE2) and antigestagen. The effectiveness of preinduction of labor in the absence of significant worsening in perinatal outcomes were achieved in 96.3%. It was found that PPROM in pregnancy 35.6 ± 1.14 weeks the duration of the latency period could reach 109.1 ± 12.63 hours. Identified objective ultrasonic criteria of the onset of labor, depending on the method of preinduction of labor in women with premature labor and PPROM. Performed an analysis the study of the characteristics of intrauterine fetus- and utero-placental hemodynamics, uterine activity, depending on the method of preinduction of labor in women with premature rupture of membranes (PROM) at 34-36 weeks of gestation.

Keywords: Preterm prelabor rupture of membranes, preinduction of labor, Prostaglandin, mifepristone.

INTRODUCTION

The problem of premature labor (PL) is a priority for obstetricians and ne-
onatologists [1, 3, 7-9] The frequency of PR in the world has reached 5 to 10% due to the modern reproductive technologies [1]. The particular problem of PL – preterm premature rupture of membranes (PPROM) [3, 8, 9]. In case of PROM at a term pregnancy the onset of labor occurs within the next 24 hours in 90 % of cases, and in preterm – only in 50%. Up to 34 weeks of pregnancy, prematurity, pulmonary hypoplasia can lead to neonatal death, therefore is justified expectant management. After 34 weeks of pregnancy, expectant management is not justified. [1, 7-9] To date there is no reliable evidence base on birth outcomes for the mother and the newborn in preinduction at PRPO and premature pregnancy [4, 7, 10, 11]. There are many methods of ultrasonic control of progress of labor, but only if a term pregnancy. In numerous publications, reviews on the preinduction / induction of labor at PRPO and PR, highlights that the research on this issue to continue to hold, there is no evidence base for uniquely interpreted findings.

The main objective of this study is to evaluate the effectiveness of preinduction of premature labor in pregnancies at 34-36 weeks of gestation.

MATERIAL AND METHODS

In the regional perinatal centers of Krasnodar examined 200 pregnant women with PPROM. Gestational age 35,6 ± 1,12 weeks (34-36 weeks). The average age 27,4 ± 2,75 years. 100 women have been made preinduction of labor. Preinduction method in the first clinical Group (Group I, 50 women) was the prostaglandin (Dinoprostone-gel, per vaginum; 0,5-1,0 mg dinoprostone in a single dose), in second clinical Group (Group II, 50 women) – antigestagen (Mifepristone, 200 mg per os daily with an interval of 24 hours, maximum 600 mg). Preinduction of labor was performed 24 hours after PPROM after complete dissection of the amniotic membranes. The Control Group included 100 women with PPROM who developed spontaneous preterm birth at 34-36 weeks of gestation. Exclusion criteria included the need for an additional method of induction (prostaglandin in case of ineffectiveness of mifepristone, oxytocin), pathology, hindering cervical ripening and preventing the advancement of the fetal head through the labor canal. The selection criteria for of preinduction drug: for mifepristone – «maturity» of the cervix at least 6 points on a scale of Bishop; markers of risk of the chorioamnionitis (rupture of membranes for more than 72 hours, leukocytosis > 15 ml·10⁹ < 18·10⁹/ml of leukocyte ore «left shift», the level of C-reactive protein > 5 mg /L < 15 mg/L or an increase of 40-50% or more from baseline in dynamics; for prostaglandin – «maturity» of the cervix more than 6 points on a scale of Bishop, the lack of markers risk of the chorioamnionitis. Evaluation «maturity» of the cervix was performed on a
scale EH Bishop (1964). Ultrasound was performed using devices expert class Volusson – 730, PHILIPS HD11 (transvaginal access and transperineal access). Were used: linear transducer with a frequency 7,5 MHz, intravaginal – with a frequency of 7,5-12 MHz, transabdominal – the frequency of 3,5 MHz). PPROM diagnosis was based on visual evaluation, «cough» sample, test system Amnisure.

RESULTS

After PPROM in women of Groups I-II symptoms of regular uterine contractions were absent. The women of the Control Group in 16 % of cases occurred mucous and bloody discharge from the genital tract in small amounts as a result of the onset of labor, 80% identified a minor, short-term labor pain. In contrast to the women’s Groups I-II women of the Control Group periodically been registered increased basal tone of the uterus, up to 10 mmHg, the development of labor occurred during the first days after PPROM: 75% of women have the latency period 12 hours or less, 21 % – more than 18 hours or less, 4% – 24 hours or less. It can be assumed that at the Control Group the PPROM was a symptom of the onset of labor. Latency period at Groups I-II was significantly more: in Group I latency period was 28,45 ± 1,5 h (19-48 hours) (p<0.01), in Group I – 109,1 ± 12,63 h (17-248 hours) (p < 0.001). For the development of a regular uterine activity required 1,44 ± 0,07 mg of Dinoprostone, 45 (90%) of women required the double dose. The average dose of Mifepristone was 336,0 ± 18,47 mg: a single dose of the drug (200 mg) was effective in 21 (42%) women, double dose (400 mg) in 24 (48%) of women, triple dose (600 mg) in 5 (10%) women. «Maturity» of the cervix with the onset of labor in women Groups I-II was 7,2 ± 0,26 points in Group I and 5,8±0,7 points in Group II (in the Control Group – 7,4 ± 0,22 points). On average ultrasound cervical length before labor preinduction in the Group I was 21,3 ± 0,15 mm (p<0.05), in Group II – 25,7 ± 0,21 mm (p<0.05) (in Control Group – 10,94 ± 0,2 mm). With the onset of labor cervical length in the Group I was 8,4 ± 0,1 mm (p>0,05), in Group II – 19,7 ± 0,16 mm (p<0.05) (in the Control Group – 8 0 ± 0,2 mm). The diameter of the internal os for women of all Groups was about 20 mm: in the Group I – 18,4 ± 0,25 mm (p>0,05), in Group II – 17,5 ± 0,22 mm (p>0,05) (in the Control Group – 22,1 ± 0,20 mm). Moreover, in the absence of the symptoms of the start of regular contractions of the uterus the internal os diameter significantly increased to 26,2 ± 1,3 mm in Group I and to 20,4 ± 2,07 mm in Group II. Consequently, in 35,6 ± 1,12 weeks of pregnancy PPROM at the onset of labor may be asymptomatic, even if preinduction. The duration of the time was from labor preinduction prior to the regular contractions of the
uterus in Group I was 18.42 ± 1.08 h (p<0.01), in Group II – 32.43 ± 1.53 h (p<0.001) in the Control Group 7.7 ± 0.28 h. In assessing fetal head descent in labor with transperineal ultrasound, it was found that the average distance from the head to the perineum before preinduction of labor in the Group I was 7.57 ± 0.08 cm, in Group II – 7.45 ± 0.07 cm; with the onset of labor in Group I – 6.65 ± 0.07 cm, in Group II – 6.74 ± 0.06 cm, in the Control Group – 5.42 ± 0.06 cm. Obviously, with the onset of labor women with PPROM and preinduction of labor at 34-36 weeks of gestation have asymptomatic an opening of the cervix, cervical shortening, fetal head descent. It was not possible to accurately determine the onset of labor. Therefore, the average duration of labor was in Group I 6.92 ± 0.22 h (p>0.05), in Group II 8.2 ± 0.11 h (p>0.05), in the Control Group 6.38 ± 0.21 h. All births occurred in the conditions of long-term epidural analgesia. In assessing uterine activity in labor found that women of all Groups have normal limits. But women in Group I mean basal tone of the uterus was significantly higher – 10.4 ± 0.20 mm Hg (p< 0.05) than in women of Group II – 9.7 ± 0.18 mm Hg, and in the Control Group – 9.5 ± 0.2 mm Hg. The frequency of uterine contractions for 10 min in Group I was 4.1 ± 0.11 (p<0.05), in Group II – 3.3 ± 0.15, in the Control Group – 3.3 ± 0.12. The duration of the contractions in the Group I was 76 ± 1.1 sec (p<0.05) in Group II 65 ± 0.6 sec, the Control Group 62 ± 0.7 sec. Duration of the systole contractions in Group I was 41.0 ± 0.8 sec (p<0.05), in Group II – 33.4 ± 0.3 sec, in the Control Group – 32.0 ± 0.3 sec. Duration of diastole of uterine contractions in Group I was 34.4 ± 0.4 sec (p<0.05), in Group II – 38.3 ± 0.3 sec, in the Control Group – 37.3 ± 0.2 sec. This means that in 35.6 ± 1.12 weeks of gestation with PPROM the preinduction of labor by prostaglandin differs significantly more significantly greater contractile activity than preinduction of labor by mifepristone. However, there were no cases of the uterine tachysystole.

Prior to the preinduction of labor in 10% of women in Group I and 8% of women in Group II by Doppler was a minor violation of the utero-placental hemodynamics (the systolic/diastolic ratio 2.18 ± 0.01 and 2.21 ± 0.01 respectively). Preinduction of labor was associated with a significant increase in the number of women with a minor violation of the utero-placental blood flow, but without a significant indicator of the change in the systolic/diastolic ratio of uterine arteries. Similar changes were found in violation of fetus – placental blood flow, identified at 8% of cases – in Group I (the systolic/diastolic ratio of the umbilical artery 3.3 ± 0.01) and in 10% of cases in Group II (the systolic/diastolic ratio of the umbilical artery 3.2 ± 0.01).

In the analysis of the method of delivery was found out that the women Groups I-II, caesarean section rates (18% and 14%, respectively) was significantly higher than in the Control Group (5%). Thus, only women in Group I in 10% of cases the indication for the cesarean section was abruptio of placenta,
in 4% of the cases – changing the position of the fetus in the oblique/transverse, in 4% of cases – the worsening of intrapartum fetal heart rate (bradycardia, long deep decelerations, the appearance of a meconium in the amniotic fluid). The women in Group II were significantly more likely (14 %) indication for caesarean section was only worsening of intrapartum fetal heart rate.

In assessing the state of Apgar scores at 1 and 5 minutes of the neonatal period at all Groups no differences were found. On the 1st minute of the neonatal period the Apgar score in Group I was $6.82 \pm 0.01$ points ($p>0.05$), in Group II $6.79 \pm 0.01$ ($p>0.05$), in the Control Group $6.85 \pm 0.01$ points. On 5th minutes the Apgar score in Group I was $7.15 \pm 0.01$ points ($p>0.05$), in Group II $7.05 \pm 0.01$ ($p>0.05$), in the Control Group $7.18 \pm 0.01$ points. In the present study it is impossible conclusively prove or disprove that that the state of the newborn was due to childbirth preinduction Dinoprostone or Mifepristone. However, none of the preinduction methods led to a significant deterioration in the newborn.

**CONCLUSIONS**

The results require further scientific research on the subject. Obviously urgent need for further research to identify changes in the cervical transcriptome in the human uterine cervix [2, 5, 6], determine Oxytocin receptor (OTR) mRNA expression has previously been demonstrated in human myometrium, decidua, chorion and amnion in term and preterm gestational tissues prior to and following the onset of labor [10] to predict spontaneous onset of regular labor, efficiency of labor preinduction and the titration of the dose of preinduction drug at PROM with the minimum effective dose, especially in preterm pregnancy.

**REFERENCES**


Local Hypothermia Skin Above Cracks Skull Fetus in the Final Period Births May Be a Symptom of Hypoxia and Ischemia of the Cortex of His Brain

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SUMMARY

We have developed a method diagnostics of the cerebral cortex hypoxia of the fetus with a thermal imager. The method is based on the following regularity revealed by us: hypoxia and ischemia decreases the intensity of radiant heat in tissues. Infrared thermometry was performed in 35 pregnant women using thermal imager ThermoTracer TH9100XX (NEC, USA) in the temperature range 26-36°C. The results showed that the local temperature in the skin parietal part of the heads of live fetuses in the final of childbirth and immediately after them ranged between 31.6°C and 36.1°C. Found that the normal on the surface of the parietal part of the head of the fetus can be detected local hyperthermia area where the temperature may be 0.5-4°C above the surrounding surface of the head. This zone has an elongated shape and is located over the central seam of cranium. Central seam of the cranium allows to evaluate the provision of oxygen cortex of brain of the fetus during labor. In this regard, the absence of periods setback temperature scalp during in moving fetus through the birth canal indicates the possibility of a healthy child in the process of physiological birth, and the emergence of local hypothermia in the projection of not fused bones the Central seam of the cranium indicates the beginning hypoxic and ischemic damage.
of cerebral cortex of the fetus and requires immediate hyper-oxygenation blood of the fetus.

**Keywords:** temperature, infrared thermography, life, birth.

**INTRODUCTION**

Hypoxia during labour is the most common cause of perinatal cerebral cortex damage and spans a leading position in the structure of morbidity, disability and mortality in children neonatal period [1]. Undeniable urgency of prompt diagnosis, and therefore prevention and early treatment of hypoxic fetal cerebral cortex. We suggest the possibility of imaging in the infrared emission spectrum of symptoms of hypoxic damage to the cerebral cortex of the fetus during labour. Continuous infrared thermography of the visible part the scalp of the fetus after its eruption is not included today in the list of mandatory studies in the management of labour, so the possibilities of the method in the diagnosis of hypoxia and / or ischemia of the cerebral cortex of the fetus during childbirth bearing-down period are unknown [2-5]. Despite the known role of general and local temperature in the preservation of life and health of warm-blooded mammals and humans [6-8], but in recent years has shown high diagnostic value of infrared thermometry and thermography different parts of the body surface in the evaluation of hypoxic, ischemic and drug lesions in adults [9-19] and children [20-24]. On this basis, we have developed a method for estimating the degree of hypoxic damage the cerebral cortex and resuscitation efficacy in adults during clinical death [25]. However, this method is not suitable for diagnosis of hypoxia and/or ischemic brain injury in the fetus bearing-down stage of labour. It was assumed that infrared thermometry of the fetal head will reveal symptoms of hypoxic damage to the cerebral cortex in the fetus. Due to the fact that hypoxia and ischemia of any portions of a human body accompanied its local cooling and a change in colour of infrared radiation multicolour red orange-yellow-green-blue to monocolour blue, the occurrence of local cooling of the skin over the skull suture fontanel and fetal the second stage of labour may be a diagnostic symptom of hypoxia his brain. Therefore thermography fetal scalp can be used to diagnose the presence of hypoxia and/or ischemic cerebral cortex fetal birth bearing-down period. Thus, the aim of our study was to develop a method for diagnosis of hypoxic-ischemic lesions of the cerebral cortex of the fetus during labour using infrared thermography.
MATERIAL AND METHODS

Infrared thermography conducted in a maternity hospital in 35 pregnant women admitted for physiological birth. From them 20 pregnant women had fetuses, with in 30-32 weeks of pregnancy demonstrated high adaptation to intrauterine hypoxia, which was confirmed by results Gauskhneht test (more than 30 seconds). Another 15 pregnant women had fetuses, with in 30-32 weeks of pregnancy demonstrated the low adaptation to intrauterine hypoxia, which was confirmed by results Gauskhneht test (less than 10 seconds). Infrared thermometry was performed using thermal imager Thermo Tracer TH9100XX (NEC, USA) in the temperature range +26 – +36°C. The temperature of the air in the delivery room is in the range +24 – +26°C.

RESULTS

In the course of the investigations it was found that the infrared thermography surface of the head of the fetus during labor ensures immediate delivery of accurate information about the dynamics of the values of its local temperature and spectrum of infrared radiation. It is shown that the range of individual values of the local skin temperature in the parietal scalp of live fetuses during labor and immediately after them in our observations was between +31.6 °C and +36.1 °C. In the absence of symptoms of placental insufficiency and there is a high stability of the fetus to hypoxia infrared portrait of the parietal part of the head is rarely significant differences in color. As some fetuses temperature above the central seam exceed the temperature of the skin over the bones of the skull on average by 2.8 ± 0.21°C (P < 0.05, n = 20).

Found that in normal pregnancy and normal physiological delivery the head of live fetal is depicted on your thermal predominantly yellow-orange-red colors. In addition, in normal the scalp of fetuses before birth has a high temperature. But the fetuses, were born in meconium waters, had a low temperature in scalp and in body of newborn. Moreover, the normal on the surface of the parietal part of the head of the fetus can be detected local hyperthermia area where the temperature may be 0.5-4.0°C above the surrounding surface of the head. This zone has an elongated shape and is located over the central seam of cranium.

In the group, consisting of 15 pregnant women with signs of placental insufficiency and with low adaptation of fetuses to hypoxia the dynamics of temperature of the visible surface of the head over the bearing-down stage of labor in 10 fetuses had no fundamental differences from the dynamics of the temperature of the fetuses in the control group mothers. But other 5 fetuses have short
periods of low temperature on the scalp in zone gaps skull. The duration of these periods ranged from 30 to 120 seconds.

We carried out an analysis of the circumstances surrounding the emergence of local hypothermia. The results showed that the immobility of the fetus in the birth canal enhances hypothermia. Found that temperature portraits of heads of these fetuses were normalized only at offset these fetuses inside the birth canal with the help of attempts.

Therefore, thermometry surface of the fetal head, carried out with the help of thermal imager in the final period delivery, allows to detect the appearance, availability and fault periods of relative local hypo- and hyperthermia on overgrown central seam skull. In our opinion temperature bare and wet surface of the fetal head during the eruption surrounded by dry air at room temperature allows to judge about the intensity of oxidative metabolism in the brain cortex, accompanied by heat. In turn, the intensity aerobic metabolism and radiation tissues to judge the adequacy of the cerebral cortex oxygenated arterial blood. Therefore, the identification normal- and hyperthermia on the entire surface of the fetal head allows to judge about the absence of threatening hypoxia and ischemia of the brain cortex.

The appearance of the period the local hypothermia over not overgrown swept seam skull fetus we offer seen as a symptom diagnosis of hypoxia and/or ischemia of the fetus, because the induced us in this period premature birth pangs and offset the fetus in the birth canal was accompanied by the increased temperature of the skin over the bone slit. Moreover, the restoration of the temperature occurred in 2-3 seconds after a successful offset the fetus.

Thus, infrared thermography the surface of the head baby don’t over overgrown central seam skull and timely management of security placement of the fetus in the birth canal using adequacy criterion in the form of preserving normal- and hyperthermia surface ensures the adequacy of the supply of the brain arterial blood oxygenated and excludes hypoxic and ischemic damage to the cerebral cortex in the newborn.

**CONCLUSIONS**

The temperature fetal head in birth demonstrates the condition of the cerebral cortex.

**REFERENCES**

Low Maternal Serum PAPP-A Levels in the First Trimester of Gestation and the Risk of Gestational Diabetes

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SUMMARY

Biochemical markers in pregnant women (maternal serum free ß-hCG and PAPP-A, as measured at 1st trimester) and their relationship with certain conditions, like preeclampsia, hypertension, gestational diabetes, etc., have been in the spotlight for some years now as several studies have addressed the issue. The present study specifically assesses PAPP-A protein as a risk factor for Gestational Diabetes (GD) – one of the most common conditions affecting 4 to 7% of pregnancies – and takes a close look to certain parameters – pregnant’s weight, age and gestational age at time of triple screening – to determine whether they may correlate with PAPP-A levels and therefore modify the risk of GD.

Keywords: biochemical, markers, pregnant, PAPP-A, weight, age, GD.
INTRODUCTION

The study’s objective was to compare first trimester biochemical marker PAPP-A (Pregnancy-Associated Plasma Protein-A) in pregnant women who developed gestational diabetes (GD) with that of a control group (pregnant women without gestational diabetes) so as to determine a significant statistical difference and whether PAPP-A may be a risk factor for GD.

MATERIAL AND METHODS

This was a retrospective cohort study. Data for the study were retrieved from the computer database at Hospital Universitario Río Hortega (Valladolid, Spain). Data were routinely recorded by healthcare personnel at the time of three clinical tests - triple screening of first trimester, glucose screening test and glucose tolerance test - during the time period 2009-2010. All available women in the database were included, except pregnant women previously diagnosed with Diabetes Mellitus or GD and women with incomplete or inaccurate data.

As per protocol, PAPP-A was measured when performing the triple screening of first trimester at week 11-13 of gestation. Afterwards, a routine glucose screening test was performed at week 24 of gestation. Pregnant women testing positive (≥ 140) on the glucose screening test underwent a glucose tolerance test to determine whether they had GD. The study sample consisted of 2,750 pregnant, among which 190 (6.9%) were diagnosed with GD at week 26-28, after the glucose tolerance test being carried out. Women were also classified by gestational age, weight and age ranges at time of triple screening to assess differences based on such parameters.

Hypothesis tests and simple bivariate analysis were performed to determine any significant difference for biochemical markers between both groups’ sampling distributions and the force association – Odds ratio (OR) – between PAPP-A and GD.

RESULTS

As it is well established, the study showed that mother’s weight and age significantly increase the risk of GD.

A statistical significant difference was found between the sampling distribution of PAPP-A in pregnant women affected by GD and that from normal pregnant women (p = 0.016). Median for gestational diabetics was 0.95 MoM. Median for normal pregnant women was 1.03 MoM.
Low PAPP-A levels correlated with a higher probability of GD: At 5th Percentile (0.425 MoM), OR = 1.85 (p = 0.024); At 10th Percentile (0.528 MoM), OR = 1.85 (p = 0.013); At 20th Percentile (0.664 MoM), OR = 1.75 (p = 0.001).

When classified by age ranges at time of triple screening, there was not a significant difference between the sample distributions of PAPP-A in both groups among women over 35 or 38 years old. However there was a significant difference among women under 35 years old (p = 0.016).

Median PAPP-A decreased in gestational diabetics as weight increased. Low PAPP-A levels (at 5th, 10th, 20th and 50th percentile) significantly correlated with a higher probability of GD when only women over 70 kg were included, and such correlation was statistically stronger as compared to the whole sample (p = 0.002 to p = 0.007): At 5th Percentile (0.403 MoM), OR = 3.59 (p = 0.002); At 10th Percentile (0.497 MoM), OR = 2.80 (p = 0.002); At 20th Percentile (0.633 MoM), OR = 2.28 (p = 0.004); At 50th Percentile (0.991 MoM), OR = 2.09 (p = 0.007).

The study found that median PAPP-A was significantly lower when measured at week 13 of gestation (0.79 MoM) by comparison to measurement at week 11 (1.01 MoM) and week 12 (0.93 MoM) in women that developed GD. Low PAPP-A levels (at 20th and 50th percentile, p value = 0.011 and 0.029, respectively) significantly correlated with a higher probability of GD when measured at an advanced gestational age (week 13 of gestation), and such correlation was statistically stronger as compared to the whole sample.

Among women over 70 kg whose PAPP-A was measured at week 13 and developed GD, only one case showed a PAPP-A value above 2.00 MoM.

CONCLUSIONS

Low levels of maternal serum PAPP-A in the first trimester correlates positively with a higher risk of GD in the second trimester of pregnancy. Such correlation is stronger if weight is higher (for pregnant women set at over 70 kg), if mother’s age is under 35 years of age or if PAPP-A is measured at a more advanced gestational age (week 13 of gestation).

Median PAPP-A in pregnant women that will develop GD seem to decrease as gestational age progresses. While it is well established that overall PAPP-A levels increase during first trimester to stay then stable through second trimester as pregnancy progresses, our results suggest that PAPP-A levels in pregnant women that will develop GD may decrease further as gestational age moves beyond week 13, so that relationship between PAPP-A levels an DG may become much stronger.

PAPP-A values over 2 MoM at week 13 and beyond, may be a strong indicator of protection against developing GD in case of women over 70 kg.
REFERENCES


Clinical Features in Low Birth Weight and Very Low Birth Weight Infants

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ABSTRACT

Introduction. Extremely low birth weight (ELBW) is defined as a birth weight less than 1000 g and infants born at less than 1500 g are termed very low birth weight (VLBW). Infants with extremely low birth weights are more susceptible to all of the possible complications of premature birth, both in the immediate neonatal period and after discharge from the nursery.

Methods. We retrospectively evaluated between 2009-2012 the risk factors and outcome among extremely low birth weight and very low birth weight (VLBW) infants born at «Bucur» Clinic of Obstetrics and Gynecology, Bucharest, Romania.

Results. Our retrospective study included 31 extremely-low-birth-weight infants (ELBW) and 65 very low birth weight (VLBW) among the 1384 premature infants recorded during the 4-year study period. Mortality rate was significantly decreased from 2009 to 2012 (43% versus 13,8%). The most common complication of pregnancy leading to premature delivery were antepartum hemorrhage (41%) and pregnancy-induced hypertension. Respiratory distress syndrome occurred in 76,8%, neonatal sepsis in 23% of infants, chronic lung disease in 35%, grade III to IV intraventricular hemorrhage in 15%, stage III to V retinopathy of prematurity in 32,6 % and necrotizing enterocolitis in 1,8%.

Conclusion. Even if extremely low birth weight and very low birth weight (VLBW) infants had severe clinical conditions such as sepsis, intraventricular haemorrhage, respiratory distress syndrome in our clinic mortality rate decreased significantly from 2009 to 2012.
INTRODUCTION

It has been proved that prematurity is the main cause of perinatal mortality and morbidity [1] being associated with risk of cognitive and behavioural disabilities. Early interventions in preterm infants may improve brain development and cognitive outcomes. We used the premature birth definition as duration of pregnancy less than 37 weeks, further categorised as moderately preterm (32-36 weeks), very preterm (28-31 weeks) and extremely preterm delivery (less than 28 weeks).

Extremely low birth weight (ELBW) is defined as a birth weight less than 1000 g. Most extremely low birth weight infants are also the youngest of premature newborns, usually born at 27 weeks’ gestational age or younger. Infants born at less than 1500 g are termed very low birth weight (VLBW). Infants whose weight is appropriate for their gestational ages are termed appropriate for gestational age (AGA). Infants who are heavier than expected are large for gestational age (LGA); conversely, those smaller than expected are considered small for gestational age (SGA) and are also usually found to be intrauterine growth restricted (IUGR) prior to birth.

METHODS

We retrospectively evaluated between 2009-2012 the risk factors and outcome among extremely low birth weight and very low birth weight (VLBW) infants born at «Bucur Clinic of Obstetrics and Gynecology», Bucharest, Romania.

Data were analyzed by the Statistical Package for the Social Sciences (SPSS for windows, version 19.0). Descriptive statistics included the mean and standard deviation for numerical variables, and the percentage of different categories for categorical variables.

RESULTS

There were 31 extremely-low-birth-weight infants and 65 very low birth weight (VLBW) among the 9367 live births recorded during the 4-year study period. The incidences of the extremely-low-birth-weight (ELBW) and very low birth weight (VLBW) infants were 21/2373 (5 ELBW + 16 VLBW) (0.008%) in 2009, 27/2280 (9 ELBW + 18 VLBW) (0.011%) in 2010 I and 31/2469 (10 ELBW + 21 VLBW) (0.012%) in 1011 and 28/2242 (7 ELBW + 21 VLBW) (0.012%).
It may be seen that in our study the incidence of ELBW has improved from 2009 to 2012, but the same trend is similar for VLBW as well. A very well defined cause couldn’t be explained from this retrospective observations. We have also studied the mortality of premature infants in this 4 years. Our results were that for ELBW and VLBW the mortality has considerably decreased from 43% to 13.8% from the first to the last year included in observation. Early neonatal mortality rate was significant decreased (43% versus 13.8%).

The infants viability was one of the most important parameters from our retrospective study. The limit of viability was improved from gestational age of 27 weeks or 850 g in 2009 to gestational age of 25 weeks or 700 g in 2012, even if it was a singular case. The mean gestational age (GA) was 27.15 weeks (standard deviation 6.13 and range, 24-34 weeks), for all the four years. Forty-six percent of the ELBW and VLBW infants were delivered by caesarean section for all 4 years, but the values were different for each period. The incidence of caesarean has increased. In our study the viability of neonates who were delivered by C-section was better than the ones who were delivered by vaginal way. The viability has improved year by year for ELBW and VLBW, and that can be correlated with C-section, but also with new techniques of neonatal intensive care. The most common complication of pregnancy leading to premature delivery was antepartum haemorrhage (41%). Regarding the respiratory complications it was observed that in prematures the respiratory distress syndrome occurred in 76.8% of infants that were included in study, but later only 23% developed chronic lung disease. Exogenous surfactant therapy was used for 57% cases, while intermittent mandatory ventilation for 89% cases.

Neonatal complications were: neonatal sepsis for 23% of infants, chronic lung disease in 35% cases, grade III to IV intraventricular haemorrhage in 15% cases, and necrotizing enterocolitis in 1.8% cases. Early neonatal mortality rate was significant decreased from 2009 to 2012 (43% versus 13.8%).

Forty-six percent of the ELBW and VLBW infants were delivered by caesarean section. Severe retinopathy of prematurity was detected in 32.6% of 89 neonates who had eye examinations. Neonates of lower gestational age had an increased development of severe retinopathy of prematurity, compared to those 30 weeks and older.

DISCUSSIONS

Preterm birth is also the dominant risk factor for neonatal mortality, particularly for deaths due to infections. Long-term impairment is an increasing issue [2]. Complications of preterm birth are the leading direct cause of neonatal mortality, accounting for an estimated 27% of the almost four million neonatal
deaths every year, and act as a risk factor for many neonatal deaths due to other causes, particularly infections [3].

Medical literature reports two types of neonatal sepsis: early-onset disease, when features of sepsis appear during the first 72 hours of birth; and late-onset disease, where the disease manifests beyond 72 hour [4]. Presenting a short report of our findings in clinical features of neonates in this article we didn’t do any references of the onset or causes of neonatal sepsis from our clinic. According to more recent data from the National Institutes of Child Health and Development (NICHD), infection-related mortality in very low-birth-weight (VLBW) infants (birth weight < 1500 g) averages 10% but can reach 40% depending on the pathogen involved [5]. It may be seen that our findings are similar, but that mortality decreased year by year.

It was reported that preterm neonates have a high risk of developing neonatal infections, resulting in high mortality and serious long-term morbidities [6]. Our study support this point of view because it was observed that in our clinic neonatal infections are more frequent in very low birth infants than normal for gestational age infants.

Worldwide it was observed that the incidence of caesarian sections has increased, as well as the incidence of preterm births, fact that was explained by sociodemographic and behavioral factors [7]. Lately, this medical change was observed in our clinic, from one year to following one. There are studies that indicate that even if the incidence of preterm birth has increased the neonatal outcomes did not worsen, and instead showed improvement [8]. ROP is a multifactorial disease involving many factors. Low-gestational age, low-birth weight, sepsis, oxygen therapy, respiratory distress syndrome and blood transfusion have been suspected to influence the incidence of ROP [9]. The most significant risk factors for development of ROP are low-gestational age and low-birth weight, as shown in many studies [10].

Extremely low birth weight survival has improved with the widespread use of surfactant agents, maternal steroids and advancements in neonatal technologies being proved in our hospital and other studies [11].

CONCLUSIONS

Even if extremely low birth weight and very low birth weight (VLBW) infants had severe clinical conditions such as sepsis, intraventricular haemorrhage, respiratory distress syndrome in our clinic mortality rate decreased significantly from 2009 to 2012.
REFERENCES

Evaluation of Fetal Vitality by Doppler Ultrasonography. Which Insonation Vascular Site is the Best to an Accurate Acquisition of This Evaluation?

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INTRODUCTION

A major concern of obstetrics today is to ensure the conditions of fetal vitality, especially in high risk pregnancies. In this aspect, the doppler velocimetry stands out as a propedeutic method that is able to detect early circulatory fetal adaptations. It’s historic initiates in 1977, when REF, combining the ultrasonography technique with the continuous Doppler device, they developed a novel method which enabled the study of arterial blood flow and in umbilical veins, in a safe and noninvasive manner, allowing to diagnose states of hypoxia and predicting adverse perinatal outcomes REF. With an increase in umbilical arteries resistance (UA), it observes a decrease in diastolic flow, and the opposite occurs in cerebral arteries, with vasodilatation of this fetal territory REF. The analysis of the combination of fetal and placental parameters seems to be a useful manner to evaluate the hemodynamic adaptation to hypoxemia and hypo-
xia. In this aspect, the cerebroplacental relation (CPR), calculated by the ratio between the pulsatility index (PI) of UA and the PI of fetal middle cerebral artery (MCA), has been suggested as a prediction method in neonatal prognostic in high risk pregnancies REF. This «centralization» phenomenon precedes in 10 to 12 days the severe fetal impairment, with acidosis and higher morbimortality REF.

However, besides the importance of the exam, the way how the analysis can be performed and, more specifically, the insonation site for this acquisition still persists as an polemical object, with authors reporting that the site does no change the assessment and even with conflicting viewpoints regarding the execution routine.

To identify truthful alterations in fetal vitality is of expressive importance defining the ideal moment to interrupt the pregnancy and avoiding that inadequate values obtained might lead to iatrogenic preterm birth.

Our study investigated what was the best insonation site to obtain an accurate evaluation that reflects the fetal status, and if there are differences in results due to the site studied in each vessel (UA – in proximal portion to placental insertion or next to fetus, in the bladder area – and MCA, if performed immediately after its origin in intern carotid or near to skullcap).

Keywords: Doppler ultrasound, Fetal blood flow, Umbilical arteries, Pulsatility index, middle cerebral arteries.

OBJECTIVES

Evaluate if the insonation local of Doppler in ultrasonography modifies the analysis of fetal vitality state, through the assessment of middle cerebral and umbilical arteries in pregnant women with low to high risk in accompanying in a reference hospital in southern Brazil.

MATERIAL AND METHODS

Prospective study, utilizing 80 patients, with analysis of three different sites of the middle cerebral artery and umbilical artery, and obtaining indexes of Doppler resistance and pulsatility, as well as the stratification of results according to the categories of pregnancy, high and low risk.

The selected pregnant were being having prenatal monitoring in the Insti-
tution and were sent for obstetric ultrasonography. The following inclusion criteria were used in the study: singleton pregnancies above 24 weeks, absence of placental disorders (placenta previa, circumvallate, suspected tumor or accretism) or umbilical cord. As exclusion criteria were used: morphological or chromosomal abnormality detected in the fetus, placental changes or umbilical previously mentioned, multiple gestation or disagreement to participate the study.

For doppler velocimetry assessment of all evaluated vessels were respected general principles (identification of the vessel with colored Doppler and pulsatile Doppler window positioning after; insonation angle below 20° (between the direction of the vessel and of the beam of pulsatile Doppler); sample volume adjusted between 1.5 and 3 mm; at least five waves of uniform flow velocity for posterior calculation; thermic index was kept below 1.5 (according with international rules).

For statistical analysis it was used descriptive tests (means, median, interquartile ranges and standard deviations) and analytical tests including Student’s t test and ANOVA for paired samples, considering significant differences where p<0.05. The study was approved by Committee of Ethics in Research of the institution.

RESULTS

From the 80 analyzed pregnant women, 31 represented the high-risk group and 49 pregnant women without the presence of pathologies (diabetes, hypertension, IUGR and drug addiction). Analyzing the results overall, with no separation of groups and high-risk stratification, there was a statistically significant difference in resistance and pulsatility indexes for umbilical artery and middle cerebral artery values (p<0.0001). It was identified two cases of significant biological implications, for the difference found, with clinical centralization by the method employed to study, if utilized vascular insonation locals for evaluation of preterm fetuses.

CONCLUSION

Despite the recognition that the Dopplervelocimetric parameters vary along the umbilical cord, having greater resistance near to fetus REF, the standardization of method is extremely necessary in order that an analysis in inadequate lead to a misinterpretation of the results and, possibly iatrogenic intervention, since prematurity remains a major determinant of neonatal mortality and its complications REF.
The objective of this study was to promote a standardization of the method and emphasize the importance of the correct site assessment for diagnostic.

There was significant difference between the different insonation points both for variables of umbilical artery and for those of middle cerebral artery. Based in our findings, the analysis should be kept in umbilical artery near the placenta and the middle cerebral artery near the skullcap, avoiding false analysis of fetal well-being and favoring unnecessary interruptions of pregnancy, precocious and iatrogenic.

REFERENCES

PreOS (Preeclampsia Open Study): a Multicenter, Prospective, Open, Non-Interventional Study Evaluating the Influence of the Angiogenic Biomarkers sFlt-1 and PlGF on Decision-Making of Physicians in Pregnant Women with Suspicion of Preeclampsia

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SUMMARY

PreOS is the first observational clinical multicenter study to demonstrate the clinical utility of the fully automated Elecsys® sFlt-1/PIGF test of Roche in maternal serum in clinical decision making in pregnant women with suspicion of preeclampsia. In women presenting with suspicion of preeclampsia, diagnosis of preeclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) or eclampsia is difficult as not all clinical signs and symptoms allow for a diagnosis or prediction of preeclampsia as described in the diverse professional or hospital guidelines. The PreOS Study aims to determine the added value of sFlt-1/PIGF testing in real world clinical practice. The consequential impact of the test on patient management with regards to hospitalization, induction of delivery and other diagnostic and therapeutic procedures is recorded along with outcomes of mother and neonate. The clinical considera-
tions and decision-making on patient management by the treating physician is captured in real time by means of an iPad® application which was specifically developed for the study. Integration of sFlt-1/PIGF testing in the diagnosis of preeclampsia in clinical practice allows well informed decisions by the treating physician, supporting better outcomes for mothers and neonates.

**Keywords**: laboratory test, patient outcome, hospitalization, delivery, clinical utility, pregnancy, clinical pathway.

**INTRODUCTION**

In 3-5% of all pregnant women preeclampsia is a major cause of maternal, fetal and neonatal mortality [1, 2]. In patients with preeclampsia increased serum levels of sFlt-1 (soluble fms-like tyrosine kinase-1), and decreased levels of PlGF (placental growth factor) cause a state of endothelial dysfunction [3-9]. The clinical pathway of preeclampsia as described in current guidelines is based on hypertension and proteinuria. In patients with an indeterminate diagnosis based on hypertension and/or proteinuria, the additional measurement of the ratio of sFlt-1 and PlGF was found a better predictor of preeclampsia than either marker alone, as the ratio reflects the concurrent dynamics in both markers [10-12]. However, the clinical utility of the sFlt-1/PIGF ratio in guiding decisions on management of patients with suspicion of preeclampsia is not established in clinical routine, yet. Therefore, a study is needed to investigate the considerations of the clinician during application of the sFlt-1/PIGF test, and to assess the effects of testing on prevention of major complications, costs of hospitalization, mortality and morbidity in both mother and neonate.

The primary objective of the PreOS Study is to assess the influence of the fully automated Elecsys® sFlt-1/PIGF test of Roche on the decision-making of the physician to hospitalize patients with suspicion of preeclampsia. The secondary objectives aim to assess the influence of the sFlt-1/PIGF ratio on the decision-making of the physician to induce delivery and further diagnostic and therapeutic procedures.

**MATERIALS AND METHODS**

PreOS is a multicenter, prospective, open-label, non-interventional study recruiting 150 pregnant women with suspicion of preeclampsia from gestational week 24+0 days until delivery. The study is conducted at five hospitals that have implemented sFlt-1/PIGF testing in routine clinical practice as an aid in diagnosis of preeclampsia.
The target population consists of pregnant women ≥ 18 years of age, from gestational week 24+0 days until delivery, presenting to the clinic with suspicion of preeclampsia for whom, according to the doctor, the determination of the sFlt-1/PIGF ratio is indicated (but not yet carried out). Written informed consent is obtained from all eligible women. Suspicion of clinical diagnosis of preeclampsia is determined when one or more of the following signs and symptoms are present: new onset of elevated blood pressure, new onset of hypertension, aggravation of pre-existing hypertension, new onset of protein in urine, new onset of proteinuria, aggravation of preexisting proteinuria, and one or more preeclampsia-related symptoms and/or findings, e.g. epigastric pain, excessive edema, severe swelling (face, hands, feet), headache, visual disturbances, sudden weight gain (> 1 kg/week in the third trimester), low platelets, elevated liver transaminases, (suspected) intrauterine growth restriction (IUGR) or abnormal uterine perfusion detected by Doppler sonography.

Women with manifest preeclampsia, eclampsia or HELLP syndrome are excluded from the study.

For all eligible women for whom the doctor thinks a sFlt-1/PIGF test is indicated, the doctor is asked to decide on the appropriate clinical procedures: «What would you do beyond the indicated serum test?» The doctor’s decisions on intended clinical procedures are recorded in a systematic manner using an iPad® application before knowledge of the result of the sFlt-1/PIGF test, as illustrated in Figure 1. The primary question is whether the patient should be hospitalized, followed by decision-making on induction of delivery and need for any further indicated diagnostic or therapeutic procedures (e.g. induction of fetal lung maturation, additional laboratory measurements, cardiotocography (CTG) scan, Doppler sonography, change of intensity of patient monitoring within one week, drug treatment).

Once the result of the sFlt-1/PIGF test has become available, the doctor is asked again to record the decisions (revised or confirmed) on the same patient. By documenting the patient management strategy before and after knowledge of the sFlt-1/PIGF ratio with an electronic timestamp, the added value of the sFlt-1/PIGF test can be evaluated in an unbiased manner. Investigators are free in their diagnostic and therapeutic decisions and further management of their patients. The consequential influence on maternal and neonatal outcomes is documented at delivery and postpartum.

The primary analysis will be performed in all patients who fulfilled the following requirements: i) eligible according the in- and exclusion criteria, ii) sFlt-1/PIGF result is available, iii) complete data on decision-making by the physician before and after sFlt-1/PIGF test result is available, and iv) adjudication by an independent experts committee is available.
The primary endpoint is the difference in proportion of correct decisions (based on outcome) for hospitalization before and after knowledge of the sFlt-1/PIGF result for patients with suspicion of preeclampsia.

RESULTS

The PreOS Study started in July 2012. Over 120 of the total of 150 eligible
patients from five obstetrical departments in Germany and Austria have been recruited until September 2013. From the investigators’ perspective, capturing of clinical decision-making by an iPad® application appears a convenient means of real time data collection. According to investigators results of sFlt-1/PIGF testing influence their decision-making in women with indeterminate clinical diagnosis of preeclampsia.

CONCLUSIONS

PreOS is the first observational clinical multicenter study to demonstrate the clinical utility of fully automated Elecsys® sFlt-1/PIGF maternal serum testing on clinical decision making in pregnant women with suspicion of preeclampsia. The study will provide evidence on the added value of sFlt-1/PIGF testing in indeterminate cases of suspected preeclampsia and guide patient management with regard to hospitalization, delivery and other clinical procedures in real world clinical practice for improved outcomes.

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REFERENCES

Effect of Plasma Lipoprotein ‘a’ Level on Outcome in Normal and Preeclamptic Pregnancy

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SUMMARY

A total of 100 patients, 50 in normal group and 50 in pre-eclampsia group, who were fulfilling inclusion/exclusion criteria who visited OPD from May 2012 to June 2013 and were finally admitted into Labour room/obstetric and gynaecology ward of Santokba Durlabhji Memorial Hospital cum Medical Research Institute, were studied in present study. The aim of the study was to study the Effect of plasma Lipoprotein ‘a’ [Lp(a)] levels on outcome in normal and preeclamptic pregnancies. In normal group 90% had Lp(a) level < 30 mg/dl while in preeclamptic group 90% had Lp(a) more than 30 mg/dl. Lp(a) levels were 2 times higher in severe pre-eclampsia as compared to mild preeclampsia pregnant patients. 8% had complication in normal group while 18% had complication in Pre eclamptic group. The mean ± sd of Systolic BP in normal patient was 121.00 ± 10.44 and in preeclamptic group was 153.92 ± 15.98. Similarly Mean ± Sd of diastolic BP in normal patient was 78.00 ± 6.00 and in preeclamptic was 93.80 ± 9.97.

Keywords: Lipoprotein ‘a’, Preeclampsia, Systolic Blood Pressure, Diastolic Blood pressure.
INTRODUCTION

Diagnosis of pre-eclampsia was based on the definition of American college of Obstetrics and Gynecologists:

a) Systolic blood pressure more than 140 mm Hg or arise of at least 30 mmHg.

b) Diastolic blood pressure more than 90 mm Hg or a rise of at least 15 mmHg.

c) Proteinuria of 300 mg or greater in 24 hour urine collection.

These criteria should satisfy at least on two occasions with six hours apart [1]. The previous studies have shown that high levels of serum lipoprotein (a) [Lp (a)] may interfere with placental circulation and may contribute to development of atherosclerosis of spiral arteries [2]. Lipoprotein (a) (Lp(a)) is recognized as a risk factor for arterial and venous thrombosis, a property which may relate to its structural similarity to plasminogen. Pregnancy is associated with a hypofibrinolytic state. Elevated Lp(a) may influence fibrinolysis and have an unfavorable role in pregnancy outcome. In this study plasma Lp(a) concentrations during normal pregnancy and pre-eclampsia in Primigravida patients (fulfilling inclusion and exclusion criteria) from 30 to 35 weeks of gestation were studied and there severity and fetomaternal outcome was seen. A standardized international reference material has been developed and is accepted by the WHO Expert Committee on Biological Standardization and the International Federation of Clinical Chemistry and Laboratory Medicine. Although further standardization is still needed, development of a reference material is an importance step towards standardizing results [3, 4].

Lipoprotein(a)-Lp(a) [5]:
Desirable: < 14 mg/dL (< 35 nmol/l).
Borderline risk: 14-30 mg/dL (35-75 nmol/l).
High risk: 31-50 mg/dL (75-125 nmol/l).
Very high risk: > 50 mg/dL (> 125 nmol/l).

MATERIAL AND METHODS

The prospective study of plasma lipoprotein ‘a’ in normal and preeclamptic pregnant patient’s was conducted in the Department of Obstetrics and Gynaecology at Santokba Durlabhji Hospital, Jaipur with the aim of studying the Effect of plasma Lipoprotein ‘a’ levels on pregnancy outcome in normal and pre-
clamptic pregnancies. All pregnant primigravida from 30 to 35 weeks were counselled and explained about the study. A woman was enrolled in this study after taking informed consent and explaining the role and benefit of Lipoprotein ‘a’ and the cost of the test was informed to the patient.

**Inclusion criteria:**
- Age group 21-30 yrs.
- Primigravida.
- Singleton pregnancy.
- Non diabetic.
- Non smoker.
- Non alcoholic.
- No other medical disease.

**Exclusion criteria:**
- Patient with cardiac disease, IHD, diabetes mellitus or renal disease.
- Multigravida.
- Multiparity.
- Pregnant patient more than 30 yrs of less than 20 yrs.
- Epilepsy.
- Uterine anomalies (ruled out by Ultrasound).

**Normal group**

In this group plasma lipoprotein ‘a’ level of the women with normal B.P were estimated between 30-35 weeks of gestation. A constant watch over their blood pressure, urine albumin and any sign and symptom of development of pre-eclampsia was made. In case patient developed PIH or pre-eclampsia, antihypertensive treatment was started and patient shifted to Pre-eclamptic group. The feto-maternal outcome of the patient with normal BP and lipoprotein level will be seen.

**Pre-eclamptic group**

Patient’s between 30-35 weeks of gestation falling in any of the inclusion criteria of pre-eclamptic group were assigned in this group. Patient assigned in this group were the one receiving antihypertensive treatment for control of blood pressure which included Nifedipine, methyldopa, labetalol depending on need of patient. Changes in plasma Lp(a) level and development of complication if any, were noted. The patient who developed some systemic disorder or any other serious illness apart from pre-eclampsia were excluded from study. The feto maternal outcome was seen. Lipoprotein ‘a’ level estimation done by immunoturbidimetric assay. The absorbance was measured at 340 nm.
RESULTS

In the present study we have observed a dose dependent relationship between elevated Lipoprotein ‘a’ level and the severity of pre-eclampsia. Whether elevated lipoprotein ‘a’ is causative factor for pre-eclampsia remains to be established. Our finding also suggest that lipoprotein ‘a’ may be an additional risk factor for pre-eclampsia and may be useful in distinguishing women with pre-eclampsia from normal pregnancy.

CONCLUSION

Hypertension is the most common medical complication of pregnancy. It has been estimated that worldwide, approximately 50,000 women die each year of eclampsia [6] . Changes in the plasma lipids during pregnancy have been recognized described and are thoughts to be done mostly to alterations in hormonal milieu. Plasma lipids and lipoproteins undergo both qualitative and quantitative changes during pregnancy. These changes revert towards normal shortly after Delivery [6] . Levels of Lp(a) appears to be lower in the normal pregnancy. The Lp(a) is a variant of LDL that is highly correlated with atherosclerosis. The hypothesis that Lp(a) levels are elevated in pre-eclampsia and associated with disease is supported by a recent observation of high Lp(a) levels in single family with two cases of severe pre-eclampsia. Lp(a) levels are elevated in pre-eclampsia and associated with severity of disease. It may serve as a marker of pathogenic process. Elevated Lp(a) may influence the fibrinolysis and have unfavorable rate on pregnancy outcome. In this background the present study was undertaken to assess the lipoprotein (a) levels in pregnancy induced hypertension. M. Mori [7] (2005) study maternal lipoprotein(a) levels in normal pregnancy and in pregnancy with evidence of vascular disease in the maternal uteroplacental circulation defined by Doppler ultrasound study. In their study they found None of the normal group had lipoprotein(a) levels greater than 30 mg/dl, a cutoff level which has been associated with increased risk of atherosclerosis. 28 of the 68 women with uteroplacental insufficiency had lipoprotein(a) levels greater than this cutoff level. In this group there was a statistically significant higher prevalence of pre-eclampsia in comparison with women with a normal lipoprotein(a) level (p < 0.001). The lipoprotein(a) level was significantly higher in severe (n = 13, median 60.5 mg/dl, P < 0.001] than in mild pre-eclampsia (n = 5, median 34 mg/dl). Those with high levels (>30 mg/dl) exhibited significantly more adverse indices of fetal outcome.
REFERENCES


Breast Cancer During Pregnancy: Three Cases Report

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SUMMARY

The incidence of pregnancy-associated breast cancer (PABC) although rare, is increasing as women are delaying childbirth. Breast cancer associated with pregnancy presents the clinician with particular challenges. The diagnosis may be delayed and difficult owing to the physiological changes within the breast and limitations on investigations. Authors report three cases of PABC diagnosed in 2011 in the Centro Hospitalar de Trás-os-Montes e Alto Douro, Portugal (in 2011 occurred 1680 births in the hospital). Two were diagnosed on the delivery’s day and one at her 14th week of pregnancy. Women were proposed to do chemotherapy (one started this treatment during pregnancy), mastectomy and radiotherapy. PABC is a rare and challenging problem. As modern women delay childbearing, this problem is expected to increase. Nowadays we know that breast cancer has an equivalent prognosis in pregnant and non-pregnant patients when matched by age and stage at diagnosis. Once PABC were significantly more likely to have more advanced stage, due to a delay in diagnosis, and presented more frequently as hormone receptor-negative tumors, they are associated with poor outcomes. In this way early detection is a significant predictor of improved outcomes. Management of breast cancer during pregnancy requires an interdisciplinary care team and careful consideration of the patient’s stage of disease, the gestational age of the fetus, and the preferences of the patient and her family.

Keywords: Breast Cancer, Pregnancy-associated breast cancer, Pregnancy, mastectomy, chemotherapy.
INTRODUCTION

Pregnancy-associated breast cancer (PABC) may be defined as breast cancer diagnosed during pregnancy or within 1 year of giving birth [1]. Currently, it is estimated that approximately one in every 3,000 births are from women who have or are being treated for breast cancer [2]. Although rare, the incidence of PABC is increasing as women are delaying childbirth [3].

Breast cancer associated with pregnancy presents the clinician with particular challenges. The diagnosis may be delayed and difficult owing to the physiological changes within the breast and limitations on investigations [4].

Over 90% of patients with breast cancer in pregnancy or during lactations present with a palpable mass, and most often (84%) these are self-reported by patients. Less frequently, breast cancer will present as breast erythema, breast swelling, bloody nipple, discharge, or local or distant metastasis [5]. The histology of tumors appears to be similar in women who are pregnant or recently delivered and in age-matched women who are not pregnant, except that estrogen receptor-negative tumors were more common in PABC women [6].

Diagnostic and treatment recommendations have been mainly based on evidence from retrospective single institutional and collective series and experts consensus, as randomized trials on this entity are understandably lacking [3]. Previous reports have suggested that PABC tends to be more advanced than non-PABC. Furthermore, some studies have suggested that the relatively poor outcome of these patients may be related of the appearance of their disease, rather than the pregnancy itself, and pregnant patients may not have a worse prognosis when age and stage are taken into account [7].

METHODS

Authors report three cases of PABC diagnosed in 2011 in the Centro Hospitalar de Trás-os-Montes e Alto Douro, Portugal (in 2011 occurred only 1680 births in the hospital).

RESULTS

Case 1: Woman, 30 years old, detected inflammatory signs of her left breast few hours after the cesarean (performed at 37th week of gestation). She also presented with a palpable axillary mass. Core biopsy showed hormonal receptors positive, Her-2 positive, grade 3, invasive ductal carcinoma of the breast. The patient did 6 cycles of chemotherapy and then she underwent left ma-
stectomy and axillary lymph node dissection. After radiotherapy, trastuzumab medication was initiated. To date, there is no evidence of local recurrence or distant metastases.

**Case 2:** Woman, 28 years old, with a history of right nephrectomy with chemotherapy for Wilm’s tumor when she was 9 years old. On the delivery’s day (vacuum extraction at 39th week) she presented with a palpable mass on her right breast (during pregnancy she noted the mass but not appreciated by associated this to changes of pregnancy). Core biopsy showed hormonal receptors positive, Her-2 positive, grade 3, invasive ductal carcinoma of the breast. She was recommended to start chemotherapy, but once she wanted to live to another country, she gave up to get treatment at our institution.

**Case 3:** Woman, 35 years old, was reported a palpable mass on her left breast at her 14th week of pregnancy. Core biopsy showed hormonal receptors positive, grade 2 invasive ductal carcinoma of the breast. She began chemotherapy at 17th weeks of gestation- 4 cycles of docetaxel. After delivery a healthy child with no apparent malformations (vacuum extraction at 36th weeks), she received chemotherapy with transtuzumab and had mastectomy with axillary lymph node dissection and then she underwent radiotherapy. To date, there is no evidence of local recurrence or distant metastases.

**CONCLUSIONS**

PABC is a rare and challenging problem. As modern women delay childbearing, this problem is expected to increase. However, in our cases women were 28, 30 and 35 years old. Young patients with PABC do not have worse prognosis compared with those with non-PABC; nevertheless pregnancy can contribute to delay in breast cancer diagnosis, evaluation and treatment [8].

The influence of pregnancy on the risk of developing breast cancer is dependent on maternal features, including age, family history, lactation post-partum, and overall parity. Advanced maternal age at first birth nearly abrogates the lifetime breast cancer protection as first pregnancy above age 35 results in loss or significant delay in the cross over effect. In terms of lifetime breast cancer risk, the age of 35 years acts as a critical point: prior to this age full-term pregnancy offers women some degree of protection, but after this age full-term pregnancy is associated with a permanent increase in breast cancer risk [9].

The physiological changes occurring in the breast during pregnancy may mean that clinical examination becomes more difficult as pregnancy progresses.
Due to physiologic pregnancy-changes in the breast, the interpretation of clinical findings, breast ultrasound and mammography is more demanding.

Contrary to popular belief, mammography with abdominal shielding can be performed during pregnancy with minimal risk. However, the increased density seen in premenopausal women, together with the physiological changes within the breast during pregnancy, may mean that mammograms are difficult to interpret. On the other hand ultrasound represents a simple, sensitive alternative to mammography in pregnant and lactating women and it has been shown to be more sensitive than mammography. In this way, although mammography is associated with a minimal risk for the fetus, rarely is necessary in the investigation of breast cancer in the pregnant woman. The samples acquired by fine needle aspiration are difficult to interpret, so core-needle biopsy may be a more appropriate initial procedure. With any interventional procedure performed on the breast of a pregnant or lactating women there is a risk of milk fistula formation, and there are higher rates of bleeding and infection. These risks can be minimized by stopping breast feeding prior to the biopsy, the use of prophylactic antibiotics and paying close attention to hemostasis.

As in non-pregnant patients, the majority of tumors are invasive ductal carcinomas (like our 3 cases), with between 80% and 100% of patients presenting with tumor of this subtype. The incidence of inflammatory tumors lies between 1.5 and 4% [4]. The prevalence of a positive estrogen and progesterone receptor status is usually lower in premenopausal compared to postmenopausal breast cancer patients, and in PABC patients this trend is even more pronounced (however in our three cases the hormonal receptor were positive) [10]. Using a variety of antibodies and scoring systems HER2 positivity has been recorded in 28-58% of PABC [4].

The treatment depends, among other things, on the gestational age at diagnosis. The general rule of cancer treatment- early diagnosis followed by appropriate management- is still the principle key to improve the treatment in PABC patients. Abortion is usually not recommended, unless opted by the woman, as it was not shown to improve outcome, except for PABC diagnosed in the first trimester when induced abortion may avoid delayed treatment. Most patients diagnosed with PABC undergo a definitive surgical treatment. Breast conservation surgical therapy (lumpectomy), with radiation treatment given after delivery or after neoadjuvant chemotherapy, is an option for women with PABC diagnosed late in pregnancy. The surgical definitive treatment is given either during pregnancy or following induced or spontaneous termination of pregnancy, depending on the time of the diagnosis. Chemotherapy as an adjuvant or neoadjuvant therapy is potentially teratogenic in the first few weeks of pregnancy and may lead to the death of the embryo [11]. However, it seems to be relatively safe after the first trimester [12]. Radiotherapy is contraindicated during pre-
Pregnancy due the potential risk for the fetus. It is still a preferred adjuvant strategy following pregnancy, as is hormonal therapy if appropriate. The proportion of congenital malformations in babies born to mothers diagnosed with PABC is apparently not higher than expected [11].

In conclusion, although the majority of palpable of breast masses are benign, breast examinations should routinely be performed in pregnant women, and identified masses should be promptly evaluated.

Nowadays we know that breast cancer has an equivalent prognosis in pregnant and non-pregnant patients when matched by age and stage at diagnosis. Once PABC were significantly more likely to have more advanced stage, due to a delay in diagnosis, and presented more frequently as hormone receptor-negative tumors, they are associated with poor outcomes. In this way, early detection is a significant predictor of improved outcomes. In this context, the patient family physician or obstetrician who performs the routine antenatal examinations can play an important role by performing a vital breast examination which may bring to light and prompt timely investigation of otherwise asymptomatic breast masses. Management of breast cancer during pregnancy requires an interdisciplinary care team and careful consideration of the patient’s stage of disease, the gestacional age of the fetus, and the preferences of the patient and her family.

REFERENCES


Prognosis Study: Prediction of Short-Term Outcome in Pregnant Women with Suspected Preeclampsia Study Using the Angiogenic Biomarkers sFlt-1/PlGF

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SUMMARY

Reliable prediction of preeclampsia and related clinical maternal and fetal adverse outcomes is still a high unmet medical need in pregnancy care. The PROGNOSIS study is addressing this unmet need and represents the first clinical study demonstrating short-term prediction of preeclampsia using fully automated Roche Elecsys® sFlt-1/PIGF maternal blood testing in pregnant women with clinical suspicion of preeclampsia. The study is the largest study conducted in this patient cohort to date. The PROGNOSIS study is designed to demonstrate ruling out of preeclampsia / eclampsia / HELLP syndrome for one week and ruling in of preeclampsia / eclampsia / HELLP syndrome within four weeks in women with clinical suspicion of preeclampsia.

Keywords: HELLP, eclampsia, signs, symptoms, rule out, rule in, diagnostic utility.
INTRODUCTION

Preeclampsia is a serious multi-system complication in pregnant women at > 20 weeks of gestation. It is a leading cause of fetal and maternal morbidity and mortality with an incidence of 3-5 % worldwide accounting for 42% of all maternal deaths per year [1].

The etiology of preeclampsia is not yet completely understood; immune factors, genetic factors and other factors may cause dysfunction of the placenta. This results in an imbalance of angiogenic factors such as sFlt-1 (soluble fms-like tyrosine kinase-1) and PlGF (placental growth factor). These factors lead to hypertension, proteinuria and other preeclampsia associated adverse outcomes [4].

Pregnant women with signs and/or symptoms of preeclampsia are often referred to high risk obstetrical departments for intensive monitoring. The current «gold standard» for the diagnosis of preeclampsia involves blood pressure measurement and determination of protein in urine. The prognostic performance of this current diagnostic standard in determining which women will develop preeclampsia / eclampsia / HELLP syndrome (hemolysis, elevated liver enzymes, and low platelet count) is quite poor. As a consequence, many women with signs and/or symptoms associated with preeclampsia are often unnecessarily hospitalized for observation, resulting in significant additional costs to pregnancy care.

The ratio between the anti-angiogenic factor sFlt-1 (soluble fms-like tyrosine kinase-1) and pro-angiogenic PlGF (placental growth factor) has been shown to be elevated in women with diagnosed preeclampsia and markedly elevated before the clinical onset of preeclampsia [2-5]. The PROGNOSIS study was designed to demonstrate the diagnostic utility of the sFlt-1/PlGF ratio in short-term prediction of preeclampsia.

MATERIALS AND METHODS

PROGNOSIS has two primary study objectives: 1) demonstrate that low ratios of sFlt-1/PlGF predict absence of preeclampsia / eclampsia / HELLP syndrome for one week (rule out); 2) demonstrate that high ratios of sFlt-1/PlGF predict diagnosis of preeclampsia / eclampsia / HELLP syndrome within four weeks (rule in).

PROGNOSIS is a multicenter, prospective, double-blind, non-interventional study with a target recruitment of approximately 1,000 eligible pregnant women. Inclusion criteria: Pregnant women ≥ 18 years, gestational week 24+0 days to 36+6 days, signed written informed consent, suspicion of clinical dia-
gnosis of preeclampsia based on or more of the following criteria new onset of elevated blood pressure, aggravation of pre-existing hypertension, new onset of protein in urine, aggravation of preexisting proteinuria, preeclampsia-related symptoms and/or findings, e.g. epigastric pain, excessive edema, severe swelling, headache, visual disturbances, sudden weight gain, low platelets, elevated liver enzymes, suspected intrauterine growth restriction (IUGR) or abnormal uterine perfusion detected by Doppler sonography. Exclusion criteria: manifest preeclampsia / eclampsia / HELLP syndrome. The study includes five consecutive visits with clinical evaluation and blood serum sampling for sFlt-1/PlGF determination at an independent measuring site. In addition, maternal and neonatal outcome at delivery and postpartum are documented.

The study is designed to i) derive and ii) validate a cut off-based prediction model for each short-term prediction primary study objective; this is done in a two-step approach consisting of a i) feasibility and ii) validation part. The feasibility part requires 500 eligible patients followed by an interim analysis to derive a sFlt-1/PlGF cutoff-based prediction model. This model will then be validated by data collected from 500 additional eligible patients.

RESULTS

The study started in December 2010 at nine sites in Europe. In March 2012, the study was expanded to thirty-one sites globally in Australia, Canada, New Zealand and South America. The interim analysis was completed in March 2013. The results so far demonstrated a prevalence of preeclampsia of approximately 20% in the target population. Study enrollment was completed at the end of August 2013.

CONCLUSION

The PROGNOSIS study is the first clinical study demonstrating short-term prediction of preeclampsia using fully automated Elecsys® sFlt-1/PlGF maternal blood testing in pregnant women with suspicion of preeclampsia.

ACKNOWLEDGMENTS

The authors thank the PROGNOSIS clinical study team for their contribution to the realization of the global PROGNOSIS study.
REFERENCES

Uterine Fundic Rupture in Nullipara – Clinical Case

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SUMMARY

Uterine rupture may develop as a result of pre-existing injury or anomaly, or it may result from labour in a previously unscarred uterus. More common in women of high parity and use of uterotonic agents. Rupture of an intact uterus in labour often involves the lower segment. The case presented is of a pregnant, 30 years old, through in vitro fertilization, who followed her pregnancy up to 20 weeks in China. She has obstetric history of 2 ectopic pregnancies, one resolved with medical treatment and another with resolution by laparoscopy salpingectomy. At 30 weeks of pregnancy was admitted at our hospital in hypovolemic shock. An emergent caesarean section was performed. At surgery was found a hemoperitoneum, with complete fundic uterin rupture, and posterior fundic placenta. Extraction of a live newborn. She develops uterine atony not responsive to medical therapy and disseminated intravascular coagulation. Life saving hysterectomy was performed. This clinical case, nulliparous with unscarred uterus, presented with ruptured of uterus fundus, rarely described in clinical literature, suggests that a past history of a gynecologic laparoscopic surgery may be considered as a risk factor for a uterine rupture during a pregnancy in an apparently unscarred uterus. Severe abdominal pain with hypovolemic shock, in a pregnant women, should raise the suspicion of uterine rupture.

Keywords: Unscarred ruptured uterus, Fundal rupture.

INTRODUCTION

Uterine rupture may develop as a result of pre-existing injury or anomaly, or
it may result from labour in a previously unscarred uterus. The incidence of spontaneous uterine rupture is calculated to be approximately 1 in 15,000 deliveries [1]. More common in women of high parity and use of uterotonic agents. The rupture of an intact uterus in labour often involves the lower segment [2]. Uterine ruptures could be divided into complete, all layers of the uterine wall are separated, and incomplete (dehiscence) ruptures, when the myometrium is disrupted but the serosa is intact. As expected the prognosis is worse when rupture is complete, with lower maternal and neonatal morbidity [3].

**MATERIAL AND METHODS**

Review of the literature about the topic and consultation process of the clinical user, a 30-year-old Chinese, pregnant woman that was admitted at our hospital in emergency service in hypovolemic shock.

**CLINIC CASE**

The case presented is a pregnant through in vitro fertilization, a chinese national, who followed her pregnancy up to 20 weeks in China and then in portuguese healthcare. She has obstetric history of 2 ectopic pregnancies, one in 2010 resolved with medical treatment (methotrexate) and another in 2011 with resolution by laparoscopy with right salpingectomy, both in China.

At 30 weeks of pregnancy, at home, she developed contractions and after about 10 min had an episode of syncope. She was admitted at our hospital in hypovolemic shock. An emergent caesarean section was performed under general anaesthesia. At surgery was found a hemoperitoneum, with complete fundic uterine rupture (figure 1), and posterior fundic placenta. Extraction of a live new-born, male, weighting 1552 g, 2000 ml of free blood with amniotic fluid was collected in a sucker bottle.

She develops uterine atony not responsive to medical therapy (rectal misoprostol and sulprostone) and disseminated intravascular coagulation. Life saving hysterectomy was performed. After stabilization in ICU (intensive care unit) ended recovery in inpatient obstetrics, discharged clinically well twelve days post labour. Macrospcopic anatomo-pathologic examination of the placenta reveals that the placenta was simple, complete with inferior weigh to what was expect for this gestational age and with advanced maturity to this gestational age, and the alterations had not pathologic signify. Her one-month follow up was uneventful.
DISCUSSION

When the rupture of a gravid uterus occurs at the fundus of an apparently unscarred uterus, prior to the onset of labour, the diagnosis is often delayed [4]. Risk factors for uterine rupture include previous cesarean sections, which is the most common, multiparty, multiple pregnancies, macrosomia, the injudicious use of oxytocics, malpresentation, an internal podalic version and instrumental deliveries myomectomy, septoplasty, metroplasty, trauma, congenital uterine anomalies, inadequate treatment of endometriosis and placental abruption. In this clinical case, none of such factors were present. Sometimes postpartum ruptures occur in patients giving birth by vaginal delivery following prior cesarean sections [1, 2, 3].

It is reasonable to think that our patient had a uterine scar from an undetected injury during the laparoscopy salpingectomy. That scar of the uterus was probably stretched with the fetus, which may have led to the bursting of the uterus at the weakest point.

Fig. 1. Complete fundic uterine rupture.
Before developing hypovolemic shock, symptomatology and physical findings in women with uterine rupture may look atypical. Symptoms of eminent uterine rupture include vaginal bleeding, sharp pain between contractions, abdominal tenderness or pain, recession of the fetal head (baby’s head moving back up into the vaginal canal), bulging under the pubic bone (baby’s head protrudes out of the uterine scar), and onset of sharp pain at the site of previous scar [3, 5, 7]. The most common sign of uterine rupture is a non reassuring fetal heart rate pattern, like variable heart rate decelerations that may progress into late decelerations, bradycardia, and death [1, 4]. Termination of pregnancy should be considered if any sudden pain is present. When the diagnosis of uterine rupture is established the way out is immediate delivery, most often by emergency laparotomy [1, 2, 7]. Successful repair of dehiscence with continuation of pregnancy have been reported [3].

Similar cases were reported by Kelly Albrecht and Albuquerque E. in their cases also there was no history of uterine surgery and like in our case rupture occurred during pregnancy or before the onset of labor [6, 7].

CONCLUSION

Uterine rupture is an uncommon but catastrophic complication of pregnancy, which is associated with significant maternal and fetal morbidity and mortality. Our clinical case, nulliparous with unscarred uterus, presented with ruptured of uterus fundus, rarely described in clinical literature, suggests that a past history of a gynecologic laparoscopic surgery may be considered as a risk factor for a uterine rupture during a pregnancy in an apparently unscarred uterus. Therefore, an acute onset of severe abdominal pain with hypovolemic shock in a pregnant women, all these together should raise the suspicion of a concealed uterine rupture.

REFERENCES

5. Arulkumaran S, Chua S, Ratnam SS, Symptoms and signs with scar rupture-value of uterine


Do We Have a Noninvasive Method to Evaluate Hemodynamics Profile in Gestational Hypertension? A Case Report

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ABSTRACT

Introduction. Noninvasive methods for monitoring hemodynamics are required to manage patients with gestational hypertension. Impedance cardiography is a noninvasive method that can provide information about hemodynamic profile being used in cardiology, intensive care, neonatology and lately in obstetrics.

Material and methods. We report the case of a 26 years of age pregnant woman who was investigated in our clinic and developed gestational hypertension. Parameters recorded in 8 seconds intervals included blood pressure, systemic vascular resistance, base impedance and stroke volume.

Results. Using ICG we performed measurements at three time points and in two maternal positions. Differences in stroke volume (SV) due to maternal position were detected by ICG in all trimesters. The blood pressure increased from the first to third period of pregnancy. There was a correlation between arterial blood pressure and systemic vascular resistance (SVR). Base impedance was an important parameter which correlated with diastolic blood pressure. Significant trends were observed for cardiac output and thoracic fluid content with advancing pregnancy stages. We observed that SVR, base impedance were correlated with cardiac output, body mass index and blood pressure.
Conclusion. This ICG device with the capability continuously providing the standard hemodynamics parameters gave us valuable information for a case with gestational hypertension but we need an extensive study to verify our findings.

INTRODUCTION

Complicating 2-3% of pregnancies, hypertension is the most common medical disorder which may appear during pregnancy. According to The National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy it is classified into the following categories: Chronic hypertension, preeclampsia-eclampsia, preeclampsia superimposed on chronic hypertension and Gestational hypertension (transient hypertension of pregnancy or chronic hypertension identified in the latter half of pregnancy) [1]. This terminology is preferred over the older but widely used term «pregnancy-induced hypertension» (PIH) being more precise [2]. In 2008, the Society of Obstetricians and Gynecologists of Canada (SOGC) simplified the classification of hypertension in pregnancy into 2 categories, preexisting or gestational, with the option to add «with preeclampsia» [3]. Pregnant patients with hypertension need noninvasive and less complicated methods for monitoring hemodynamics. Among the several methods proposed, impedance cardiography (ICG) has gained particular attention [4].

MATERIAL AND METHODS

We report the case of a 26 years of age pregnant woman who was investigated in our clinic. That was the first pregnancy of the patient, she had no other medical disorder such as obesity, diabetes, kidney disease, rheumatoid arthritis, lupus or scleroderma, mother or sister with preeclampsia or high blood pressure before becoming pregnant. She developed gestational hypertension during the 35 week of gestational age. She had the prenatal care including the standard investigations recommended in our clinic. Furthermore we performed recordings of hemodynamics parameters using ICG-M501. The registrations were made in the first, second and third trimester of pregnancy. Parameters recorded in 8 seconds intervals included blood pressure, heart rate, cardiac index, cardiac output, index of myocardial contractility, mean arterial pressure, stroke volume, stroke index, systemic vascular resistance, base impedance and we calculated body mass index.
RESULTS

The 26 years of age Romanian woman had no significant medical history, no termination of pregnancy or stillbirth in the past and she didn’t smoke or used medication in recent time. She developed gestational hypertension from the 35 week of pregnancy, without proteinuria.

Using ICG we performed measurements at three time points (12, 21 and 36 weeks of gestation) and in two maternal positions (right and left lateral decubit). We obtained values for each second during a period of eight seconds. In Table 1 we present the mean value for each measurement. Differences in stroke volume (SV) due to maternal position were detected by ICG in all trimesters, but with no statistical significance. In this case we observed that CO, CI, SV, SI decreased from the first to third trimester. The blood pressure increased from the first to third period of pregnancy. There was a correlation between arterial blood pressure and systemic vascular resistance (SVR). Significant trends were observed for cardiac output and thoracic fluid content with advancing pregnancy stages. Base impedance has significantly increased due to amniotic fluid or in correlation with blood pressure but we need further study with more patients to conclude. We observed that SVR, base impedance were correlated with cardiac output, body mass index and blood pressure.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>1&lt;sup&gt;st&lt;/sup&gt; trimester</th>
<th>2&lt;sup&gt;nd&lt;/sup&gt; trimester</th>
<th>3&lt;sup&gt;rd&lt;/sup&gt; trimester</th>
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<td>58</td>
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<tr>
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<td>33,3</td>
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<td>1434</td>
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</tr>
<tr>
<td>DABP</td>
<td>70</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>MABP</td>
<td>80</td>
<td>82</td>
<td>90</td>
</tr>
</tbody>
</table>

CI-cardiac index, CO-cardiac output, HR-heart rate, IC-an index of myocardial contractility, MABP-mean arterial blood pressure, SI-stroke index, SV-stroke volume, SVR-systemic vascular resistance, Z-base impedance.
DISCUSSION

Hemodynamics during pregnancy is characterized by dramatic physiologic changes: mean arterial pressure drops between 16th and 20th weeks of gestation, with a greater decline in diastolic pressure than that in systolic pressure, but after 20th week mean arterial blood pressure slowly returns to prepregnancy levels. In the same time cardiac output increases, and it’s apogee is typically at least 40% greater than the baseline. Stroke volume and heart rate increase to achieve this rise in the quantity of blood into the pulmonary and systemic circulations. Using Swan-Ganz catheters hemodynamic assessments of untreated primiparous hypertensive patients were detailed by Visser and Wallenburg who found that cardiac outputs and intravascular volumes get lower and systemic vascular resistance and cardiac afterload higher in these patients. In the same time it was proved that using impedance techniques compliance of the large conduit arteries is reduced [5].

Even if it may seen that treatment options have not significantly changed in recent years, insight on the pathogenesis of gestational hypertension has been remarkable. Nicolaides described in a study that women with preeclampsia had slower heart rate and long heart cycle and it is the result to a longer diastolic duration than the ejection duration time [6].

Mustafa and colleges recently published a review about gestational hypertension including physiology, hemodynamics, endothelial dysfunction, angiogenic factors emphasizing on recent advances in this field and diagnosis tools [7]. Our interest in this article was to evaluate only hemodynamic changes in a patient who developed gestational hypertension in the 35 gestational week of pregnancy without including other diagnosis or evaluation devices. That type of noninvasive method proved as similar literature reports that in this medical condition the CO is lower and SVR is elevated as compared to normotensive pregnancy [8]. As other studies have already identified [9] the onset of preeclampsia and gestational hypertension is more frequent in late pregnancy, the same happened to our patient.

Burlingame presented that ICG and echo demonstrate significant correlations in some but not all measurements of cardiac function, ICG detect small changes in SV associated with maternal position change. ICG measurements reflected maximal cardiac contractility in the antepartum period [10].

Because of the noninvasive nature of impedance cardiography, this method is gaining popularity in the obstetric field especially in measurements for contractility parameters ‘acceleration-’, ‘velocity-’ and ‘heather-index’ in the 3rd trimester pregnancies and pre-eclamptic pregnancies [11].

Parrish MR showed in a recent study which involved late gestation hypertensive pregnant patients who underwent ICG evaluation that ICG hemodynamic
profiling of late gestation hypertensive patients can rapidly and reliably identify those with severe or superimposed preeclampsia [12].

CONCLUSION

This ICG device with the capability continuously providing the standard hemodynamics parameters gave us valuable information for a case with gestational hypertension but we need an extensive study to verify our findings.

REFERENCES

Effectiveness of ‘George’s Intrapartum Monitoring Strategy’ on Operative Delivery and Perinatal Outcomes at a Teaching Hospital in London: a 5 Year Experience

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SUMMARY

‘George’s Intrapartum Monitoring Strategy’ (use of fetal ECG (STAN) with intensive training in process based CTG Interpretation and mandatory competency testing for all staff) has significantly reduced our intrapartum caesarean sections over the last five years. We have the lowest emergency caesarean section rate in London (6.1-8.9%) and one of the lowest HIE rates in the United Kingdom. In addition, we have one of the lowest failed instrumental vaginal births in the United Kingdom. There was a continued reduction in the rate of neonatal metabolic acidosis, HIE and NND.

Keywords: GIMS, Fetal ECG (ST-Analyser or STAN), Competency testing, hypoxic ischaemic encephalopathy (HIE), emergency caesarean section.

INTRODUCTION

Cardiotocograph (CTG) Interpretation is riddled with inter and intra-obser-
ver variation due to dependence of pattern recognition. Even experts in CTG Interpretation do not agree on basic features observed on the CTG Trace and if pattern recognition is solely used for CTG Interpretation, false positive rate of CTG may be up to 60%. In addition, the positive predictive value of an abnormal or pathological CTG for intrapartum hypoxia has been reported to be below 30%. Recent Cochrane Review has suggested that use of CTG in high risk labour has not reduced cerebral palsy rate or perinatal deaths but has contributed to a significant increase in caesarean sections and operative vaginal delivery rates. The only benefit appears to be a 50% reduction in neonatal convulsions, the long term benefit of which is still uncertain.

St George’s Healthcare NHS Trust is a world leader in intrapartum fetal monitoring which has pioneered a process-based CTG Interpretation based entirely on deeper understanding of fetal physiology. Intrapartum Fetal monitoring Group at St George’s Hospital conducts over 20 CTG and fetal ECG Masterclasses (ST-Analyser or STAN) in the United Kingdom, Europe, Middle East, Singapore, Asia and Australia. In addition, it is the first Maternity Unit in the United Kingdom to introduce a mandatory process based CTG testing and a policy on competency testing on intrapartum fetal heart rate monitoring for all obstetricians and midwives as well as minimum requirements for mandatory attendance at weekly CTG meetings as part of continuous professional development. Moreover, all trainee obstetricians have to undergo a mandatory Hands-on Training in Instrumental Vaginal births as part of their induction into the department.

Process Based CTG Interpretation involves understanding the fetal physiological response behind the patterns observed on the CTG Trace and to prospectively predict the changes on the CTG Trace based on fetal physiology. Understanding the types of hypoxia during labour and the resultant patterns on the CTG Trace aids clinicians providing intrapartum care to determine the potential rate of fall in the pH based on the patterns observed on the CTG Trace to take corrective action. Therefore, it enables midwives and obstetricians to treat the fetus in-utero rather than to treat the CTG Trace by using terminologies such as ‘pathological trace’. Moreover, the decelerations are determined based on whether they are caused by baro-receptor stimulation or chemo-receptor stimulation and the fetal response to ongoing hypoxic or mechanical stress.

Use of fetal ECG using ST-analyser (STAN) helps clinicians to understand central organ oxygenation (i.e. myocardium) and to determine compensatory changes within the myocardium (glycogenolysis) secondary to ongoing hypoxia. This has immensely helped our maternity unit to avoid unnecessary operative
interventions for suspected fetal compromise based on pattern recognition. We have stopped performing all peripheral tests of fetal wellbeing, including fetal scalp blood sampling (FBS) based on better understanding of physiology behind fetal response to hypoxic stress. A fetus diverts all the oxygenated blood from peripheral tissue such as the fetal scalp, which is a non-essential organ in response to ongoing hypoxia. In addition, deeper understanding of the blood supply to fetal scalp demonstrates that blood supply is very minimal at the usual site of scalp puncture for FBS or lactate. Therefore, peripheral tests such as fetal scalp blood sampling have very little anatomical and physiological justification and have been shown by recent Cochrane Systematic Review that they don’t reduce operative interventions or improve neonatal outcomes.

MATERIAL AND METHODS

To determine the impact of our ‘George’s Intrapartum Monitoring Strategy’ (use of fetal ECG (ST-Analyser), an intensive ‘process-based’ training in CTG interpretation and a mandatory competency-testing) on intrapartum interventions and perinatal outcomes over a five year period (2008-2012). We analysed our emergency caesarean sections, failed instrumental vaginal births, neonatal metabolic acidosis, hypoxic ischaemic encephalopathy (HIE) and early neonatal death (NND) rates were compared between 2008-2009 and 2010 to 2012, when GIMS was implemented.

RESULTS

Despite increasing in number of births over the five year period (2008-2012), neonatal metabolic acidosis reduced from 1.37% in 2008 to 0.76% in 2012. Rate of HIE also decreased from 1.2/1000 in 2008 to 1.1/1000 in 2012, NND from 1.7/1000 in 2008 to 1.3/1000 in 2012. There has been a significant decrease in our emergency caesarean section rate from 15% in 2008 to 9% in 2012.

CONCLUSIONS

George’s Intrapartum Monitoring Strategy’ (use of fetal ECG (STAN) with intensive training in process based CTG Interpretation and mandatory competency testing for all staff) has significantly reduced our intrapartum caesarean sections over the last five years. We have the lowest emergency caesarean section rate in London (6.1-8.9%) and one of the lowest HIE rates in the United
Kingdom. In addition, we have one of the lowest failed instrumental vaginal births in the United Kingdom. There was a continued reduction in the rate of neonatal metabolic acidosis, HIE and NND.

Our results demonstrate that use of fetal ECG (STAN) for intrapartum fetal monitoring with intense training on physiology based CTG interpretation and mandatory competency testing has significantly reduced our emergency intrapartum interventions, including emergency caesarean section rates. Contrary to misconception regarding the usefulness of fetal blood sampling in reducing operative deliveries, we have demonstrated that despite stopping FBS based on lack of anatomical and physiological justification as well as lack of robust scientific evidence (only 3 were performed out of 5300 births in 2012), we were able to continuously reduce our emergency caesarean section rate as well as instrumental vaginal delivery rates. This indicates that when a test of oxygenation of a central organ (fetal ECG or STAN) is being carried out, a peripheral test such as FBS or scalp lactate is not essential, even if the CTG appears ‘pathological’, provided there is fetal compensatory response (a stable baseline fetal heart rate and reassuring baseline fetal heart rate variability).

Our study illustrates that the use of GIMS (George’s Intrapartum Monitoring Strategy) not only reduces unnecessary operative interventions during labour, it also has reduced our neonatal metabolic acidosis rate from 1.37% to 0.76% over the last 5 years, despite of St George’s Maternity Unit being a tertiary referral centre with a regional fetal medicine service. Our hypoxic ischaemic encephalopathy rate (HIE) is also one of the lowest in the United Kingdom (1.1/1000). Some studies have not demonstrated a significant benefit in reducing operative delivery rates by using fetal ECG (STAN). However, these studies have appeared to rely on pattern recognition for CTG Interpretation and have continued to use fetal scalp blood sampling (FBS) if the CTG was considered to be ‘pathological’ based on pattern recognition. Lack of intense training of all staff prior to introducing the technology also has detrimental effects as we reported in 2007. We reported that failure to adhere to guidelines and human factors contributed to almost all our poor perinatal outcomes in 2007. These findings helped our unit to focus on intense staff training and use of physiology in interpreting CTG traces, even whilst fetal ECG is used for intrapartum fetal heart rate monitoring. Such physiological, ‘process-based’ interpretation of the CTG to determine fetal compensatory response to ongoing hypoxic stress based on features observed on the CTG trace coupled with avoidance of FBS for ‘pathological CTG’ and intense training of midwives and obstetricians on CTG Interpretation with mandatory competency testing, as per GIMS has contributed to continued reduction in operative interventions and HIE rates in our unit.
Therefore, we strongly advocate the use of fetal ECG (STAN) for intrapartum fetal heart rate monitoring, along with intense training in physiology-based CTG Interpretation and Mandatory Competency Testing for all midwives and obstetricians working in labour ward to improve perinatal outcomes as well as to reduce unnecessary operative interventions. Clinicians also have a responsibility to revisit the anatomical and physiological basis for peripheral tests of fetal wellbeing such as fetal blood sampling or fetal scalp lactate, especially in view of the recent Cochrane Systematic Review that has suggested that FBS does not reduce caesarean section rates or improve any neonatal outcome.

In view of rising caesarean section rates and financial costs as well as resultant complications of caesarean sections including morbidly adherent placentae, there is an urgent need to re-visit intrapartum fetal monitoring strategy to reduce unnecessary operative interventions, whilst ensuring a reduction in hypoxic ischaemic encephalopathy. Use of ‘pattern recognition’ and peripheral tests of fetal wellbeing have not been found to be useful in reducing operative interventions or improving neonatal outcomes. Whereas, our 5 year data clearly demonstrates that the use of George’s Intrapartum Monitoring strategy (GIMS) that involves the use of fetal ECG (ST-Analyzer), an intensive ‘process-based’ training in CTG interpretation and a mandatory competency-testing for all obstetricians and midwives working in the labour ward, has resulted in a significant reduction in emergency caesarean sections and hypoxic ischaemic encephalopathy (HIE) rates.

REFERENCES

The Clinical Usefulness of the Continuous Electronic Fetal Monitoring with Computerized Analysis of Fetal Heart Rate Tracings in Intrapartum Assessment of Fetal Condition

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SUMMARY

We have assessed the usefulness of the continuous electronic fetal monitoring with computerized analysis of fetal heart rate tracings during a labor in predicting clinical state of the neonate based on the postpartum umbilical gas analysis. The analysis has been performed for the 90 patients who have been monitored during first period of a labor in Labor Room with continuous electronic fetal monitoring with computerized analysis of FHR tracings. Additionally we have performed Doppler ultrasound examination in selected vessels of fetoplacental area. Just after the labor we have collected gas analysis from umbilical cord. We have found that there is no statistically significant correlation between postpartum pH, pCO₂, HCO₃ parameter of gas analysis and parameters of computerized analysis of CTG tracings. We have performed ROC analysis which has proven the low value in differentiation of fetal condition for all parameters of computerized analysis of CTG tracings. Only in the group of patients with abnormal outcomes of computerized analysis of FHR tracings confirmed by abnormal intrapartum Doppler ultrasound examination we have found lower pH values of postpartum gas analysis from umbilical cord. Parameters of the computerized analysis of CTG tracings are not statistically significant in clinical usefulness in intrapartum differentiation of fetal condition. Doppler flow para-
meters can specify the fetal condition and reduce the operative methods of a labor.

**Keywords**: cardiotocography, labor, Doppler ultrasound examination, gas analysis.

**INTRODUCTION**

The rate of cesarean delivery has recently significantly increased [1, 2]. One of the most common indication to performing cesarean section is intrauterine fetal asphyxia [2]. Cardiotocography is the basic method of noninvasive prenatal diagnosis. The use of computerized analysis of fetal heart rate tracings has reduced the meaning of subjective factors in CTG evaluation and enabled the assessment of variability [3]. The aim of the study is the assessment of the usefulness of the continuous electronic fetal monitoring with computerized analysis of fetal heart rate tracings during intrapartum monitoring in predicting clinical state of the neonate based on the postpartum umbilical gas analysis.

**MATERIAL AND METHODS**

We have made the analysis for the 90 patients hospitalized in the 2nd Department of Gynecology and Obstetrics Wroclaw Medical University. The patients have been monitored during first period of a labor in Labor Room with continuous electronic fetal monitoring with computerized analysis of FHR tracings, called MONAKO system. We have used telemetry heads which enabled the women full comfort of movements. In analysis we have used baseline fetal heart rate, the number and type of accelerations, the number and type of decelerations, the average value of oscillation, percentage of each type of oscillation, LTV, STV and moment analysis (Fig. 1). Additionally we have performed Doppler ultrasound examination in fetal umbilical artery (UA), middle cerebral artery (MCA) and ductus venosus (DV) using portable ultrasound system Voluson I. Just after the labor we have collected gas analysis from umbilical cord. In statistical analysis we have used Doppler ultrasound parameters: S/D; PI; RI UA and MCA, CPR (PI MCA/PI UA) and parameters of postpartum umbilical cord gas analysis: pH; pCO₂; HCO₃⁻; BE. All the parameters have been statistically analized with Statistica 10.0 (StatSoft Poland, Cracow) with Medical Set 2.0.
Fig. 1. Computerized analysis of FHR tracings.
RESULTS

We have found that there is no statistically significant correlation between postpartum pH, pCO₂, HCO₃ parameter of gas analysis and parameters of computerized analysis of CTG tracings. We have performed ROC analysis which has proven the low value in differentiation of fetal condition descending respectively for: the number of decelerations, baseline fetal heart rate, moment analysis, number of accelerations, LTV, STV and the medium value of the oscillation. All the parameters of computerized analysis of CTG tracings have exhibited very low sensitivity in the range of 0-7% and high specificity 98% (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Area under the ROC curve</th>
<th>Standard error</th>
<th>-95% - left end of confidence interval</th>
<th>+95% - right end of confidence interval</th>
<th>CUT point for the ROC curve</th>
<th>Sensitivity with 98% specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>The number of decelerations</td>
<td>0,571</td>
<td>0,063</td>
<td>0,447</td>
<td>0,695</td>
<td>45</td>
<td>7%</td>
</tr>
<tr>
<td>bFHR</td>
<td>0,559</td>
<td>0,078</td>
<td>0,405</td>
<td>0,712</td>
<td>117,1</td>
<td>0%</td>
</tr>
<tr>
<td>Moment analysis</td>
<td>0,464</td>
<td>0,09</td>
<td>0,289</td>
<td>0,64</td>
<td>1,9</td>
<td>7%</td>
</tr>
<tr>
<td>The number of accelerations</td>
<td>0,454</td>
<td>0,097</td>
<td>0,264</td>
<td>0,644</td>
<td>0</td>
<td>7%</td>
</tr>
<tr>
<td>LTV</td>
<td>0,43</td>
<td>0,092</td>
<td>0,25</td>
<td>0,611</td>
<td>24,2</td>
<td>7%</td>
</tr>
<tr>
<td>STV</td>
<td>0,428</td>
<td>0,092</td>
<td>0,244</td>
<td>0,612</td>
<td>3,7</td>
<td>7%</td>
</tr>
<tr>
<td>The average value of oscillation</td>
<td>0,422</td>
<td>0,081</td>
<td>0,262</td>
<td>0,581</td>
<td>8,4</td>
<td>7%</td>
</tr>
</tbody>
</table>

For Doppler flow parameters the sensitivity has reached higher values 64% for S/D UA, 57% for RI UA (Fig. 2), 50% for PI UA and 43% for CPR with slightly lower specificity 93% (Table 2). Only in the group of patients with abnormal outcomes of computerized analysis of FHR tracings confirmed by abnormal intrapartum Doppler ultrasound examination we have found lower pH values of postpartum gas analysis from umbilical cord.

CONCLUSIONS

Parameters of the computerized analysis of CTG tracings are not statistically significant in clinical usefulness in intrapartum differentiation of fetal condition. The continuous electronic fetal heart rate monitoring with the computeri-
Tab. 2. ROC analysis for UA, MCA and CPR parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Area under the ROC curve</th>
<th>Standard error</th>
<th>95% - left end of confidence interval</th>
<th>95% - right end of confidence interval</th>
<th>CUT point for the ROC curve</th>
<th>Sensitivity with 98% specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>UARI</td>
<td>0.9</td>
<td>0.038</td>
<td>0.825</td>
<td>0.975</td>
<td>0.68</td>
<td>57%</td>
</tr>
<tr>
<td>UAS/D</td>
<td>0.896</td>
<td>0.039</td>
<td>0.82</td>
<td>0.972</td>
<td>2.96</td>
<td>64%</td>
</tr>
<tr>
<td>UAPI</td>
<td>0.887</td>
<td>0.039</td>
<td>0.811</td>
<td>0.964</td>
<td>1.22</td>
<td>50%</td>
</tr>
<tr>
<td>CPR</td>
<td>0.852</td>
<td>0.043</td>
<td>0.768</td>
<td>0.935</td>
<td>1.082</td>
<td>43%</td>
</tr>
<tr>
<td>MCARI</td>
<td>0.622</td>
<td>0.08</td>
<td>0.465</td>
<td>0.779</td>
<td>0.56</td>
<td>7%</td>
</tr>
<tr>
<td>MCAS/D</td>
<td>0.609</td>
<td>0.084</td>
<td>0.445</td>
<td>0.774</td>
<td>2.29</td>
<td>7%</td>
</tr>
<tr>
<td>MCAPI</td>
<td>0.59</td>
<td>0.076</td>
<td>0.441</td>
<td>0.74</td>
<td>0.85</td>
<td>7%</td>
</tr>
</tbody>
</table>
Computerized analysis of CTG tracings is still characterized by too high value of false positive outcomes. This leads to the increase of unnecessary cesarean sections rate [4]. One of the main targets of intrapartum care should be proper supervision of fetus and fast detection of intrauterine fetus hypoxia. So we need other methods which could verify the abnormal parameters of the computerized analysis of CTG tracings. Doppler ultrasonography is a method widely used in monitoring fetal condition during pregnancy, especially in high risk pregnancies [5]. There are works proving the usefulness of Doppler examination in the assessment of intrapartum fetus well-being [6, 7, 8, 9]. Doppler flow parameters can specify the fetal condition and reduce the operative methods of a labor.

REFERENCES

Impact of Personal Characteristics of Perinatal Care Specialists on Perinatal Indicators’ Relevance Selection

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SUMMARY

Perinatal indicators – a tool to trigger perinatal health care’s improvement – are compared between countries and regions. However, some differences can be explained by possible confounders, such as population size, risk factors and decision makers/health professionals. In this way, and knowing that physicians and researchers have a key role on perinatal health care, we assessed, by a mean of a web-based survey, if their personal characteristics influenced the relevance they give to 47 perinatal indicators. Our results show that characteristics such as age or years of experience can influence the relevance given to an entire group of indicators and that some particular indicators are seen differently according to several personal characteristics. Thus, we can conclude that these characteristics of health professionals and other decision makers might bias collection, evaluation and policy-making on perinatal health care, as well as hamper its improvement.

Keywords: Health care, Quality indicators, Perinatology, Europe.
INTRODUCTION

Promotion of perinatal health improvement is a top priority to European health care providers [1]. To achieve that, decision makers (mostly non-specialists but also health professionals) and those who can also change healthcare services, as the media and general public, must get access and understand health data, motivating the first ones to develop and implement perinatal health programmes [2, 3] and pinpointing areas requiring action [4]. That data is based on measurements of health care systems – indicators. A good indicator should be representative, unbiased, valid, precise, sensitive, specific, reliable and, as a marker of progress, based on general usefulness and importance, technical qualities and practical concerns [2, 5]. This representativeness only can be achieved with little or without intra and inter-observer variability of the measurement [6]. To set the above mentioned policy priorities, international comparisons are very important and should be encouraged [7]. However, Europe is very heterogeneous in countries’ population size or risk factors and in health professionals or decision makers. This can bias differences or variations of perinatal indicators [2,7]. Being health professionals and researchers crucial to collect and evaluate perinatal indicators, the main goal of this study was to understand if the personal characteristics of specialists influence the importance they give to perinatal indicators.

MATERIAL AND METHODS

This cross-sectional study designed to assess the opinion of specialists on perinatal medicine and regarding perinatal indicators, comprised all indicators from the 2010 and 2003 European Perinatal Health Reports [3,8] and suggestions from our previous work [9]. By mean of a web-based survey, 89 respondents – European expert physicians and/or researchers who have published in perinatal medicine between January 2009 and August 2011 – evaluated indicators as having no, low, medium or high relevance (scored as 0 to 3) or selected «do not know» (valued as 0) if they had not enough information. Indicators were distributed in the same four groups used by EURO-PERISTAT: «Population characteristics/risk factors», «Health care services», «Maternal health» and «Fetal, neonatal and child health». The importance given to each group of indicators was assessed by the mean of the relevance of indicators attributed by the respondents - mean score. They were also asked to answer several personal questions such as age, gender, country of work, profession, specialty, years of experience and if they were head of department. Data was extracted from Medquest (a web-based survey tool) and descriptive statistics, t-tests, correla-
tions, ANOVAs, Chi-square and Fisher’s exact tests were performed using IBM SPSS Statistics 20™.

RESULTS

The population (89 participants) had a mean age of 49.6 years (SD: 10.9), 66% were male and represented 17 European countries (37% from Northern Europe; 30% from Southern Europe; and 33% from Western Europe). 84% were physicians and, from these 33% were head of department, being 45% specialists of child care and 55% of mother care. They had an average of 22.2 years of experience (SD: 10.7). The mean scores of each group of indicators were: «Population characteristics/risk factors» – 2.5 (SD: 0.3); «Health care services» – 2.1 (SD: 0.5); «Maternal health» – 2.3 (SD: 0.6); and «Fetal, neonatal and child health» – 2.4 (SD: 0.5). The relevance given to the second group of indicators – «Health care services» – had a significant positive correlation of 0.27 with age (p = 0.016) and 0.29 with years of experience (p = 0.008). These characteristics had not significant correlation with other groups’ relevance. Gender, country of work (by European region), heading a department and specialty (mother care vs. child care) were not significant in each groups’ relevance. Although, some particular indicators’ relevance varied significantly according to these personal characteristics (see Table 1).

CONCLUSIONS

To have a better view on health care quality we should look at groups of indicators [5]. In that way, after analyzed them, we were able to conclude, unlike what might be expected, that some personal characteristics, such as age or years of experience, can influence the relevance given to an entire group of perinatal indicators – «Health care services» group. Moreover, other personal characteristics, such as gender, country of work, heading a department or specialty, influence the importance given to several particular perinatal indicators (Table 1). So, although we knew that apparent differences of indicators can be explained by confounders [2], we did not know that they might be biased according to personal characteristics of health professionals and researchers. This can lead to different capital expenditures on perinatal health care services, leading to an exacerbation of differences and maybe becoming a complex web based on personal characteristics. In conclusion, health professionals and researchers might affect and bias, according to personal characteristics, collection, evaluation, and health policy-making on perinatal health care.
Tab. 1. Indicators significantly influenced by personal characteristics. p-values were obtained using Chi-square or Fisher tests (p-values from Fisher test are indicated with *). † values of 0, 1 or 2 were merged and compared to «High relevance».

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Respondents who gave more relevance</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population characteristics/Risk factors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distribution of maternal age</td>
<td>Younger specialists</td>
<td>0.045*</td>
</tr>
<tr>
<td>Distribution of parity</td>
<td>Specialists with more experience</td>
<td>0.038†</td>
</tr>
<tr>
<td>Distribution of parents’ occupational classification</td>
<td>Specialists of child care</td>
<td>0.004</td>
</tr>
<tr>
<td>Distribution of mothers’ prepregnancy body mass index (BMI)</td>
<td>Specialists of mother care</td>
<td>0.002†</td>
</tr>
<tr>
<td>Maternal use/abuse of drugs or alcohol</td>
<td>Specialists from Western and Southern Europe</td>
<td>0.025†</td>
</tr>
<tr>
<td>Multiple birth rate by number of fetuses</td>
<td>Specialists from Northern Europe</td>
<td>0.047†</td>
</tr>
<tr>
<td><strong>Health care services</strong></td>
<td></td>
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<tr>
<td>Births without obstetric intervention</td>
<td></td>
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<tr>
<td>Younger specialists</td>
<td></td>
<td></td>
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<tr>
<td>Older specialists</td>
<td></td>
<td></td>
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<tr>
<td>Distribution of place of birth by volume of deliveries</td>
<td>Specialists from Western and Southern Europe</td>
<td>0.008*</td>
</tr>
<tr>
<td>Mode of delivery by parity, plurality, presentation, previous cesarean section and gestational age</td>
<td>Specialists of mother care</td>
<td>0.048†</td>
</tr>
<tr>
<td>Neonatal screening policies</td>
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<tr>
<td>Heads of department</td>
<td></td>
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<tr>
<td>Male specialists</td>
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<tr>
<td>Number of caregivers involved in prenatal and natal care</td>
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<tr>
<td>Heads of department</td>
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<td>Older specialists</td>
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<tr>
<td>Percentage of all pregnancies following treatment for subfertility</td>
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<tr>
<td>Older specialists</td>
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<tr>
<td>Percentage of infants breast fed at birth</td>
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<td>Older specialists</td>
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<tr>
<td><strong>Maternal health: morbidity and mortality associated with fetus</strong></td>
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<tr>
<td>Incidence of tears to the perineum</td>
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<td>Older specialists</td>
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<tr>
<td>Maternal mortality by cause of death</td>
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<td>Older specialists</td>
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<tr>
<td><strong>Fetal, neonatal and child health</strong></td>
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<td>Distribution of Apgar score at 5 minutes</td>
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<td>Older specialists</td>
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<td>Distribution of birth weight by vital status, gestational age and plurality</td>
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<td>Older specialists</td>
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<tr>
<td>Distribution of gestational age by vital status and plurality</td>
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<td>Older specialists</td>
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<td>Fetal heart rate distribution</td>
<td></td>
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<td>Older specialists</td>
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<tr>
<td>Fetal mortality rate by gestational age, birth weight and plurality</td>
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<td>Older specialists</td>
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<td>Fetal and neonatal deaths due to congenital anomalies</td>
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<td>Younger specialists</td>
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<tr>
<td>Infant mortality rate by gestational age, birth weight and plurality</td>
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<td>Older specialists</td>
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<tr>
<td>Prevalence of cerebral palsy</td>
<td></td>
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<td>Older specialists</td>
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<tr>
<td>Prevalence of selected congenital anomalies</td>
<td></td>
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<tr>
<td>Older specialists</td>
<td></td>
<td></td>
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<tr>
<td>Prevalence of late induced abortions</td>
<td></td>
<td></td>
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<tr>
<td>Older specialists</td>
<td></td>
<td></td>
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<tr>
<td>Severe neonatal morbidity among babies at high risk</td>
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</tbody>
</table>
REFERENCES

Lower Obstructive Uropathy in a Male Fetus: a Case Report

S. Mineiro*, A. Codorniz, L. Caseiro, E. Pereira, A. Costa, Z. Carpinteiro, J. Carvalho, V. Caeiro

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SUMMARY

We report the case of a 23-year-old primiparous woman that at 28th weeks of pregnancy had the suspicion of a male fetus with posterior urethral valves syndrome.

The follow-up ultrasound at 30th week confirmed a lower urinary tract obstruction with severe bilateral hydronephrosis (left RDP 16,2 mm; right RDP 12,7 mm) with enlarged bladder (sagittal diameter 49 mm) and ureters, in a 50th/75th percentile fetus but still with no oligohydramnios (amniotic fluid index of 120 cm). These findings suggested urethral obstruction.

The pregnant woman was sent to a skilled obstetric center with pediatric surgery at 30th week to perform lung maturation with corticosteroids. Pregnancy termination was suggested when renal injury appeared translated by oligohydramnios.

Keywords: hydronephrosis, urinary tract, obstruction, posterior urethral valves.

INTRODUCTION

The Obstructive Uropathy includes a wide variety of different pathological conditions characterized by dilatation of part or all of the urinary tract. When it occurs in the second half of pregnancy, hydronephrosis may result and the
severity of renal damage will depend on the degree and duration of the obstruction.

The Obstructive Uropathy includes a wide variety of different pathological conditions characterized by dilatation of part or all of the urinary tract. When it occurs in the second half of pregnancy, hydronephrosis may result and the severity of renal damage will depend on the degree and duration of the obstruction.

Fetal hydronephrosis is defined by varying types of pelvicalyceal dilatation and is a common finding in 1% of fetuses. Mild hydronephrosis or pyelectasia is defined by the presence of an anteroposterior diameter of the pelvis greater than 5 mm at 20-29 weeks and greater than 7 mm at 30-40 weeks of gestation.

Urethral obstruction can be caused by urethral agenesis, persistence of the cloaca, urethral stricture or posterior urethral valves. In this condition sonographic findings can be enlarged bladder, hydroureters, hydronephrosis, different degrees of renal hypoplasia and dysplasia, oligohydramnios and pulmonary hypoplasia.

MATERIAL AND METHODS

A 23-year-old primiparous woman with irrelevant personal or family history had a normal obstetric follow-up consultation since 16th week. The first trimester combined screening was not performed. Instead, it was realized the mor-

Fig. 1. Axial ultrasound image of abdomino-pelvic cavity of a fetus at 28th weeks of gestation showing bilateral hydronephrosis and partial atrophy of the renal cortex.
phological ultrasound study at 21\textsuperscript{th} week. There were no anomalies in the male fetus, with a regular growth in 50\textsuperscript{th} percentile, normal amniotic fluid and placentation. However, at 28\textsuperscript{th} week in the routine consultation, the ultrasound revealed a bilateral enlargement of renal pelvic diameter (RPD) and distended bladder with amniotic fluid at the lower limit of normal. Weekly follow-up ultrasound enabled the measurement of amniotic fluid index and fetal biophysical profile in order to finish the pregnancy when there was renal function impairment.

RESULTS

At 34\textsuperscript{th} weeks gestation labour was induced and a boy was born of eutocic birth delivery with 2250 grams of weight and Apgar score 9/10. The diagnosis of posterior urethral valves was confirmed and the newborn was transferred to a pediatric surgery center to be corrected surgically.

Fig. 2. Coronal ultrasound image of pelvic cavity of a fetus at 30\textsuperscript{th} weeks of gestation showing distended bladder suggesting urethral obstruction.
CONCLUSIONS

Posterior urethral valves are obstructing membranous folds within the lumen of the posterior urethra that occur only in males, and are the most common cause of bladder outlet obstruction, being the presumptive diagnosis in this case-report. Bilateral involvement and renal pelvic diameter larger than 15 mm during the third trimester are at the greatest risk of impaired postnatal function. Postnatal surgical treatment is the gold standard and consists in primary ablation during cystoscopy or vesicostomy as an alternative choice.

REFERENCES

GYNECOLOGY
Emergency Contraception and the Scottish Sexual Health Strategy: Can Rates of Unintended Pregnancy Be Reduced?

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SUMMARY

Unintended pregnancy is a global sexual health problem. Outcomes of unintended pregnancy include unwanted childbirth and abortion which may be associated with negative physical and psychosocial health implications for women. In Scotland, the Scottish Sexual Health Strategy has the stated goal of improving the sexual health of the people of Scotland. One aim of the Strategy is to reduce rates of unintended pregnancy, and one policy designed to achieve this is ‘widening access to emergency contraception’. This paper examines the success of this policy with reference to the implicit link it makes between expanding access to emergency contraception and increasing its effective use, aiming thereby to reduce rates of unintended pregnancy. Since there evidence that past policies and strategies expanding access to EC have failed to reduce such rates, alternative approaches are discussed.

Keywords: unintended pregnancy, abortion, teenage pregnancy, emergency contraception, long-acting reversible contraception, key clinical indicators, Scottish sexual health.
INTRODUCTION

The goal of the Scottish Sexual Health Strategy is to improve the sexual health of the people of Scotland, with one specific aim to reduce rates of unintended pregnancy. A subsequent annual report identifies «widening the availability of emergency hormonal contraception» (EC) as a key policy in the reduction of unintended pregnancies [1]. Implicit in this policy is the assumed link between expansion of access to EC, increases in its effective use, and reduction in rates of unintended pregnancy.

Since licensing of EC in the UK in 1984, deregulation in 2001 and the removal of charge in 2008 have expanded access to EC in Scotland. Deregulation may have increased use of EC and certainly provides women with faster access, but has had no measurable effect on rates of unintended pregnancy [2]. It is too early to ascertain what effect the removal of charge has had on use of EC.

Local strategies to expand access have included advance provision schemes and Patient Group Directions (PGDs), which provide a licence for allied health professionals to dispense EC without a doctor’s prescription. The evidence shows advance provision of EC increases its use by women, but with no subsequent measurable effect on rates of unintended pregnancy [3]. Patient Group Directions have expanded access to EC by removal of the barriers of age and cost, but have not been applied universally. There is no evidence that PGDs increase use of EC by women, and therefore it is unlikely they have influenced rates of unintended pregnancy.

MATERIAL AND METHODS

A comprehensive literature search of online databases was conducted to review the evidence on the impact of widening access to emergency contraceptives on their effective use, and subsequent effects on rates of unintended pregnancy. Fifty seven papers were included for final review. Statistics on use data and proxies for unintended pregnancy were taken with permission from publicly available national statistics records at the Information Services Division (ISD), Scotland.
RESULTS

The ISD has defined ‘Key Clinical Indicators’ (KCIs) designed to monitor the development of health service provision in Scotland [4]. While it is acknowledged that not all unintended pregnancies end in abortion, and not all teenage pregnancies are unintended, both can be used as proxies for rates of unintended pregnancy to examine the success of a policy of expanding access. However, despite the relevant information being available and collected, these are not highlighted as KCIs.

Historically, absolute numbers of abortions have risen consistently since the passing of the Abortion Act in 1967. However, the most recent ISD data have shown a reduction in absolute numbers of terminations in Scotland between 2008 and 2011, with rates of 13.3 per 1000 in 2008 and 12.0 per 1000 in 2011 respectively [5]. This represents a measurable downward direction in a proxy for unintended pregnancy since the introduction of the Sexual Health Strategy. Of particular note is that the fall in numbers has been greatest in women under the age of 20.

In women aged under 20, rates of teenage pregnancy fluctuated between around 54 to 58 per 1000 between 1994 and 2006. The most recent data from the ISD show a decrease in the rate among the same subgroup of women from 52.9 per 1000 in 2009 to 50.2 per 1000 in 2010 [6].

CONCLUSIONS

The obvious but flawed conclusion from these statistics is that a downward trend exists in the numbers of abortions and teenage pregnancies, and therefore the policy of widening access to EC must be working. However, there are myriad confounding factors which do not permit this. These include the independent effects of different policy arms of the Strategy as well as economic, social and demographic changes which cannot be accounted for. A three year ‘trend’ is not long enough for firm conclusions to be drawn, and indeed such small reductions have been observed in the past in the context of an overall trend of rising numbers of abortions.

There has been considerable political enthusiasm for expanding access to EC in Scotland. The failure of advance provision and other local strategies to reduce rates of unintended pregnancy points to a problem with national policy: with a likely efficacy of only around 50% if used within 72 hours and with no
guarantee that women will use EC effectively, why commit scarce political and economic resources to expanding access? This leads us to the conclusion that resources may be better invested in contraceptive methods with better proven effectiveness in reducing unintended pregnancy.

Few unintended pregnancies result from true failure of a contraceptive method; most are the result of incorrect or inconsistent use of contraception [7]. It follows that methods of contraception in situ before intercourse are likely to be more effective in preventing pregnancy than post-coital methods like EC. The typical-use failure rate of long-acting reversible methods of contraception (LARC) is between 0.05% and 0.1%. This is in contrast 15% and 8% for the combined oral contraceptive pill and condoms respectively [7].

Crucially, there is evidence that LARC is effective in reducing rates of unintended pregnancy. In a study in the USA, lower rates of pregnancy were found amongst teenage mothers using long-acting methods to prevent pregnancy [8]. This and other studies led the UK-based National Institute for Clinical Excellence to recommend that increased uptake of LARC would lead to reductions in unintended pregnancy, a recommendation adopted by the Scottish Sexual Health Strategy.

Data on long-acting methods are a Key Clinical Indicator for the Strategy. Despite the recent reported reductions in both abortions and rates of teenage pregnancy, the uptake of LARC has been fluctuant in Scotland in recent years. Rates of uptake per 1000 women aged 15-49 of ‘very long acting methods’ (intrauterine device, system and implant) were 56.7 in 2009/10, 60.6 in 2010/11 and 55.4 in 2011/12 respectively [4]. Promotion of these methods has failed to procure an increase in use in the short term and therefore no effect on rates of unintended pregnancy can be demonstrated. The advantages of a public health vision with an emphasis on long-acting methods therefore remain theoretical and time will be required for a more complete analysis.

There are however challenges to such a vision. From a reproductive rights perspective, there is an ethical question in asking individual women to use long-acting methods which arguably restrict their freedom to reproduce at the time of their choosing. This is particularly true of implantable methods. This issue could perhaps be mitigated through strong emphases on individual choice, the importance of sex within stable relationships, and the intention of the policy to improve access to the method without necessitating its use.

Additionally, LARC is a heterogeneous group of products with variable
durations of action (between one month for oestrogen-progestin ‘injectables’ and several years for progestin-only implants and devices) and side effects such as weight gain and bleeding patterns. Thus, their acceptability to women is relevant. There is some evidence in Scotland that some of these barriers may be difficult to overcome [9].

From a public health perspective, the case for promotion of long-acting methods has been made. However, given the lack of robust data available in Scotland on the use of emergency contraceptives generally, it is possible EC could yet be proven to make a positive contribution to the contraceptive options for women. Promoting more effective use of EC as an adjunct to other methods, including greater uptake of post-coital implantable methods, may signal a future policy direction, and there is some evidence that repeated use of pre- and post-coital hormonal methods may be effective [10].

It is therefore arguable the most effective Scottish sexual health strategy for the future would combine several of the options discussed above, coupled with a renewed focus on more robust data collection for a wider ranges of contraceptives. Such an approach would resonate with international prescriptions to provide the widest range of safe, effective contraceptive options for women, and represent further progress toward the goal of better sexual and reproductive health for all.

REFERENCES


Case Report – Multidose Methotrexate As an Approach to the Management of Ovarian Ectopic Pregnancies

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SUMMARY

Ovarian ectopic pregnancies (OEPs) occur in 0.03-0.06% of all pregnancies. They carry the potential risk of rupture resulting in haemoperitoneum and exsanguination if not treated promptly. This recent case in a peripheral Melbourne hospital highlights the use of multidose methotrexate (MTX) at 50 mg/m² as a management option in a clinically stable OEP case. We describe a case of a 22 year old primigravid woman who was strongly in favour of avoiding surgery and its potential complication of an oophorectomy. Her OEP successfully receded following 3 doses of MTX. Our literature review reveals a paucity of data in the medical management of OEPs hence this case is an important addition to the literature. The majority of cases of OEPs have been managed surgically and the gold standard is regarded as laparoscopic ovarian wedge resection. However, in stable OEPs in women requiring future fertility, we have demonstrated that multidose MTX is a viable option.

Keywords: medical management ovarian ectopic pregnancy multidose methotrexate.

CASE

Miss X was a 22 year old primigravid woman who presented to the emergen-
The transvaginal and transabdominal ultrasound scan done in the community showed no evidence of an intrauterine gestational sac, but a small amount of fluid in pelvis was present. The right ovary measured 28x28x33 mm and within it there were heterogeneous echoes. This cystic structure was considered as a possible corpus luteum. There was no abnormal vascularity seen but an ectopic pregnancy could not be ruled out. Her left ovary measured 29x21x28 mm and was normal in appearance. On presentation her blood tests revealed Hb 142, WCC12.4, Neut 8.2, Pl 277, and serum BHCG 3743.

The next day Miss X’s BHCG was repeated to reveal a drop to 2212. Vital signs stabilised and she was no longer febrile. A repeat USS at our institution confirmed the absence of an intrauterine pregnancy, a moderate amount of free fluid in the pouch of douglas, and the cervix was long and closed. Superolateral to the right ovary was a 19x16x13 mm echogenic mass with a central cystic area strongly suggestive of an adnexal ectopic pregnancy. Assuming it was a gestational sac it corresponded to gestation of 5 weeks. There was no increased vascularity, yolk sac or fetal pole seen.

In light of the presentation, investigations and her haemodynamically stable state Miss X was treated medically using methotrexate (MTX) as per hospital protocol. Her body surface area was calculated as 1.9 m² and at a dose of 50 mg/m² she was given 95 mg IM. Miss X was observed for one hour post dose and discharged with follow up BHCGs on day 4 and day 7 post MTX. She was
advised to re-present if there was increased bleeding or pain.

On day 3 post-MTX Miss X represented with increased bleeding and pain. Her pain required regular panadeine forte and diclofenac and was interfering with her sleep. Her PV bleeding required her to change pads 2 hrly but she was not passing clots. On examination Miss X was haemodynamically stable. Her abdomen was soft, with generalised lower abdominal tenderness especially in RIF with guarding. Her BHCG had dropped to 1798. An ultrasound the next day demonstrated normal ovaries but her right adnexa had a 59x28x39 mm heterogeneous mass within which was a 15x15x15 mm cystic region. BHCG on day 4 rose to 1904.

Miss X remained in hospital, by day 5 post-MTX she was pain-free and the bleeding had stopped. Her abdomen was soft and non-tender. On day 6, a vaginal exam revealed no adnexal mass or cervical excitation. A repeat US showed the size of the ovarian mass to be unchanged. Miss X experienced fresh PV bleeding and pain later that evening with mild tenderness in RIF. There was no peritonism or cervical excitation and the patient remained haemodynamically stable. In view of the mass being unchanged, BHCG not dropping adequately and the patient preference for conservative management, a 2\textsuperscript{nd} Dose of MTX (90 mg IM) was administered 6 days after her initial dose. Her symptoms settled overnight and she was discharged home next day.

Miss X re-presented day 7 following her second MTX dose with PV bleeding and some mild pain. In light of her BHCG on day 4 and day 7 not dropping by more than 15% she was counselled about the failure of medical management. Her options at that stage were to have a 3\textsuperscript{rd} dose of MTX or to proceed to a laparoscopy (and therefore potentially an oophrectomy). Miss X strongly preferred conservative management and opted for a further dose of MTX. A repeat ultrasound prior to her 3\textsuperscript{rd} dose showed fluid in POD and the ectopic size unchanged at 26x16x20 mm. The 3\textsuperscript{rd} dose of MTX 95 mg IM was administered the next day, day 15 post 1\textsuperscript{st} dose MTX, and she was discharged following one hour of observation.

Day 2 post 3\textsuperscript{rd} dose of MTX, Miss X re-presented to ED with acute onset RIF pain worsening over time. She reported very light PV bleeding and some mild nausea. Miss X had RIF tenderness and guarding but no cervical excitation. She was afebrile and haemodynamically stable hence was observed closely overnight. Day 3 post 3\textsuperscript{rd} dose of MTX, her pain resolved and her BHCG dropped significantly to 238. By day 6 post 3\textsuperscript{rd} dose of MTX, her BHCG was 100. She was counselled about the risk of rupture even though BHCG was dropping. Miss X was observed another two days before being discharged and having her BHCG followed up in an outpatient setting where it normalised by day 13 post 3\textsuperscript{rd} dose MTX, 27 days after initial presentation.
DISCUSSION

An ovarian ectopic pregnancy (OEP) is a rare and potentially fatal condition where a pregnancy implants in the ovary. These make up 2-3% of ectopic pregnancies (EP) (1-3) and 0.03-0.06% of all pregnancies (1, 3). EPs accounted for 13 maternal deaths in the UK between 1997-99 (4). The incidence of EPs per live births, OEPs per EPs, and OEPs per live births have all increased between 1989 and 2009 (1). Several papers have shown intrauterine devices and assisted reproductive technology to be associated with higher risk of OEPs (3, 5).

OEPs continue to be a diagnostic challenge. In a recent study of 110 OEPs, 89% presented with intermittent lower abdominal tenderness, 47% with vaginal spotting, 38% with a palpable adnexal mass, 26% with shoulder pain and 12% in shock, while 8% were asymptomatic (1). Similar presentations were noted in other studies (2, 6). Originally, OEPs were diagnosed histologically according to the Spiegelberg criteria (7): the fallopian tube and fimbria should be intact and separate from the ovary, the gestational sac should occupy the normal position of the ovary, the gestational sac should be connected to the uterus by the ovarian ligament, and ovarian tissue must be present in the specimen attached to the gestational sac. However, advances in ultrasound technology, especially transvaginal scanning, has allowed OEPs to be largely diagnosed preoperatively (2), rendering the Spiegelberg criteria largely obsolete in the diagnostic stage of current practice. US diagnosis allows for medical and expectant management in suitable patients. Differentials for an OEP include corpus luteum and endometrioma. Features suggestive of OEP are a wide echogenic ring with an internal echoluscent area on the ovary (8), or a hyperechoic trophoblast surrounded by thickened hypoechoic ovarian tissue (9).

OEPs can be managed surgically, medically or expectantly. Due to the rarity of the condition, OEPs do not have an individual management guideline but many institutions extrapolate the tubal ectopic pregnancy guideline to OEPs. This is not ideal as OEPs can behave differently to tubal EPs, which prompted us to undertake this review of the literature. The vast majority of OEPs reported in the literature have been surgically managed. Between 1990 and 2010, 250 OEP cases were reported, of which only 8 were managed medically (5).

The commonest management option described in the literature is surgical resection of the ovarian ectopic. Prior to the availability of reliable transvaginal USS data, most OEPs were diagnosed at laparotomy or diagnostic laparoscopy which then favoured surgical management. Another factor leading to higher prevalence of surgical management is due to patients presenting in haemodynamic instability. In ruptured ectopics with haemoperitoneum, emergency laparotomy is the commonest approach due to urgency (10). Surgical interventions are
predominantly ovarian wedge resections but a smaller percentage of oophorectomies are also reported (1, 2). Since 1996 laparoscopic ovarian wedge resection seems to be the gold standard in surgical management (2, 3, 5). The reported benefits of laparoscopy over laparotomy for tubal ectopics are shorter operating times, less intraoperative blood loss, shorter hospital stay, and reduced requirement for post op analgesia (11-13). It is reasonable to believe similar benefits will apply in the situation of OEPs.

The medical management of OEPs is still an evolving area. There are several case reports of treating OEP successfully with systemic methotrexate (MTX) (14, 15) although in one series of 8 OEPs, 50% failed MTX and required rescue surgery (5). There are several different MTX protocols, as well as protocols using other medications to treat OEPs. The most commonly used method in tubal ectopics is a single intramuscular dose of MTX at 50 mg/m² (10) where the body surface area is calculated using square root of height (cm) x weight (kg/3600). Serum BHCG is then checked on Day 4 and Day 7 where day one is the day of injection. If there is a drop of 15% or more between D4 and D7 the therapy is deemed a success otherwise a repeat dose is given (10, 16, 17). The IM 50 mg/m² MTX dose was developed for EPs in general (18). This regimen was successfully applied in an OEP case in 1994 (14). In unruptured OEPs, MTX is favourable as it avoids surgery and the associated complications of possible oophorectomy, haemorrhage, and later pelvic adhesions (19).

In another successful OEP case, the patient received a multidose regimen of four IM MTX injections according to body weight alternating with 0.1 mg/kg of leucovorin calcium per vagina after 30 hrs until BHCG decreased by 15% (20). Other approaches describe using transvaginal US guided aspiration and injection of MTX (21). Non-MTX based treatment has also been described in an assisted reproductive technology (ART) population where all the cervical ectopic, corneal ectopic and tubal ectopics in a series were treated using US guided injection of potassium chloride into the ectopic gestation sac or fetus, with success in 25 of 27 patients (22).

Most of the data involving the use of MTX is based on the general EP population as data describing medical management of OEP is scarce. With regard to tubal ectopics in stable patients, a variety of medical treatments have been shown to be as effective as surgery (23). Randomised controlled trials comparing MTX with laparoscopic surgery in tubal EPs have found that 15.8-20.6% of women will require more than one dose of methotrexate and between 5.3-14.7% of women will require surgical intervention, while a case series of 315 EPs showed a rate of 17.5% requiring multiple MTX doses and 5.2% requiring surgical intervention (16, 17, 23, 24). A single dose of IM 1 mg/kg MTX has been found to be a safe choice that does not compromise future reproductive outcomes in a cohort of women diagnosed with EP after ART (25).
After MTX treatment in tubal pregnancies most women will experience abdominal pain (18), but verifying the source of pain as tubal abortion or tubal rupture can be difficult. As a result some women will need to be admitted for observation and assessment by transvaginal ultrasound (23, 26). This increase in abdominal pain has also been reported in cases of OEPs (14).

Use of MTX needs to take into account the patient’s clinical status and practical factors. The patient needs to be haemodynamically stable, their initial serum BHCG and presence of fetal cardiac activity are considered factors in the rate of success. High initial BHCG in tubal EPs is associated with longer follow up, further doses of MTX and high chance of surgical management (24). In tubal EPs MTX has been successful in BHCG as high as 6418IU/L (17) but quality of life data suggest MTX is only attractive in women with BHCG below 3000IU/L (16, 27). Fetal cardiac activity in EPs has a reduced chance of success (28), which is therefore considered a relative contraindication for MTX therapy. MTX has possible side effects of myelosuppression, hepatotoxicity and nephrotoxicity and is renally cleared therefore prior to each MTX dose a full blood count, liver function tests and urea and electrolytes need to be checked for abnormalities which would then exclude use of MTX. Blood group and cross match need to be undertaken in case of ectopic rupture. Due to the risk of rupture, candidates for MTX need to be within close proximity of the hospital and be reliable to present should they experience any increase in abdominal pain or any PV bleeding. As MTX has a teratogenic risk, women will require reliable contraception for the next 3 months.

In certain scenarios expectant management is also an option. Often this is advisable if the BHCG has already begun dropping and or the ovarian mass is small and or the BHCG is very low.

CONCLUSION

OEPs are a rare but potentially fatal condition. This literature review demonstrates that the majority of reported cases have been managed laparoscopically. Surgical management has the advantages of being able to achieve rapid haemostasis in an unstable patient and the ability to pathologically confirm the diagnosis. Medical management of OEPs is seldom described in the literature, particularly regarding the use of multiple doses of MTX. MTX offers a non-invasive choice that avoids the risks and ramifications of surgery in a young nulliparous woman. Ultimately the management of any condition depends upon the benefits and risks of each option for the individual patient and the patient preference. Our case demonstrated that in a stable OEP in a young patient, MTX can successfully treat the condition however the course of the treatment and respon-
se of the OEP can differ from that of a tubal EP. We have demonstrated that despite initial failure of MTX therapy, further doses can be successful. Hence multiple attempts are valid if the patient is stable and still keen for medical management. Further investigation into medical management of OEP is required in order to develop a specific treatment protocol and further elucidate the risk benefit profile of this therapy.

REFERENCES

Single Incision Laparoscopic Surgery in Pregnancy: a Case Report and Review of the Literature

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**SUMMARY**

Laparoendoscopic single-site surgery (LESS), also known as single incision laparoscopic surgery (SILS), is advancing the minimally invasive surgical approach and used as surgical technique in gynecologic surgery. Herein, we describe our experience with a case report utilizing the SILS in pregnancy and discussing relevant peer-reviewed English literature. A 42-year old female, who was pregnant at 17-week gestational age, presented with sudden onset right lower abdominal pain. Her lab results were: white blood count (WBC) 13,000, and hemoglobin (Hb) 106 g/L. Pelvic ultrasound characteristic revealed right large adnexal cyst measuring 12x12x7 cm with torsion. Diagnosis of ovarian cyst with torsion was made, which indicated surgical intervention during pregnancy. SILS was performed using single-port through 2 cm umbilical incision to the peritoneal cavity. Uterus and left ovary with adnexa were normal. Right ovarian cyst with torsion was identified. Un-twisting of the torted pedicle and ovarian cystectomy was performed, the patient had a spontaneous vaginal delivery at 38-weeks. Thus, we report our successful experience of SILS in pregnancy without any complications. This report is one of the early experiences using SILS in pregnancy without any complications, and likely be the first from the Arab World (Middle-East) experience. This case highlights the possibility of considering
SILS option for intervention in gynecologic surgeries during pregnancy. Further studies would be desirable to determine short- and long-term outcomes.

**Keywords**: Single incision, Laparoscopy, Pregnancy, Gynecologic surgery, Case report, Literature review.

**INTRODUCTION**

Over the years, there have been many advances in the field of minimally invasive surgery (MIS). Particularly, laparoendoscopic single-site surgery (LESS), which is also referred to as single-incision laparoscopic surgery (SILS), describes the use of one small skin incision to complete laparoscopic surgical procedures where traditionally multiple incisions are created.

Although, SILS was first described over two-decade ago [1], but gynecologists have been slower in adopting the SILS approach for wider applications/indications. The SILS is a promising technique that is feasible and relatively safer when performed by experienced laparoscopic surgeons, and can be offered for a variety of gynecologic procedures, including for the treatment of select malignancies [2].

Recent comparison series and two randomized controlled trials suggested some improved cosmetic results, reduced post-operative pain and analgesic when SILS was compared with the traditional laparoscopic approach 3-7]. However, in the peer-reviewed English literature, there is a limited reported experience using the SILS (LESS) approach in pregnancy. Kim et al [8] reported a similar case using LESS to describe a surgical approach for the management of a pregnant woman with an ovarian tumor using cyst exteriorization outside the umbilical incision. Dursum et al [9] reported the feasibility of LESS approach for the management of huge adnexal cysts and adnexal torsion during pregnancy in two patients. These early observations concluded that LESS in pregnant patient with adnexal mass is feasible and might be a better surgical option for the pregnant patients with adnexal pathology [8,9].

Herein, we report our successful experience of a case utilizing SILS in pregnancy without any complications, which highlights the possibility of considering this approach for future interventions in gynecological surgeries during pregnancy. Notably, this is one of the early experiences of SILS in pregnancy, and likely be the first report from the Arab Word (Middle-East) in the peer-reviewed literature.
MATERIALS AND METHODS
(WITH CASE PRESENTATION)

A 42-year old female, who was pregnant at 17-week gestational age, presented with sudden onset right lower abdominal pain at our clinic. She had no nausea, vomiting, anorexia, or vaginal bleeding. All her previous pregnancies were normal vaginal deliveries without any reported complications. Her past medical and surgical history was unremarkable.

On examination, she was found to be febrile and tachycardic. Her body mass index (BMI) was 40 kg/m², with abdomen tenderness over the lower abdomen (mainly right side), and no rebound tenderness. Her lab results were: white blood count (WBC) 13,000, and hemoglobin (Hb) 106 g/L. Pelvic ultrasound characteristic revealed right large adnexal cyst measuring 12 x 12 x 7 cm with torsion. Therefore, the diagnosis of ovarian cyst with torsion was made, which indicated surgical intervention during the pregnancy.

Options of laparotomy versus laparoscopy were discussed with the patient (and also with the appropriate family members) at length, including the potential risks such as abortion, injury of the gravid uterus, post-operative pain, and prolonged hospitalization. The option of SILS approach was also discussed with her due to the cyst site and the limited space available for using the conventional laparoscopy, hence no space for multiple ports. She eventually agreed on SILS option, which was performed on an emergency basis.

Single-port was introduced through 2 cm umbilical incision to the peritoneal cavity (Fig. 1). A 2-cm vertical incision was made in the umbilicus at the beginning of the operation. Layer-by-layer, the peritoneal cavity was entered; then 0 polyglactin 910 (Vicryl) sutures were placed at each side of the fascia (as a stay sutures to help in final closure at the end of the procedure). The SILS device was inserted into the wound opening transumbilically. After insertion of the SILS device into the abdominal cavity, three 5-mm sheaths were inserted through the device. CO₂ gas was insufflated through the special connector to the device [Fig. 1(B)].

Intra-abdominal pressure was maintained at 12-mm Hg. Once pneumoperitoneum was achieved using 2.5 L of CO₂, no leakage of CO₂ from the connected portions was noted. Intra-abdominal visualization was obtained with a 5-mm 0-degree laparoscope (Pano-view; Richard Wolf GmbH, Knittlingen, Germany) inserted through a 5-mm cannula. No intra-uterine instruments were used. Carefully and slowly, the patient was placed in the Trendelenburg position. At the abdominal entry, initial inspection revealed no adhesions, a gravid uterus 17 gestational weeks in size, a normal left adnexa, and the whitish glistening smooth surface of a torted right ovarian tumor. The tumor was grasped using conven-
tional laparoscopic atraumatic grasping forceps and un-torsion of the cyst was achieved. Then, the ovarian mass was pulled towards the umbilical incision. The ovarian cyst was punctured with a curved Metzenbaum scissors, and its content was aspirated using a suction irrigation apparatus. To prevent cyst spillage, the puncture site on the cyst was held and pushed-up against the abdominal wall with a forceps. Then, the deflated cyst was exteriorized through the umbilical wound, at the same time the SILS device was removed from the umbilical wound. After the ovarian tumor was removed, CO₂ insufflations were stopped. A right ovarian cystectomy was then performed using the traditional technique outside the abdominal cavity, with preservation of as much ovarian tissue as possible [Fig. 2(C)].

The right ovary was placed back in the peritoneal cavity, the SILS device was re-attached to the umbilical wound, and pneumoperitoneum was re-established. Irrigation and hemostasis were then performed under laparoscopic control. The peritoneum and fascia were closed layer-by-layer with the 0 polyglactin 910 (Vicryl™) sutures, and the skin was closed with 4-0 polyglactin 910.
sutures. The fetus tolerated the operative intervention well, with a fetal heart-beat of about 150 bpm (both before and just after the operation).

Throughout the procedure, we did not encounter any bleeding or intra-operative complications. The operative time was 40 min with minimal blood loss (< 100 mL). Notably, intensive care unit service was not required, and the patient was discharged for home day-2 post-operatively with positive fetal heart, no signs of uterine contraction, reporting minimal pain, and tolerating a regular diet. No tocolytic agent was required, and the post-operative surgical wounds demonstrated satisfactory cosmetic results. Final pathology came as benign serous cyst adenoma with evidence of torsion. The patient had a spontaneous vaginal delivery of a healthy baby boy at 38 weeks.

CONCLUSIONS

In this report, we described our successful experience of SILS in pregnancy.
without any complications, which highlights the possibility of considering SILS option for interventions in gynecological surgeries during pregnancy. The SILS approach was not only feasible in this pregnant woman, but also turned out to be much better choice since the patient was morbidly obese with pregnancy and twisted ovarian mass, which was located in the right upper abdomen because of the pregnancy effect. The surgical options were either to do a large incision laparotomy with its morbidity, or to perform pannectomy for the patient’s large pannes at the time of laparotomy. Alternatively, we could have opted to perform a conventional multi-port laparoscopy, which would have resulted in a very limited access inside the abdomen and may not have been able to use the skin incision of the SILS device to complete the cystectomy and remove the specimen. We achieved good cosmesis with minimal discomfort, and the patient is currently having a better quality-of-life with the pregnancy outcome. Since this report supports the previous limited experiences using the SILS (LESS) in pregnancy, further prospective studies that compare traditional laparoscopy with SILS would be desirable to determine short- and long-term clinical outcomes.

REFERENCES

Effects of Black Cohosh on the Plasminogen Activator System in Vascular Smooth Muscle Cells

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SUMMARY

BcEx induces PAI-1 protein expression in the VSMCs likely via an oxidant mechanism. It also stimulates the enzyme activity of PAI-1 and reduces that of free t-PA. These findings suggest that black cohosh might exert a negative influence on fibrinolysis.

Keywords: Black cohosh, Plasminogen activator inhibitor 1, Vascular smooth muscle.

OBJECTIVE

The rhizome of the Cimicifuga racemosa plant (commonly known as black cohosh) has been used for menopausal complaints [1, 2]. Studies regarding the cardiovascular effects of black cohosh are lacking. We investigated the effect of black cohosh on the plasminogen activator system in cultured vascular smooth muscle cells (VSMCs).
METHODS

VSMCs were isolated from rat aortae. Expression of plasminogen activator inhibitor type 1 (PAI-1) and tissue-type plasminogen activator (t-PA) proteins were evaluated by Western blot analysis and enzyme-linked immunosorbent assay, respectively. The activities of PAI-1 and t-PA in the conditioned media were assessed by fibrin overlay zymography. A 40% 2-propanol extract of black cohosh was used [3].

Fig. 1. Effects of black cohosh extract (BcEx) on the plasminogen activator system in cell lysate. Representative blots are shown in the upper section of each panel. The intensity of the bands was densitometrically determined, and normalized to that of corresponding β-actin bands. Graphic data are depicted in the lower part of the panel. Time-course effects (A) and dose-response effects of BcEx (B) on PAI-1 as determined by Western blot analysis. (C) Time-course effects on t-PA as assessed by ELISA. *P < 0.05 vs. control at 0 h.
Fig. 2. Mechanism for PAI-1 effect of black cohosh extracts (BcEx). (A) Effects of treatment with vitamin E and ICI 182,780 for 8 h, respectively, as determined by Western blot analysis. (B) Intracellular production of reactive oxygen species (ROS) detected by dichlorofluorescin fluorescence under confocal microscopy (100×). (C) Kinetics of intracellular ROS production as assessed by flow cytometry analysis.

*P < 0.05 vs. vehicle-treated control; †P < 0.05 vs. baseline. EtOH, ethanol; DMSO, dimethyl sulfoxide.
RESULTS

1. **BcEx induced the expressions of PAI-1 protein in the cell lysate of VSMC**

   In Western blot analysis, the expression of PAI-1 increased in a time-course experiment with 100µg/mL BcEx, showing a peak at 8h (Fig. 1A). In addition, the PAI-1 expression also increased in a dose-dependent manner when checked at 8h of treatment with BcEx, and significant increments in PAI-1 expression were found at a BcEx concentration of 40µg/mL or higher (Fig. 1B). As assessed by ELISA, t-PA production in the VSMC remained unchanged, and it was not altered by BcEx treatment (Fig. 1C).

2. **Up-regulation of PAI-1 expression was ROS-dependent**

   To explore possible mechanisms for BcEx action, involvement of oxidative stress was examined first. EtOH (0.1%) did not make a difference, but vitamin E (50µM), a potent antioxidant, significantly suppressed BcEx-induced PAI-1 expression (Fig. 2A). Under confocal laser microscopy, BcEx treatment induced intracellular ROS production (Fig. 2B). Flow cytometry presented the kinetics of ROS formation by BcEx (Fig. 2C). Estrogen receptor (ER) mediation was also checked. Neither ICI 182,780 (1µM), an ER antagonist, nor DMSO (0.1%) affected PAI-1 expression (Fig. 2A).

3. **BcEx stimulated PAI-1 activity and reduced free t-PA activity in the CM**

   Reverse fibrin overlay zymography for assessing PAI-1 activity showed that BcEx stimulated PAI-1 activity (Fig. 3A). A significant increase was observed at 4 h, and a peak was reached at 12 h. As assessed by ELISA, t-PA secretion into CM increased with time in vehicle-treated controls. However, t-PA secretion by BcEx treatment did not differ from controls at each time points (Fig. 3B). Fig. 3C demonstrates that free t-PA activity increased with time in the control, and BcEx reduced it after 12 h in fibrin overlay zymography. BcEx also increased higher molecular weight complexes from 8h on, which were thought to be inactive t-PA bound to PAI-1.

CONCLUSION

Black cohosh does not affect t-PA expression but induces PAI-1 expression likely via oxidative stress in VSMC. In addition, black cohosh stimulates the enzyme activity of PAI-1, and reduces that of free t-PA by inducing a binding
Fig. 3. Time-course effects of black cohosh extracts (BcEx) on the t-PA secretion and enzyme activities of the plasminogen activator system in the conditioned media. Representative zymographs are shown in the upper section of each panel. The intensity of the bands was densitometrically determined, and graphic data are depicted in the lower part of the panel. (A) Effects on PAI-1 evaluated by reverse fibrin overlay zymography. Effects on t-PA as assessed by ELISA (B) and fibrin overlay zymography (C). *P < 0.05 vs. vehicle-treated control; †P < 0.05 vs. control at 0 h.
to PAI-1. Further studies are needed to elucidate the impact of black cohosh on the cardiovascular system.

REFERENCES


Woman’s Contraceptive Needs and Preferences in the Postpartum Period

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SUMMARY

The level of knowledge women have about contraceptive methods and their personal preferences, can strongly influence this choice. The aims of this cross-sectional study are to describe women knowledge about postpartum contraception and to identify their related needs and expectations. During pregnancy and postpartum, 45.5 per cent of the women reported that they had received adequate information about contraception. Of these ones, 64.3 per cent reported their intention to use contraception, even if they did not always have appropriate knowledge about fertility and the use of contraceptive methods. Women’s intention to use contraception was proportional to their level of education. Women need more and appropriate information about postpartum contraception, to make a conscious choice in relation to their needs and without putting their health at risk. To promote the awareness of the choices related to postpartum contraception, it is important to understand the personal characteristics that influence or hinder this choice.

Keywords: post partum contraception, knowledge, post partum period.

INTRODUCTION

Post-partum period presents some risks of unwelcomed pregnancies and new mothers, who deal with the problem of beginning or resuming contraception, have to choose either the right contraceptive method or the appropriate time to start it [1, 2, 3]. If they breastfeed, the chosen contraceptive method should not
interfere with breastfeeding and such choice could often involve any questions and doubts about both lactation and baby development and growth.

Although most women are nursed by a health professional during pregnancy, over half of them has not received any information about restarting sexual intercourse and about contraception. [4, 5]. Women’s knowledge level about the different contraceptives, [6] their wish whether or not to have a baby, their personal preferences and whether or not using long term contraceptive methods can influence the setting in of a pregnancy [1].

Most women are inclined to use a contraceptive when restarting sexual intercourse in the post-partum period but their knowledge about reproductive health and family size planning are scarce and they do not know the real advantages and disadvantages in using the different options [4]. Women should be thus informed and advised about post-partum contraception during pre-birth visits, immediately after birth and later in the post-partum period [7, 1, 8, 9, 3, 10, 11], this information is but too often omitted [4].

In Italy the issues of sexual intercourse restarting and puerperium contraception are not mentioned in the recommendations for the physiological pregnancy, among information to provide pregnant women with [12]. Only one survey about post-partum contraception and related needs has been found out [5] and the current studies of the literature on the topic focus mainly on specific targeted population and do not always identify puerperal women needs.

**MATERIAL AND METHODS**

This research aims to describe knowledge and expectations of new mothers about contraception in the post-partum period: to detect their intention to use contraception and to illustrate their knowledge, opinions and expectations on this matter. Furthermore it would identify the related informative and educational needs of the involved women. The cross sectional study was conducted from November 2011 to February 2012 at a third level midwifery center. 300 new mothers, over 18 and with a good knowledge of Italian language, were subsequently recruited to express their consensus to the interview, before their discharge.

They were submitted a questionnaire of 38 items, structured on this survey and describing: personal characteristics (age, education level, job, civil status) and midwife history; received information and knowledge about contraception and its use in the post-partum period, related needs and preferences. The study had been authorized by the Administration of the Clinic where we conducted the survey.
RESULTS

The average age of women is of 33, Dev. Std 5.12, (range 18-45). 20 per cent of them is foreign, only 1 per cent is single. 50 per cent owns a school leaving certificate, 32.3 per cent owns a degree and 249 of them (83 per cent) work. 160 new mothers (53.3 per cent) are at their first pregnancy and 295 (98.3 per cent) have been nursed during their pregnancy, 151 (50.3 per cent) have attended birth training course. 256 women are currently breastfeeding (85.3 per cent) and 136 (53.1 per cent) would go on with breastfeeding over the six month standard period. 74.3 per cent of them has previously used contraception.

Information and knowledge about contraception in post-partum period

135 women (45.5 per cent) state to have received clear and adequate information during their pregnancy. 72 of them (53.3 per cent) have received information during their pre-birth visits at the family advice bureau and 47 (34.8 per cent) from their trustworthy gynecologist. 123 women (41 per cent) have received explanation about the different contraceptive methods. In the post-partum period only 46 (15.4 per cent) have received any information about contraception. 170 (56.7 per cent) who have been interviewed, show to have correct information about whether or not to get pregnant during breastfeeding, 211 have been informed (70.3 per cent) about condom or IUD use and 38 (12.7 per cent) about using pill during breastfeeding.

137 women (46 per cent) think, on the whole, to own adequate information about contraception and 103 (76.3 per cent) of them have received such information during their pregnancy. Those ones who have attended birth training course show to have more knowledge than those ones who did not attend such courses.

Contraception in the post-partum period and related expectations

193 women (63.3 per cent) (I.C. 58.6 per cent-69.8 per cent) aim to use contraception in the post partum period.

The highest frequency in using contraception in the post-partum period is among Italian women between 31 and 35 (45 per cent), with a degree 78 (80.4 per cent) and with two children.

98 women (32.7 per cent) would use pill and 70 (23.3 per cent) condom.

256 women are breastfeeding (85.3 per cent) and of these ones, 136 (53.1 per cent) think to go on with it over the six month standard period; 164 (64.1 per cent) of them would use contraception and 62 (37.8%) would prefer to use hormonal methods, 49 (29.9 per cent) would use condom; among the 37 women (14.5 per cent) who have not decided to use contraception, 14 (48.3 per cent)
would prefer to use hormonal methods as well. 149 women (49.7 per cent) would use a contraceptive whose using is easy, 128 (42.7 per cent) would like to find a contraceptive offering a long term protection.

CONCLUSIONS

Personal characteristics and midwife history of the interviewed women are nearly superimposable to the national ones [13].

74.3 per cent of the interviewed women used contraception before pregnancy, and this data is similar to the previous studies on this topic [5, 9] but superior to those studies developed in different contexts, especially the African ones, [14, 2] because they could be ascribed to a better knowledge level of contraception and to social, economic and cultural differences [15]. 64.3 per cent of the new mothers think to use any contraceptive after birth, in a percentage which is similar to that one of other surveys [16, 5, 17, 2], but which is still inferior to the data of the survey by Cwaik and other people [1] about American women. That could be explained by the social and cultural differences among different populations, and especially when these ones are compared to the Anglo-Saxon countries.

The aim to use contraception is proportional to the considered age [15, 18, 19, 9], up to 35 in our study and in women with a medium-high level of instruction, that are 80 per cent in this study [18, 19, 9, 2]; a high education level increases in fact knowledge, choice skills and service access.

Hormonal methods (32 per cent) together with condom (23 per cent) represent the most preferred contraceptives in the post-partum period [5, 1, 2]. The formers present but a questionable safety moreover, in the post-partum period, the estroprogestin pill is not recommended and the pill with only progestin can be used only after the six weeks standard period [20]. Data could also indicate women’s need of information and education and their subsequent necessity to be supported in their choice, that has to respect both their own preferences and the most appropriate recommendations for contraceptive methods. This choice can be influenced by previous knowledge; only 45.5 per cent of women affirms to have received adequate information about contraception before delivery and only 41 per cent about the different available options, although they had been nursed during pregnancy; this data are however higher if compared to the 37.11 per cent of the survey by Donati & Gandolfo [5] and to 23.9 per cent of the survey by Omololu & Okunowo [2]. The women who have received more information and counselling during pregnancy tend to use more contraception in the post-partum period [1, 21, 2]. On the other hand any knowledge about contraception can increase, once specific information have been received [5,
22], as a consequence, any information mediated by the interpersonal relationship between midwives and women could represent an efficient strategy.

According to the interviewed women the two main reasons to use post partum contraception are: not to have other pregnancies (46%) or to space them out 22 per cent [15, 2].

Over about 40 per cent of the involved women has shown any doubt or inadequate knowledge about fertility and pill use in the post-partum period [5]. Data about increasing use of contraception related to age or education level together with the wish to limit pregnancies is present in similar studies [16, 18, 23, 2], but not in the one by Oye-Adeniran et al. [14].

Women knowledge about contraception and their use could be partly inadequate because of the few or scarce information they receive from the health professionals, as a consequence, only women who have more information are able to use more contraception in the post-partum period. Knowledge about contraception is necessary, although it is not the only element which ensures its use [6]. Knowledge can be developed through information about contraception and counseling for its use.

To make women and couples aware of their choices, it is necessary not only to provide them with required knowledge, but also to detect their real knowledge, expectations and motivations, to develop adequate support and educational intervention, to safeguard reproductive health and procreative choices, to develop their problem solving skills in order to compare the different characteristics of the contraceptive methods to women’s preferences, values, life experience and last but not least, to promote women empowerment.

REFERENCES


Contraception and Sexual Behavior of Female Medical Students in Portugal

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SUMMARY

Sexual risk behaviors place adolescents and young adults at risk for sexually transmitted diseases (STDs) and unintended pregnancy. It is therefore important to study different populations in order to develop strategies aimed to reduce these risk behaviors and help young people adopt lifelong attitudes that promote their health. The authors designed this study in order to evaluate sexual and contraceptive behavior of Portuguese female medical students, as the existent information on this subject is very scarce. The results revealed a low risk sexual behavior with high use of effective contraception, despite the utilization of emergency contraception by nearly one third of the students.

Keywords: Contraception, sexual behavior, dyspareunia, sexual transmitted diseases.

INTRODUCTION

Sexual behavior in women influences the risk of cervical cancer, infection with STDs, unwanted pregnancy and voluntary abortion. A survey made to United States of America (USA) high school students in 2011 [1] revealed that 40% did not use a condom the last time they had sex, 77% did not use any type of contraceptive method regularly and 15% had sex with more than four people during their life. According to Centers for Disease Control (CDC) reports, an estimated 8300 young people aged 13-24 years in the USA had Human Immu-
nodeficiency Virus (HIV) infection in 2009 [2] and nearly half of the 19 million new STDs each year are within this age group.

Adequate sexual education programs with emphasis on contraception and on the avoidance of risk behaviors play a fundamental role in the prevention of these conditions. The knowledge of sexual and contraceptive habits in a population allows the timely design and effective placement of prevention strategies and allocation of resources to treat the complications that arise from risk behaviors. However, there are few studies under this subject in Portugal. This study was designed in order to better understand the sexual behavior and contraceptive habits of Portuguese medical students.

MATERIALS AND METHODS

An anonymous voluntary questionnaire was handed to female medical students of the University of Coimbra, to collect gynecological and obstetrical history information. On the day of the delivery of the questionnaires there was a brief informative session about the aim of the study with clarification of eventual doubts. Pain was recorded using the World Health Organization (WHO) numeric scale. In order to avoid false answers to sensitive questions, the questionnaires were distributed in an individual envelope that was sealed by the student after answering.

All answers were recorded in a database and statistical analysis was made using IBM SPSS version 20.

RESULTS

A total of 670 questionnaires were collected. The sample was composed by 14.7% first grade, 16.9% 2nd grade; 24.3% 3rd grade; 16.6% 4th grade; 13.9% 5th grade and 13.6% 6th grade students. The average age was 21.4 years (18-35). Race was Caucasian in 98.8%.

Regarding sexual orientation (n=669), 97.8% reported being heterosexual, 0.7% homosexual and 1.5% bisexual. Twenty six percent reported being virgins. The age of first intercourse was 17.7 years (14-29), and the average number of partners was 2.1.

Concerning frequency of intercourse (n=479), 50.7% reported less than 1 per week, 44.5% reported more than twice a week and 4.8% reported daily activity. Dyspareunia was reported by 27% of the students, with an average pain score of 1.7.

Post-coital bleeding was reported in 18%.
Only 1% of the students reported history of previous pregnancy, unwanted in 0.4% and subject to voluntary interruption. Contraception was used by 79% of students, and most used a combination of methods. Globally 73.4% used condoms, 72.5% the withdrawal method, 72.2% oral anticonceptives, 2.24% vaginal ring, and 0.15% used contraceptive implants and transdermic systems. About 5% of students reported not having used contraception on the first sexual intercourse and 34.5% reported having used emergency contraception, with an average of 1.5 usages (1-5).

The vaccination rate for human papilloma virus (HPV) was 54%.

CONCLUSION

This study revealed that medical students represent a low risk group in what is concerned with sexual habits and contraception, reflecting the educational profile and medical culture of these women.

The studied population presented a high use of effective contraception, resulting in a low rate of unwanted pregnancies. Nevertheless, emergency contraception was used by one third of the individuals. The HPV vaccination rate in this population is low but, since the reported rate of unprotected sexual intercourse is also low, the cervical cancer risk was considered minimal. Sexual education strategies should focus on promoting stable, effective contraception to lower emergency methods usage.

REFERENCES

Compliance with the Dosing Scheme among 8416 Women Using Oral Contraceptives Designed for Non-Stop Dosage in Daily Clinical Practice in Poland

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SUMMARY

Patient adherence to oral contraceptives regimen influences the safety and efficacy of this family planning method, and therefore understanding of non-compliant behaviors is essential. To evaluate patients compliance with the dosing scheme we conducted cross-sectional, non-interventional, study in 8416 women on oral contraceptives, used in the non-stop regimen (28 pills for 28 days). 450 investigators reported women’s demographic and socio-economic characteristics and dosage errors in the period of three consecutive cycles. The observation included 24788 menstrual cycles (1901 women/years). The descriptive statistics was prepared. The relations between selected factors and dosage errors were assessed using the single factor ANOVA test and the non-parametric ANOVA Kruskal-Wallis test. In total, 1265 (15.5%) women delayed or/and missed 2093 Pills during 3 cycles. The respective numbers of women with dosage errors were 688 (8.2%), 528 (6.4%), 388 (4.8%) and corresponding numbers of Pills were 979, 634, 480, in the first, second and third cycle. The significant increase in the number of dosage errors were observed depending on education level, place of residence and working status. It was also demonstrated that the
number of dosage errors decreased over time, which can suggest the learning effect. The observed relations between dosage errors and particular socio-economic factors may have practical implications (e.g. identification of non-compliance groups and a need for a better medical consulting).

**Keywords:** regimen, dosage error, dosage mistake.

**INTRODUCTION**

In the development of the Pill we observe two opposite trends. The first one, aiming to increase safety, is achieved by reduction of estrogens activity/dose, the second one, aiming to increase contraceptive efficacy, is achieved by shortening the hormone free interval.

But the patient adherence to treatment remains a basic element which impacts pharmacotherapeutic effectiveness. As far as oral contraceptives are concerned, it influences the safety and efficacy of this family planning method. The dosage errors may lead to spotting/bleeding and lack of contraceptive efficacy [1, 2] and prompt requests for medical advice [3]. The patient’s noncompliant behavior contributes to vast difference between perfect and typical use of the Pill – the respective percentage of unintended pregnancies in the first year of use is 0.3 and 9.0 [4]. The potential to improve compliance is traditionally seen in adequate counseling [1, 5, 6].

We perform this study to better understand the noncompliant behavior among Polish women using oral contraceptives in clinical praxis.

**MATERIAL AND METHODS**

The cross-sectional, non-interventional, study was conducted in 8416 women on oral contraceptives used in the non-stop regimen (28 pills for 28 days) in 450 centers. Investigators reported women’s demographic and socio-economic characteristics, and dosage errors in the period of three consecutive cycles. The observation included 24788 menstrual cycles (1901 women/years). The descriptive statistics was prepared. The relations between selected factors and dosage errors were assessed using the single factor ANOVA test and the non-parametric ANOVA Kruskal-Wallis test.

**RESULTS**

The mean age of the women was 28,4 (median 27) years. 49% of women
were married, 50% reported previous pregnancy, 92% were on combined oral contraceptives and 42% took the first hormonal contraceptives at the study start. In total, 1265 (15.5%) women delayed or/and missed 2093 pills during 3 cycles. The respective numbers of women with dosage errors were 688 (8.2%), 528 (6.4%), 388 (4.8%) and corresponding numbers of pills were 979, 634, 480, in the first, second and third cycle. The significant differences in the number of dosage errors were observed depending on education level, place of residence and working status (Table 1).

\[\text{Tab. 1. Socio-economic factors of significant relation to dosage errors.}\]

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>%</th>
<th>Dosage errors (mean)</th>
<th>P</th>
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<tr>
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<td>434</td>
<td>5.2</td>
<td>0.27</td>
<td>0.00714</td>
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<td>Secondary</td>
<td>3779</td>
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<td>30.7</td>
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<td>4043</td>
<td>50.0</td>
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</table>

It was observed that the higher level of education was related to the lower average number of errors (Fig. 1), location in the town with less than 50 000 inhabitants and in the big city was related to higher average number of errors (Fig. 2), similarly to part time job (Fig. 3).

The following factors were NOT significantly related to the number of dosage errors: marital status, previous pregnancy, children under 2 years of age, user status, experience status, pill choice and pill intake time (Table 2).

With regard to safety, 136 adverse events were reported, including 127 adverse drug reactions (ADR), 32 serious ADR (vaginal bleeding all but one case) and in 23 women contraceptives were permanently discontinued.
Fig. 1. Pill errors and education level.

Fig. 2. Pill errors and place of residence.
Fig. 3. Pill errors and working status.

Tab. 2. Socio-economic factors of NO relation to dosage errors.

<table>
<thead>
<tr>
<th>Variable</th>
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<td>59,4</td>
<td>0,25</td>
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POP – Progestogen Only Pill
COC – Combined Oral Contraceptive
CONCLUSIONS

The study describes the relations between the selected characteristics of Polish women using oral contraceptives taken for 28 days of the cycle and dosage errors. It also demonstrated that the number of dosage errors decreased over time, which can suggest the learning effect. The observed relations between dosage errors and particular socio-economic factors may have practical implications (e.g. identification of non-compliance groups and a need for a better medical consulting).

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REFERENCES

Carcinoid of the Appendix During Caesarean Section

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ABSTRACT

The carcinoid tumour of the appendix is one of the most common tumours of the appendix, but one of the rarest anatomic locations of carcinoids. The appendix carcinoid tumour has a low aggressive profile and rarely is associated in about 10% with a carcinoid syndrome, which includes diarrhoea, abdominal cramps, malabsorption and flushing. This described as appendiceal lesion and is most frequently discovered incidentally in the removed organ. The diagnosis of appendiceal carcinoid is difficult when is combined with pregnancy because there are no particular signs before surgical intervention and in some cases the clinical symptoms are similar to pregnancy. General the diagnosis of appendix diseases is considered more difficult in pregnant than in non pregnant women. We present a case of a 32-year old female patient who was found to have a carcinoid tumour of the appendix found incidentally during a secondary caesarean section. The tumour was discovered as a result of the routine exploration of the whole abdomen. After detail investigation, which proved no evidence of appendiceal disease spread, the patient was treated by simple appendectomy and peripheral lymph nodes sampling. No postoperative complications were observed.

Keywords: pregnancy, caesarean section, appendix carcinoid.
INTRODUCTION

Carcinoid tumours are the most common tumours of the appendix, while they are ileum’s uncommon neoplasms in the diffuse peripheral endocrine system [1]. They are derived from cells in the wall of various organs like: lungs, mediastinum, thymus, liver, pancreas, bronchi, appendix, ileum, ovaries, prostate and kidneys [2]. Most of them usually are grown slowly and often they are not associated with any other symptoms [3]. We present a case of appendix tumour, which was an incidental finding during caesarean section.

CASE REPORT

A 32-year-old female II Gravida, II Para, was presented with amniotic sac membrane rupture in the 40+th week of pregnancy. Her past medical history was unremarkable, but she reported recurrent three episodes of appendicitis with right upper quadrant abdominal pain, intolerance to fatty food and diarrhoea for the last two years. She was given the diagnosis of irritable bowel syndrome and had been advised for her diet by her internist. In the gynecological pregnancy ultrasound the appendix was not visualized. The patient had undergone caesarean section according to Misgav-Ladach due to obstructed labour depending on cephalo pelvic disproportion. She has delivered a male fetus of 4650 g with an APGAR score 9/10/10, height 59,0 cm, head conference 40 cm and ph 7,28 BE + 1.8. No gross abnormality was detected in the examination. Before the closure, the usual exploration of the abdomen revealed an edematosus, firm and enlarged distal appendix resembling an chestnut in shape. During the operation, appendix was visualized with hart consistence, no moving capability highly suspicious for infection and with hart bowel adhesions. No grossly palpable lymph node was observed. With the suspicion of appendicitis an appendectomy during the caesarean section was performed. The specimen was examinated in frozen section and the histopathological examination during the surgery revealed a appendix carcinoid tumour. Macroscopically in the base of appendix a tumour with yellow appearance was found. The patient during the caesarean section underwent combined procedure of simple appendectomy and peripheral lymph nodes sampling. Appendectomy and lymph nodes sampling added 20 minutes to the operation time and extended the hospital stay by about one day.

Macroscopically the appendix was 4 cm in length and 1 cm in width in the distal part and associated with a small mass of 1.0 cm diameter tumour and hyperaemia. The appendix was removed and the final histological examination revealed the findings of the frozen section examination and demonstrated a tumour occurred in the borders of distal third to base of the appendix, with the
typical histologic signs of a carcinoid tumour. Histopathological examination confirmed insular and alveolar structures, formed by round ovalar cells with round central nuclei. The mucosa, submucosa were all infiltrated by tumoral structures which has a solid growth pattern extended to muscularis propria without invasion to the serosa.

The surgical margins were free of tumor, with no mesoappendix and vessel invasion and well differentiated pT2 pNxM R0LoVoG1. No residual tumour was observed. Immunohistochemistry of tumour cells showed strong granular cytoplasmic positivity for chromogranin A and synaptophysin, protein S-100, positive to neuron specific enolase (NSE), Leu-7 antigen (prediluted CD-57), CEA and negativity for epithelial membrane antigen, ki-67 profile and cytokeratins 7 and 20 confirming the diagnosis of carcinoid tumour. No microscopic traces of tumor were found between the six (6) removed pericecal lymph nodes. The macroscopically finding of tumor – hyperemia – could be explained due to serotonin-induced vasodilatation of the vascular bed in the appendix base. The postoperative course was uneventful, no wound infections or serious morbidity were observed. The neuroendocrine markers like as Serotonin 190 ng/ml (normal values 40-200 ng/ml), chromogranin A 48 pg/ml (normal values 40-100 pg/ml and its metabolite the urinary 5-hydroxy-indoleacetic acid (5-HIAA) 6.3 mg/ml (normal values 2-9) were normal by the primary surgical intervention. Three, six and twelve months later the serum markers were still normal and the 111 Indium Octeotide scan, body osteoscan, which performed 6 months postoperatively was negative, consequently indicating a favorable prognosis. The patient remained asymptomatic at follow up visit.

**DISCUSSION**

The histopathological distribution of appendiceal tumours include: very aggressive adenocarcinomas, papillary mucinous cystadenocarcinoma and benign tumours which enclose the appendix carcinoide or neuroendocrine tumours [4, 5]. Despite of the relatively benign nature of the carcinoid tumours (giant cells, anaplasia, rarity of mitoses), however there are appendiceal malignant tumours in about two-thirds of the malignancy category which histological confirmed as carcinoid. Most of the appendicial carcinoids are diagnosed in adults female and reaches a peak incidence in middle age, the median age is 29.8 years [6]. Appendiceal carcinoids are histopathological confirmed in 0.16-up to 0.7% of all appendectomy specimens, account for 1.8% of all intestinal tumours, for 85% of appendicial tumours, occurring in 1.08-1.3% of the excised specimens [7, 8, 9, 10]. The simultaneous presence of this tumour during the pregnancy is much less common [11]. A review of the literature reveals 20 cases of carcinoid
of the appendix during pregnancy in the last 67 years [12, 13]. According to appendix anatomy, 75% of the carcinoids are located in the apex, 20% in the middle part, and 5% in the base of the appendix as in our case [14]. The best prognosis have the apex appendix carcinoids. There are controversies about the appropriate management for the observation of carcinoid appendix patients. The main factor of the prognosis in appendiceal carcinoids is the size with a cut-off diameter value of 2 cm and no macroscopically metastatic disease, while other authors doubt this finding [15, 16]. They maintain that there is possibility of coexistence of tumour size less than 2 cm and metastases to regional lymph nodes. According to some authors lymphatic invasion is a difficult histologic criterion and the tumour extension in mesoappendix could be a valuable index of regional lymph node spread [17]. Our case was treated with appendectomy and lymph node sampling. The relationship between appendiceal carcinoid and pregnancy has not satisfactory elucidated. In the few published cases was recommended depending of pregnancy age simple appendectomy and in histological confirmation of aggressive type as second surgical step the hemicolectomy [18]. No fetal malformations were reported [19].

Generally the appendiceal carcinoid is not malignant. The follow up included serum markers, osteoscan performed 3 months postoperatively then every 6 months for 1 up to 3 years. These follow up examinations are very useful for the good prognosis of the described tumour.

Appendectomy is not recommended generally to be performed during a caesarean section, if there are not macroscopically suspicious signs. However all appendices should be inspected routinely in caesarean section and those found to be diseased must be removed [20].

In cases of carcinoid suspicion should be mandatory the remove of appendix and the explore of the whole abdomen for suspicious lymph nodes after histopathological examination in frozen section of the appendix.

REFERENCES


Age at Menarche and Menstrual Cycle Attitudes in a Population of Medical School Girls


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ABSTRACT

Objective: menarche is one of the most important biological signals in the life of a woman. The first cycles tend to be anovulatory and vary widely in length. Menarche occurs between the ages of 9 and 16 years in most girls in developed countries. The aim of this study was to examine the association between the menarcheal age and the menstrual cycle abnormalities.

Material and methods: in this study medical students of Alexandroupolis, aged 18-22 years, were screened by completing a questionnaire concerning their menarcheal and menstrual attitudes experiences. The results of detail endocrinologic workup were appropriate. A transvaginal sonographic examination, accompanied if necessary by a transabdominal examination was performed on all participants.

Results: the average age of menarche was 11.4 +/- 1.5 years. The participants were enrolled in two groups: Group A = 45 with early menarche < 14 years and Group B = 58 with late menarche > 15 years. All study participants completed the questionnaires according to current menstrual attitudes. The participants of the group B were reported more menstrual cycle abnormalities, especially oligomenorrhoea and we found about 29% PCOS (Polycystic Ovary syndrome) frequency.

Conclusion: the diagnosis and management of menstrual disorders in young girls is a particular challenge. A detailed and confidential medical and psycho-
social history are necessary, contributing to investigation and finding of cycle abnormalities.

**Key words:** Menarche, Menstrual cycles, Teenagers medical school, PCOS.

### INTRODUCTION

Adolescence is associated with menstrual disorders which included: polymenorrhea, oligomenorrhea and dysmenorrhea [1]. Menstrual abnormalities are more common among younger girls, becoming less frequent as they grow older, 3-5 years after menarche and depending on the maturation of the hypothalamus, pituitary gland and ovary and their interactions [2, 3]. The menstrual attitudes in teenagers according to literature, in most cases of the third year after menarche have a timing interval between bleeding periods in the range of 21-34 days [4, 5]. The prevalence of menstrual irregularities is reported approximately 22.7% [6]. During the first years of menstruation it is not rare for a girl to present with an irregular menstrual pattern. Menstrual disturbances are quite common during this phase of life and are not only associated with occasional deviations from normal endocrine gynaecological functional axis but with various reasons which include the following factors: psychological or physical stress, polycystic ovary, endometriosis, hypogonadism or cancer [6, 7, 8]. Menstrual problems in teenagers can depend on pathological organic reasons or have temporary causes and lack of suitable investigation and treatment, resulting in chronic condition of these problems and more difficulties with the treatment at a later date. Menarche is a significant event in the course of puberty. In this study, we investigated the possible relationship between the menarcheal age and the menstrual cycle abnormalities.

### MATERIAL AND METHODS

We studied 103 healthy, medical school girls, aged 18-22. They were recruited from Paedagogic Academy and Medicine school in Alexandroupolis. All participants have received and completed a questionnaire about menstrual attitudes from their menarche during the time from October 2003 until October 2006. The study respondents were agreed to participate in the follow up study for the next six months after the first doctor visiting. Excludable criteria were: premature pubarche defined as the appearance of pubis hair in girls younger than 9 years, use of oral contraceptive, administration of any medication, presence of chronic conditions such as genetic syndrome, liver, cardiac and renal
disease. Irregular menstrual cycles are: an average cycle length between 22 and 41 days or two or more cycles with a length of 22 or 41 days during the past year or oligomenorrhea is an average cycle length between 42 and 180 days. Transvaginal sonography was performed between cycle day 9 and 12 to determine the follicle number and revealed more than 10 follicles of 2-8 mm in diameter. The following parameters were analyzed: age at time of menarche and kind of menstrual cycle abnormalities. The study participants provided informed consent and gave their assent before entering the study.

RESULTS

The age at menarche ranged from 9 to 16 years. The average age of menarche was 11.4 +/- 1.5 years. The participants, 103 in total were enrolled in two groups: Group A = 45 with early menarche ≤ 14 years and Group B = 58 with late menarche ≥ 15 years. All study participants completed the questionnaires according to current menstrual attitudes. The participants of the group B were reported more irregular menstrual cycles, in the most cases oligomenorrhea, cycle length more than 35 days. Most girls with oligomenorrhea had a cycle duration of 35-70 days and 4.6% reported periods more than 6 months. We observed that about 29% (Subgroup C=17) of the participants from this group B fulfilled the Rotterdam criteria for PCOS. The criteria for polycystic ovaries (PCOS) as defined by the 2003 Rotterdam consensus are based on the follicle number and ovarian volume, which decrease with age. In this subgroup C hormone levels (FSH, LH, DHEAS) were measured on day 6 or 7 after menses.

Tab. 1. Laboratory findings of participants Group C.

<table>
<thead>
<tr>
<th>Hormones</th>
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<tr>
<td>LH</td>
<td>&gt; 16 IU/L in 7/17 cases (41.17%)</td>
</tr>
<tr>
<td>LH/FSH ratio</td>
<td>&gt; 2.5 in 12 /17 cases (70,58%)</td>
</tr>
<tr>
<td>DHEAS</td>
<td>&gt; 8.9 µmol/l in 15/17 cases (88,23%)</td>
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According to our results was found that elevated LH levels were associated with high androgen levels and the prevalence of PCOS in our population was 29%.

In the participants of Group A were confirmed no serious menstrual cycle abnormalities and none case was observed suspicious for PCOS. If the menar-
Menstrual cycle anomalies are the main gynaecological problems amongst teenagers and young women [9]. According to Slap menstrual irregularities are a leading reason for visits to gynecologist approximately 75% in adolescent females [10]. Body weight and nutrition pay an important role in pubertal development and for the occurrence of menarche is required a critical body fat mass [11]. In the developed world the age of menarche has gradually fallen depending on improving nutrition and increasing obesity [11]. Due to the confirmation based on literature research ports that a certain percent of young girls report menarche before their 12th birthday and should be provide adequate health information using age-appropriate education material in the primary school [11, 12]. It is of great meaning to learn the relationship of teenagers obesity and age of menarche. Wiksten-Almström et al reported, that menstrual cycle abnormalities are often the result of malfunction of the hypothalamic pituitary ovarian axis. And in 58% of the cases the analysed young women suffered from a secondary amenorrhoea [13].

According to Quint and Smith menstrual problems in adolescence are occurred commonly 2-5 years after menarche depending on the slow maturation of hypothalamus pituitary ovarian axis [14].

In our study in participants with a menarche at the age of ≤ 14 years were administrated hormones, however fewer 15% than in the group with a menarche at the age ≥ 15 years. In the last group hyperandrogenemia and polycystic ovary syndrome are responsible for the oligomenorrhoea and treatment with hormones in 90% of the cases was necessary.

This retrospective analysis was intended to describe the association between the age of menarche and the development of menstrual cycle abnormalities in young medical school girls. All participants in this study after carefully gynaecological examination underwent in cases of suspicion for a hormonal dysfunction in a hormonal analysis. Hormonal problems like hyperandrogenemia have often been the underlying reason for the development of menstrual cycle abnormalities [15].

General adolescents seem reluctant to seek medical help for the menstrual cycle disorders [16]. Further studies should be performed to determine the reason for these common problems in this young population and newer strategies need to be employed.
REFERENCES

5. Fraser IS, Critchley HO, Munro MG, Broder M. Can we achieve international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding? Hum Reprod. 2007 Mar; 22(3):635-43. Epub 2007 Jan 4.
Group A Streptococcal Toxic Shock Syndrome After Uterine Instrumentation

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SUMMARY

A case report of intrauterine group A streptococcal (GAS) infection following an endometrial pipelle biopsy complicated by toxic shock syndrome (TSS).

Our aim is to elicit the awareness of the possibility of acquiring GAS as a cause of Endometritis that can be complicated by toxic shock syndrome. GAS infection is a possible cause of endometritis with the risk of invasive disease that can lead TSS, which carries a high mortality.

A case report of a 65 years old woman who developed isolated GAS following endometrial pipelle biopsy for postmenopausal bleeding (PMB). The patient developed TSS that warranted admission to the ITU for management of toxemia and correction of the electrolyte imbalance.

A literature review of the reported cases of GAS pelvic infection concluded that invasive disease could occur in adult women with a rapidly progressive course with high mortality rates.

Keywords: Group A strep. Infections, pelvic infections, post uterine instrumentation infection.

CASE

A 65 years old patient developed progressive abdominal pain and backaches 24 hours after a pipelle biopsy for postmenopausal bleeding. Four days later, the
patient complained of vaginal bleeding, discharge and shoulder pains. She presented to A&E systemically unwell with a 38-degree pyrexia, hypotension, tachycardia and tachypnea.

Generalized abdominal distension and tenderness with absent bowel sounds was noted on examination.

The patient was resuscitated and had a sepsis work up including high vaginal swabs (HVS). Acute surgical pathology was ruled out with chest/abdomen X-rays and abdominal/pelvic CT scan. Following a review of the patient, the Gynaecology team suggested the possibility of a pelvic infection with septicemia secondary to the pipelle biopsy.

The patient was admitted to ICU for TSS management. She was treated with IV Tazocin and clindamycin. The electrolytes imbalance and acidosis were corrected.

The patient was switched to oral antibiotics after two days and made a satisfactory recovery in three days. HVS revealed a GAS infection. An MRI for persistent abdominal and backaches suggested a fundal intrauterine querying a mass or a uterine malignancy. The Blood culture had negative result for any septicemia.

On the MDT discussion with microbiology team concluded a GAS infection with toxemia.

The patient was discharged on oral antibiotics. A diagnostic Hysteroscopy revealed a benign looking polyp and otherwise normal cavity. The histology revealed a chronic endometritis.

**DISCUSSION**

Group A streptococci (GAS) are bacteria flora in the throat and on the skin. GAS spread is mainly by direct contact with nose and throat discharges of an infected individual or with infected skin lesions. The majorities of GAS infections are mild and present as strep throat or impetigo. However, they can cause severe and even life threatening diseases such as necrotizing fasciitis and streptococcal TSS (a rapidly progressing infection causing shock and multi-organ failure). TSS may presents with fever, dizziness, confusion, hypotension, rash and abdominal pain. Approximately 20 percent of patients with necrotizing fasciitis and 60 percent with TSS die\(^1\).

TSS is due to exotoxins produced by GAS. Exotoxins are proteins, which have two polypeptide components one is responsible for binding the protein to the host cell and one is responsible for the toxic effect. In our case there was no septicemia identified and clinical shock state was a result of exotoxin pro-
duction. GAS exotoxins can cause shock and organ damage as well as enzyme mediated tissue destruction. Patients with chronic illnesses like cancer, diabetes, kidney dialysis, and who use medications such as steroids are at higher risk.

Group A streptococcal Vulvo-vaginitis was reported in prepubertal girls with a prevalence of between 11% and 20%. In adult women, a vaginal carrier state was described, but symptomatic vulvovaginitis is rare. It has been reported that out of 1,010 cases of adult women with recurrent vaginal discharge, 49 (4.9%) cases had GAS in the culture.

GAS Pelvic infection could be complicated by toxic shock syndrome. A case of an adult endometritis 5 days following IUCD presented with nausea, vomiting, and diarrhea as well as diffusely tender and rigid abdomen with free fluid on bedside sonogram. The IUCD was removed. The cultures (IUCD and blood) grew out GAS.

Another case described a 43-year-old presenting with fevers, vaginal bleeding and abdominal pain after an office endometrial biopsy. She was found to be in septic shock, abdominal tenderness and erythematous rash. She was managed with intravenous antibiotics and fluid resuscitation. She underwent a total abdominal hysterectomy and bilateral salpingo-oophorectomy, with findings of acute pelvic infection. Blood cultures grew out GAS.

Primary peritonitis has been reported to be caused by GAS with asymptomatic genital tract colonization as a portal of entry. Two patients reported with signs and symptoms of a toxic shock-like syndrome, including rapid onset of fever and shock, skin rash, desquamation of palms and soles, and multisystem involvement with vomiting, diarrhoea, myalgia, renal failure, and severe disorientation without focal neurological deficits. Peritoneal fluid and blood culture revealed GAS.

Oral sex has been reported to be a risk factor for invasive GAS disease. GAS peritonitis and TSS in an otherwise healthy woman was found to be secondary to IUCD as a portal entry and oral sex with an asymptomatic GAS carrier in his throat.

Penicillin is the treatment of choice for treating mild disease. For penicillin-allergic patients with mild illness, erythromycin can be used. Clindamycin can be added to the treatment in cases of necrotizing fasciitis or TSS. In serious infection a combination of clindamycin and ceftriaxone or meropenemum is recommended.

**CONCLUSION**

Despite the majority of pelvic infections is not GAS however raising the suspicion of GAS as a possible source of infection will lead to early recognition.
and treatment of the patients prior to the development of established TSS. Subsequently, the risk of poor outcome of invasive GAS disease will be markedly reduced.

REFERENCES

Proliferative Activity of Various Cells in Ovarian Endometriosis: What Cell Population Is Leading?

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SUMMARY

Histopathological and immunocytochemical studies of ovarian samples obtained through laparoscopic resection of damaged ovaries in 17 women (29.18 ± 1.29 years old) were carried out. Ovarian endometrioid heterotopias were detected simultaneously in two morphological variants: endometrial glands with their subsequent cystic transformation and cytogenetic stromal overgrowth along the vessels with active neoangiogenesis. Endometrial stromal cells have the highest proliferative activity compared to epithelial cells lining endometrial cysts and endothelial cells of blood vessels. Endometrial stromal cell proliferation resulting in formation of endometrial glands/cysts, neoangiogenesis, and stromal cell accumulations (periglandular or perivascular) induce significant remodeling expressed in ovarian fibrosis which is accompanied by inhibition of folliculogenesis and development of primary infertility.

Keywords: ovarian endometriosis, cell proliferation.

INTRODUCTION

Ovarian endometriosis is a disease that more often affects women of childbearing age and in some cases results in infertility. Currently the nature of this disease is more controversial. Some authors suppose that endometriosis is asso-
associated with increased risk of ovarian cancer (Mandai M. et al., 2009; Munksggaard P.S., Blaakaer J., 2012). This study was designed to evaluate the role of proliferative activity of various ovary cells in promoting ovarian endometriosis.

**MATERIAL AND METHODS**

Ovarian samples were obtained through laparoscopic resection of damaged ovaries in 17 women (29.18 ± 1.29 years old). Histopathological and immunocytochemical studies of ovarian endometriosis and assessment of proliferative activity of different ovarian cell populations (epitheliocytes of endometrioid glands, stromal cells and smooth muscle cells of blood vessels) were carried out using the proliferation marker Ki-67 (Spring Bioscience, USA).

**RESULTS**

In all patients the unilateral (76.5%) or bilateral (23.5%) endometrioid ovarian cysts were revealed. Severe pelvic pain was significantly associated with cysts ≥ 4 cm. Ovarian endometriosis was characterized by highly heterogeneous morphological structure. We observed both endometrioid glands with secretory epithelium and endometrioid cysts with endothelial-like (flattening) epithelium, as well as the bands of endometrial stromal cells infiltrating cortical and medullary zones along blood vessels. An important characteristic of ovarian endometrioid heterotopias was a wide range of metaplastic transformation of the epithelium in endometrioid cysts. In most of the endometrial cyst epithelium was flattened (endothelial-like) or cubic, but in all cysts foci of ciliary or mucoid metaplasia were observed. Metaplastic transformation of the epithelium is considered as an adaptive response to pathogenic conditions. These transformations are not associated with cell transdifferentiation but represent a coordinated nuclear-cytoplasmic reorganization in response to changes in functional activity and hormonal regulation (Nicolae A. et al., 2011). In the areas of significant stretching of cyst walls because of their filling by blood we observed endothelial-like metaplasia of the epithelium, but in the areas with folded cyst structure epithelial cells looked like mucoid or ciliary cells.

Endometrioid heterotopias caused pronounced remodeling of cortical and medullary zones. In the ovary cortex, inhibition of folliculogenesis with the destruction of oocytes and fibrosis development was observed. The number of primordial (primary) follicles (40.04 ± 3.24 µm in diameter) was small in all cases (1 to 3 in the field of view). In the ovaries of women with primary infertility, destruction of oocytes in primary follicles was observed. In the ova-
ries of women with normal reproductive function, few secondary follicles (124.41 ± 4.26 µm in diameter), yellow and white bodies were detected. In the yellow bodies near the ovary capsule foci of hemorrhage were observed. The destruction of oocytes in ovarian endometriosis may be due to changes in their local microenvironment, including modified ratios of pro- and antiapoptotic factors and the accumulation of cytokines in the follicular fluid inducing cytotoxic effect (Ekimova M.V. et al., 2010).

The most significant changes were recorded in the stroma of the medulla. In the medulla, proliferation of endometrial stromal cells was accompanied by neoangiogenesis, significant fibrosis. Proliferation of endometrial stromal cells led to significant changes in the architectonics of the ovaries accompanied by neoangiogenesis with the formation of sinusoidal blood vessels. Arterial smooth muscle cell hyperplasia and hypertrophy were observed along the vessels and in arterial muscle layer. Neoangiogenesis was seen in areas of hyperplasia and hypertrophy of the smooth muscle cells associated with cytogenetic stromal invasion. A simple explanation for that might be found in enhanced mitotic activity of endometrial stromal cells migrated to the ovaries with blood. The newly-formed vessels were oriented along the smooth muscle cells and were represented mainly by endothelial lining. In all cases, fresh hemorrhages were found. In two cases, there were small predominantly perivascular mononuclear infiltrates and neutrophils in the blood vessels.

Immunocytochemical analysis (using Ki-67 marker) of the proliferative activity of different cells showed the most intensive label in endometrial stromal cells and in the endothelium of newly formed vessels. Ki-67-labeled stromal cells were located predominantly around endometrioid glands/cysts and along blood vessels. The highest index of labeled stromal cells was detected in the areas of pseudodecidualization (31.6 ± 4.9%) and around the newly formed blood vessels (9.3 ± 2.1%).

In the epithelial lining of endometrial cysts only single cells were labeled, in most cases the label was absent. Low labeling index of endometrioid epithelial cells in ovaries indicated the end of morphogenetic processes. Proliferative activity of stromal cells may reflect the progression of the morphogenetic processes and is likely to be a poor prognostic marker. Pronounced stromal cell invasions may be considered as a ‘malignant’ form of external endometriosis. In addition to the proliferative activity of endometrial stromal cells, we observed hyperplasia, hypertrophy and occasional Ki-67-labeling of smooth muscle cells in the ovarian medulla.

**CONCLUSIONS**

Thus, ovarian endometriosis is characterized by histological heterogeneity:
presence of endomertioid glands/cysts with metaplastic transformation of epithelial cells and massive endometrial stromal cell invasion. Endometrial stromal cells demonstrate the highest proliferative activity compared to the epithelial lining of endometrial glands/cysts and endothelial cells of blood vessels. We conclude that endometrial stromal cells are the main cellular population inducing formation of endometrial heterotopias and resulting in active neoangiogenesis and smooth muscle cell formation.

REFERENCES

An Experience on Non-Venereal Acute Genital Ulcers in Adolescence

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SUMMARY

Lipschutz Ulcer – or Ulcus Vulvae Acutum – encompasses acute genital ulcers in young women – often without previous sexual relationships – which tend to spontaneous recovery. Its incidence is low and diagnosis is made by exclusion or differential diagnosis, after ruling out all other possible causes (sexually transmitted diseases, autoimmune causes, traumas, etc.). This is a rare and underdiagnosed condition which cause remains unknown, although it has been associated with Epstein Barr Virus in recent studies. We report the case of a young girl suffering a sudden onset of painful ulcerated lesions on the vulva accompanied by low-grade fever.

Keywords: vulvar, Lipschütz, ulcus, vulvae, acutum.

INTRODUCTION

Non-venereal acute genital ulcers are uncommon in adolescence. These ge-
nital ulcers are sometimes accompanied by systemic symptoms requiring a differential diagnosis. We present the case of a 14 years old girl who came to the emergency service some 48 hours after a sudden development of painful ulcerated lesions on the vulva and low-grade fever. She had never had sexual relationships nor had ever drugged herself; neither had made use of topical medication for the vulvar region nor had suffered any vulvar trauma of late.

**MATERIAL AND METHODS**

The medical exploration showed three unilateral violaceous necrotic ulcers on the inner side of the labia minora – as in a «mirror» disposition. The ulcers presented irregular and necrotic edges surrounded by an erythematous halo and a background of fibrin, being painful to palpation (Fig.1). No palpable lymphadenopathy was noted. Her medical history had two remarkable issues: mouth ulcers in childhood (at age of three) and a suspected lumbosciatica – at the time under examination for about one year, as a result of a sport injury. A number of tests were performed to find the source of her lesions: blood count, biochemistry, urine culture, vulvar ulcers culture to test for bacteria and viruses, vaginal and endocervical cultures, multiple peripheral blood serologies and ulcers biopsy. Suspecting *Bechet’s disease* because of the canker sores in childhood and the lumbosciatic, HLA B27 and HLA B51 antigens tests were run. While waiting for results, a treatment based on local and systemic anti-inflammatories and topical and systemic antibiotic was administered.

![Fig. 1. Unilateral violaceous necrotic ulcers on the inner side of the inner libs.](image-url)
RESULTS

An objective and significant improvement was observed after 5 follow-up days -the persisting ulcerations showed reparative changes. Cultures tested negative and the analytical results seemed mostly normal. HLA B27 and HLA B51 antigens tested negative, as well as serology for hepatitis B, C, syphilis, HIV, Epstein Barr Virus, etc. However, serology for Cytomegalovirus (CMV) infection tested positive, and the vulvar biopsy seemed to show – yet not conclusively – evidence of an inflammatory process. Two weeks later, flu symptoms had disappeared and the ulcers showed significant improvement and re-epithelisation (Fig. 2). The clinical picture was completely resolved after three weeks from diagnosis without relapse (Fig. 3).

CONCLUSIONS

Lipschutz Ulcer diagnostic should come after ruling out other potential causes, particularly venereal – such as chancroid, syphilis, Herpes Simplex, Herpes zoster, etc. – as well as other non-infectious causes: physical and chemical traumas, systemic conditions like Behcet’s disease, adverse drug reactions, inflammatory bowel diseases, lymphoma, typhoid fever, paratyphoid fever, vulvar pemphigoid or idiopathic recurrent aphthous. Its etiopathogeny remains unknown. Lipschutz Ulcer has been associated with Epstein Barr Virus primoinfect-
tion – diagnosed by PCR lesions’ biopsy – as well as with Salmonella paratyphi. Similar lesions have been observed in HIV infected women, and it’s also been associated with Cytomegalovirus, which was detected in this young patient.

*Lipschutz Ulcer’s* most common clinical picture is one which starts by showing a febrile syndrome commonly accompanied by odynophagia, muscle pain, asthenia and, sometimes, adenopathy and headache. Shortly after, ulcers appear on inner lips and even outer lips. These ulcers might be several, deep and painful, while might or not be accompanied by inguinal lymphadenopathy. The process is self-limited and recovery comes spontaneously. Topical treatment to relieve local symptoms is recommended and antibiotic treatments are often administered. Fever disappears after few days from onset, and ulcers tend to heal in about 15 days, not leaving any consequences after three weeks approximately.

A most remarkable feature in this case was the necessary differential diagnosis to discard Behcet’s disease, which had to be suspected because of patient’s medical history. *Behcet’s disease* and any connection to infectious diseases were finally ruled out. CMV detection reinforced the possibility of this being a case of *Lipschutz Ulcer*.

REFERENCES

1. Lampert A, Assier-Bonnet H, Chevallier B, Clerici T, Saiag P. *Lipschutz’s genital ulceration:*


Large Splenic Cyst Misdiagnosed as an Ovarian Cyst

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SUMMARY

We are reporting a case of a 27 year old female with large splenic cyst which was misdiagnosed as a benign left sided ovarian cyst. The patient was booked for urgent laparoscopic ovarian cystectomy with Palmer’s point as a method of entry due to the size of the cyst. She underwent laparoscopy when the diagnosis of a mass of non-ovarian origin was made. Both ovaries and uterus were normal. Surgical team was involved but the nature of the mass could not be determined at the time. After the surgery the patient had CT Abdomen with contrast which showed malformation of abdominal viscera and malposition of the upper abdominal structures. There was a large cyst arising from inferior border of the spleen extending into the left side of the pelvis. Laparoscopy showed to be a safe diagnostic and operating option providing that the entry point is thoroughly considered.

Keywords: Ovary, spleen, laparoscopy, Palmer’s point of entry.

MATERIAL AND METHODS

A 27 year old married lady presented to our Gynaecology clinic with a history of abdominal pain and distention.
Her menstrual cycle was normal and regular. She had her first cervical smear taken 1 year ago and it was normal. There was no previous gynaecological history.

On examination the abdomen was uniformly distended with a cystic mass reaching 4 cm above the umbilicus. The patient was booked for an urgent pelvic and abdominal ultrasound scan and CA12-5 was taken.

The scan suggested a left sided ovarian cyst measuring 14x10x9 cm, possibly haemorrhagic. There were no septations or increased flow on Doppler. The uterus was anteverted, normal in size, with endometrial thickness of 9 mm. Right ovary appeared to be normal. The CA12-5 was reported as normal.

The patient was booked for an urgent laparoscopic ovarian cystectomy with the possibility of oophorectomy. During laparoscopy, Palmer’s point was used as an entry point in order to avoid the cyst that was extending above the umbilicus. On introduction of the laparoscope, the cyst looked purple in colour and was soft to touch (Fig. 1). It was quite uncommon for ovarian cysts which are usually tensed. The cyst was extending deep into the left side of the pelvis lying in close proximity to the left ovary. On closer examination both ovaries and uterus appeared to be normal (Fig. 2). The cyst was arising from a structure in

Fig. 1. Splenic cyst.
the middle of the upper abdomen. Surgical input was requested but the nature of the cystic structure could not be determined at that point. Peritoneal wash was taken and sent for cytology.

After the surgery the patient was booked for CT scan with contrast. The result showed congenital abnormality of the upper abdominal viscera with the spleen situated in the mid abdomen. The cyst was arising from its inferior border. The pancreatic body and tail appear to lie anterior to the stomach, the rest of the abdominal structures were normal.

The patient is currently waiting for elective surgery to be performed by the surgical team.

RESULTS

This case shows that all cases of large ovarian cyst should be investigated by either MRI or CT scan to confirm their nature and exclude malignancy potential. If this is not possible then avoiding the cyst on entry would be prudent.
CONCLUSIONS

Some abdominal structures or their malpositions are quite often misdiagnosed as ovarian tumours [2, 3]. However, there are only few cases of splenic tumours that were initially diagnosed as of ovarian origin described in the literature [1]. In this case the diagnosis was made based on ultrasonographic assessment.

During laparoscopy, if direct entry method would have been contemplated, this could have resulted in heavy bleeding. We recommend thorough radiological assessment for all cases of large ovarian masses.

REFERENCES

The Use of Intrauterine Endoceptive of Levonorgestrel-Releasing – Mirena® Bayer (MIR VE01-04/Jul 09. Reg. MS 1.0020.0087), As Adjuvant Treatment in Vulvar Hidradenitis Suppurativa (HS) – Case Report

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ABSTRACT

Vulvar hidradenitis suppurativa is a chronic and recurrent infection that affects women of childbearing age; manifests as multiple and recurrent skin abscesses. Major surgical excisions in vulva and perineum have been proposed without mitigating its recurrence. Etiological factors are unknown, but the literature suggests correlation with menstrual cycle and androgenic activity. We report a case where there was a reduction of relapses in patient with oligomenorrhea user of intrauterine device releasing levonorgestrel (Mirena® Bayer).

Keywords: sweat gland diseases; hidradenitis suppurativa; levonorgestrel endoceptive.

INTRODUCTION

HS is a chronic infection, manifesting as follicular occlusive lesion involving inguinal, perianal and vulvar skin (1). The literature suggests a relationship with peripheral androgen levels or hypersensitivity to androgens (2). Clinical course
is highly variable, and the average duration of a single lesion is seven days and the average injury is two per month, with a variable range of one per year, up to 30 per month. Etiology and pathogenesis are not fully understood. Several clinical observations sustains the hypothesis of HS compared with sex hormones (3). The hyperandrogenism has been suggested as a causal factor of HS in women (4, 5, 6) but the association between androgens and HS remains controversial.

MATERIAL AND METHODS

A 46 years-old woman complaining recurrent painful lesions in inguinal region. She had no episodes of HS during amenorrhea period stated by the endoceptive use.

RESULTS

HS is the occlusion of the apocrine glands due to a defect in their terminal differentiation, which prevents the detachment of the follicular epithelium (4, 7, 8, 9). Keratinocytes of acroinfundibulum express the key enzymes involved in the metabolism of androgens in situ (for in situ synthesis of androgens weak, its transformation into testosterone and its reduction to dihydrotestosterone – DHT) (10). Treatment include non-pharmacological measures, topical and systemic medications, and surgical procedures. A retrospective study shows16 of the 29 women treated with antiandrogenic drugs (55%) versus 6 of 23 women treated with oral antibiotics (26%) responded positively to treatment (11). Still others report that the increased levels of hormonal agents, such as androgens contribute to the development of HS (12).

CONCLUSION

By the present case, we observed less episodes of HS during the use of levonogestrel endoceptive. The hormonal effect is not understood. We propose use of levonogestrel endoceptive as adjuvant treatment, minimizing recurrences of HS.

REFERENCES

Efficiency of Application of Aromatase Inhibitors in Combined Treatment of Genital Endometriosis

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SUMMARY

Nowadays there is no regimen or scheme of genital endometriosis (GE) treatment that can guarantee absence of recurrence of the disease, which indicates the necessity of searching for new drugs for treatment of the disease. We found that the square of aromatase expression in endometriotic heterotopies is 34 times higher than in endometrium of healthy women, that is pathogenetic rationale for the use of aromatase inhibitors in the treatment of patients with endometriosis. We have found that patients with GE after surgical interventions on ovaries, have a significant reduction of ovarian reserve, which should also be considered when choosing a hormonal therapy. Performed combined treatment of 85 patients with GE (surgery followed by application of aromatase inhibitors, letrozol, combined with progestagen orgametril in continuous regimen) was effective for the relief of pain in 97.6% of patients and allowed to overcome infertility in 30.6% of women with severe endometriosis and repeated previous ineffective courses of hormonal therapy. We had determined that the use of aromatase inhibitors in combination with supplements of calcium and vitamin D3 does not lead to decrease of BMD. However, undoubtedly, application of aromatase inhibitors requires further in-depth study.

Keywords: genital endometriosis, aromatase, immunohistochemical study, reduced ovarian reserve, bone mineral density, aromatase inhibitors.
INTRODUCTION

Endometriosis is the disease, characterized by growth of endometrial tissue outside its normal localization. It is generally accepted that violations of hormonal and immune homeostasis are the most important pathogenetic factors of development and progression of this disease [1]. Modern research is focused on studying epigenetic aspects of endometriosis, metabolism of sex hormones, and molecular peculiarities of endometriotic heterotopias [3]. One of the priority directions is the study of aromatase activity in patients with endometriosis [2]. Aromatase is the enzyme responsible for conversion of androgens into estrogens. Nowadays none of therapeutic approaches can guarantee absence of disease recurrence [4]. This fact proves necessity of search for new groups of medications for endometriosis treatment [6].

OBJECTIVE

To study the efficacy of aromatase inhibitors in combination with progestagens from the group of 19-nortestosterone derivates, in combined treatment of patients with genital endometriosis (GE).

PATIENTS AND METHODS

Inclusion criteria for the study were: females from 18 to 45 years of age, presence of genital endometriosis, verified during laparoscopy and confirmed by the results of histological examination, chronic pelvic pain and/or infertility. The severity of endometriosis was estimated in scores based on R-AFS classification. Determination of serum levels of FSH and AMH was performed by ELISA. Evaluation of aromatase expression in endometriotic heterotopias and in eutopic endometrium of healthy women was conducted by immunohistochemical analysis with the use of mouse monoclonal antibodies to aromatase. Determination of bone mineral density (BMD) was performed by double-energy X-ray absorptiometry of lumbar spine, proximal femur and distal forearm regions.

RESULTS

Immunohistochemical study was performed in 45 patients with GE and in 9 women of comparison group, in whom gynecological pathology was excluded
during laparo- and hysteroscopy. Accordingly to score estimation of severity of GE, I stage was found in 15.6% of patients, II stage in 15.6% of women, III stage in 17.8% and IV stage in 51.1% of patients, respectively.

On the basis of performed immunohistochemical analysis it was shown that expression of aromatase in eutopic endometrium of healthy women was practically absent, the average square of expression was \(0.35 \pm 0.35\%\). In endometriotic heterotopies level of aromatase expression was increased in all the samples, the average square of expression was \(11.94 \pm 2.11\%\). Thus, the square of aromatase expression in endometriotic heterotopies reliably differed from the square of its expression in endometrium of healthy women (\(p < 0.01\)), significant differences in aromatase expression depending on the degree of severity of the disease were found.

The average brightness of aromatase expression in the foci of endometriosis was \(139.96 \pm 8.17\) units of brightness, as in the endometrium of patients of control group, it was \(134.10 \pm 16.93\) units of brightness. When evaluating the expression of aromatase in endometriotic heterotopies, optical density was \(0.2778 \pm 0.0274\) average units, though in the endometrium of patients of the control group it was \(0.2943 \pm 0.0616\) respectively. There were no significant differences in the average brightness and optical density of aromatase expression between patients with GE and patients of the control group. Thus, the most reliable marker is determination of the average size of aromatase expression. The obtained data on the reliability of the increase of expression of aromatase in endometriotic heterotopies served as pathogenetic rationale for the use of aromatase inhibitors in combined therapy of GE [5, 6].

After surgical treatment, 85 patients with GE aged from 22 to 43 years (mean age 32.85 \(\pm 0.56\) years) were appointed aromatase inhibitors in combination with progestagens from the group of 19-nortestosterone derivates. We prescribed letrozole, 2.5 mg, 1 tablet a day and lynestrenol, 5 mg, 1 tablet 2 times a day in a continuous regimen for a period of 4 to 6 (mostly) months. It is known that aromatase inhibitors are applied in combination with hormonal preparations with antagononadotropic effect, as severe deficit of aromatase leads to formation of functional ovarian cysts.

First stage of endometriosis according to R-AFS classification was revealed in 2.4% of patients, II stage – in 7.1%, III stage – in 27% and IV stage – in 63.5% of women. Endometrioid cysts were diagnosed in 77.7% of patients. In 61.2% of patients we revealed retrocervical endometrioid infiltrate from 1.5 to 5.5 cm in diameter. Extranational endometriosis was found in 13.0% of patients: 8.2% of patients had endometriosis of colon, 4.7% of patients – endometriosis of appendix, 5.9% – endometriosis of diaphragm, 2.4% of patients – endometriosis of umbilicus. Pelvic adhesive disease was found in 85.9% of patients. The prevalence of adhesive disease, evaluated in scores of R-AFS classification,
was estimated from 1 to 64 scores (average 43.4 ± 3.4 points). Obliteration of Douglas pouch was observed in 45.9% of patients (in 23.1% it was partial obliteration, of 76.9% it was total obliteration).

Adhesiolysis was carried in 86% of patients during the operation with subsequent application of adhesion barrier. Upon detection of endometriotic heterotopies, we performed their excision, which, in 36.0% of cases, was supplemented with coagulation of surface foci. Upon detection of ovarian cysts we performed cystectomy. When the diagnosis of retrocervical endometriotic infiltrate was stated, we performed its excision. In 4.7% of patients appendectomy was performed due to endometriosis of appendix. In 4.7% of patients with endometriosis of sigmoid colon, accompanied by a narrowing of the bowel lumen, pain on urination or intestinal bleeding, we performed device resection of rectosigmoid fragment.

On the basis of intraoperative examination, 74.1% of patients had a recurrence of the disease. It was noted that 48.0% of patients with recurrence of GE previously received GnRH agonists (from 1 to 3 courses, each for 3-6 months). Prior to therapy, chronic pelvic pain was observed in 68.2% of patients, dyspareunia in 32.9%, infertility in 72.9% of women.

Based on examination of serum levels of FSH and AMH during follicular phase of the cycle, preserved ovarian reserve was found only in 9 patients (10.6%), in 28 patients (32.9%) we revealed hypergonadotropic ovarian insufficiency, 56.4% of women of the main group had significant reduction of ovarian reserve. Mean value of AMH in patients with GE was 0.6 ± 0.1 ng/ml, the average level of AMH in healthy women with regular menstrual cycle was 3.8 ± 1.1 ng/ml. The average level of FSH in blood of patients with GE and hypergonadotropic ovarian failure was 41.6 ± 4.1 IU/ml, which was significantly higher than basal level of FSH in blood of healthy women (6.9 ± 0.5 IU/ml). Peculiarities of ovarian reserve in patients with GE confirm necessity of search for new groups of drugs for treatment of recurrence of the disease.

Taking into consideration possible negative impact of aromatase inhibitors on bone tissue, as well as the fact that 48.2% of patients had previous courses of GnRH agonist treatment which also reduces bone mineral density, all the patients were performed dual energy X-ray absorptiometry before the appointment of aromatase inhibitors. Based on the results of dual energy X-ray absorptiometry prior to application of aromatase inhibitors, in 48.2% of patients with GE indicators of BMD were within the range of normal values, in 40.1% of women osteopenic syndrome was diagnosed, in 8.2% of women we identified severe osteopenia of lumbar spine and forearm. In 3.5% of patients the reduction of BMD in the regions of lumbar spine and middle third of forearm was interpreted as osteoporosis. Reduction of BMD in proximal femur was not revealed. During application of aromatase inhibitors all the women were prescri-
bed supplements of calcium 500 mg with 200 IU of vitamin D3 and microelements (calcemin advance, 1 tablet 2 times per day) for prophylactics of reduction of BMD. Patients with osteoporosis and severe osteopenia received bisphosphonates (ibandronate, 150 mg 1 time per month orally or 3 mg once in 3 months intravenously) for the period from 4 to 6 months.

On the basis of the results of repeated DXA we determined that the use of aromatase inhibitors in combination with daily intake of calcium and vitamin D3 supplements does not lead to reduction of BMD. In the group of patients who received antiresorption therapy with ibandronic acid, positive dynamics (increase in BMD up to 2.9% ± 3.5%) was noted.

During therapy with aromatase inhibitors in combination with progestagens pain syndrome was absent in 97.6% of women. Though during previous courses of GnRH pain syndrome was observed in 18.7% of cases. None of the patients had signs of recurrence of GE, based on physical and laboratory examination, pelvic ultrasound examination. Control laparoscopy was performed in 5.9% of cases which confirmed regression of endometriotic foci. Among side effects the most common were: spotting (38.8%) and acne (15.3%). During treatment, 9.4% of patients noted hot flashes up to 5 times a day, in these patients we revealed hypergonadotropic ovarian failure prior to therapy. A slight weight gain had 22.4% of patients, on average 2.46 ± 0.56 kg. None of the above mentioned side effects was the reason for early termination of treatment.

After the completion of therapy course with aromatase inhibitors, 30.6% of patients with infertility, GE and repeated courses ineffective hormone therapy became pregnant. Pregnancy occurred spontaneously in 21.1% of women, after stimulation induction with the use of gonadotropins in 26.3% of patients. In IVF protocol with the use of own oocytes pregnancy occurred in 15.8%, with the use of donor oocytes in 36.8% of patients. 63% of women delivered healthy babies in time, other women are still pregnant. The duration of the observation period of patients with GE, who received therapy with aromatase inhibitors, ranged from 1 to 3.5 years.

**CONCLUSION**

Based on the results of immunohistochemical study we determined that endometriotic heterotopies are characterized by increased aromatase expression, the square of its expression in endometriotic foci is 34 times higher than in eutopic endometrium of healthy women. These data can be taken into consideration as a pathogenetic rationale for the use of aromatase inhibitors in patients with GE [2, 5]. On the basis of serum levels of FSH and AMH, we determined that after surgical interventions on ovaries in patients with GE significant reduc-
tion of ovarian reserve is noted, which should also be considered when choosing a group of drugs for hormonal therapy of these patients. It was shown that combined therapy of GE (surgery followed by use of aromatase inhibitors in combination with progestagens in continuous regimen) leads to elimination of pain syndrome in 97.6% of patients and allows to overcome infertility in 30.6% of women with severe endometriosis and previous ineffective courses of hormonal therapy. It was also noted that the use of aromatase inhibitors in combination with supplements of calcium and vitamin D3 doesn’t lead to decrease of BMD. However, the use of aromatase inhibitors, of course, requires further in-depth study.

REFERENCES

Is Lactobacillus Rhamnosus BMX 54 Vaginal Application a Good Strategy to Counteract Bacterial Vaginosis Recurrences?

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SUMMARY

Bacterial Vaginosis (BV), the most prevalent vaginal infection in reproductive-age women, results from the replacement of the lactobacilli dominated vaginal flora by a predominant anaerobic bacteria. Recurrent Bacterial Vaginosis, generally defined as 3 or more episodes of Bacterial Vaginosis per year, is a worldwide clinical emergency since the long term recurrences rate is up to 40% of women within 3 months after initiation antibiotic therapy and up to 50% of women after 6 months. Since BV is associated with many adverse clinical outcomes including pelvic inflammatory disease, unfavourable pregnancy outcomes and, recently HIV and since the CDC (Center for Diseases Control) recommended antibiotic therapy with metronidazole or clindamycin results in a high recurrences rate, there is a need to find some new therapeutic options for BV prevention.

We have collected the recent clinical data belonging to a specific probiotic (Lactobacillus rhamnosus BMX 54 – deposited with the CNCM under the Budapest Treaty, accession number CNCM I-4497) vaginal application to understand if a combined approach (CDC recommended antibiotic therapy followed by Lactobacillus rhamnosus BMX 54 long lasting vaginal application) might work better. Suprisingly, a sample of 475 women enrolled in controlled (363 patients) and uncontrolled (112 patients) clinical trials shown a significant improvement for the combined approach versus antibiotic alone in the long time management.
Keywords: Bacterial Vaginosis recurrences, Lactobacilli, metronidazole, clindamycin, Lactobacillus rhamnosus BMX 54, combined approach, NORMOGIN.

INTRODUCTION

Bacterial Vaginosis is the commonest worldwide vaginal infection with a prevalence that varies from 9% to 50% in women of reproductive age [1] and may reach 70% among female sex workers [1]. The aetiology of BV is still unclear but it is currently considered to be a synergistic polymicrobial syndrome, characterised by depletion of Lactobacillus spp and an intense increase (100 to 1,000 – fold above normal levels) in vaginal anaerobic bacteria like Gardnerella vaginalis, Mycoplasma hominis, Atopobium vaginae, Prevotella spp, Veillonella spp and Mobiluncus spp [2]. Lactobacilli exert a protective function against opportunistic and pathogenic organisms by a competition with other microorganisms for nutrients and for adherence to the vaginal epithelium [3], by a stimulation of the immune system [3], by a reduction of vaginal pH (production of organic acids like lactic acid) [3] and by a production of antimicrobial substances such as bacteriocin and Hydrogen peroxide [7]. BV often represents a silent pathology for women (almost 50% of the women affected by BV are asymptomatic) [4] and the clinical diagnosis could be made according to the presence of at least three of the following four criteria (Amsel Criteria) [4]:

1) thin, homogeneous vaginal discharge; 2) vaginal pH higher then 4.5; 3) «fishy» odour of vaginal fluid after addiction of 10% KOH (Whiff test); 4) presence of clue cells on microscopic evaluation of saline wet preparation.

Since the CDC recommended antibiotic therapy (metronidazole or clindamycin administered orally or intra-vaginally) [5] failed to control relapses of BV (40% of recurrences at three months and 50% of relapses after six months) [3] B.V. recurrences could be considered a «drug-free pathology», for which a combined approach is firmly hoping [6] (Figure 1). Lactobacillus rhamnosus BMX 54, the active principle of the drug NORMOGIN, has recently been tested on a totally sample size of 475 women affected by B.V. recurrences [4,5,9-13]. Aim of this report was to assess the effectiveness of a long-lasting Lactobacillus rhamnosus BMX 54 vaginal tablets application in colonizing the vaginal microenvironment and in preventing B.V. recurrence rate.

MATERIALS AND METHODS

Lactobacillus rhamnosus BMX 54, the active principle of the drug NORMOGIN, has recently been used in a lot of clinical trials [4,5,9-13] with the aim to
evaluate its effectiveness in control B.V. relapses in combination with CDC recommended therapy [5]. A Lactobacillus rhamnosus spp has been selected because of its ability to colonize the vaginal microenvironment and to survive up to seven weeks after exogenous implantation, showing a positive clinical prevention in urogenital tract infections [4]. 363 patients have been enrolled in controlled clinical trials versus metronidazole (metronidazole alone – 500 mg twice day by oral route for seven days – 150 women and the same metronidazole scheduled followed by a two or a six months Lactobacillus rhamnosus BMX 54 vaginal application once weekly – 153 women) [5,9-12] or versus no
treatment in pregnancy [sixty pregnant women assigned randomly to the untreated arm (n=30) on to receive vaginal application of one tablet containing Lactobacillus rhamnosus BMX 54 (NORMOGIN) once a week for 12 weeks (n=30)] [13]. 112 women have been enrolled in open uncontrolled clinical trials in which Lactobacillus rhamnosus BMX 54 administration time was quite different lasting from three alternate days vaginal application to 24 months [7,8]. Each NORMOGIN vaginal tablet contained at least $10^6$ colony-forming units (CFU) of live and lyophilized Lactobacillus rhamnosus BMX 54 [4].

RESULTS

The results obtained in controlled trials [5,9-12] clearly substantiated the effectiveness of the combination therapy (metronidazole 500 mg twice daily for 1 week following by a once weekly vaginal application of Lactobacillus rhamnosus BMX 54 for six months in preventing B.V. relapses, not only during the treatment time (6 months) but also during the 6 months follow-up without lactobacilli treatment [5] (Figure 2). In the same time the controlled clinical trials performed in pregnant women versus no treatment, in which Lactobacillus rham-
nosus BMX 54 has been vaginally administered for 12 weeks once weekly, supported its effectiveness in preventing the development of abnormal vaginal microflora and in control the cervical parameters that could represent a risk-factors of vulnerability to preterm delivery [13]. Interestingly in an open, uncontrolled trial, Lactobacillus rhamnosus BMX 54 vaginal application for 2

Fig. 3. Effect of vaginal treatment with Lactobacillus rhamnosus (Normogin®) for 24 months on vaginal pH of forty women affected by Bacterial Vaginosis.
(A) Mean pH values of vaginal swabs taken before (diagnosis) and after 12 and 24 months of therapy.
(B) Scatter plot of individual pH values. Each point represent a patient, horizontal lines indicate mean ± SD (n=40).
*** P < 0.001; ** P < 0.02 (ANOVA for repeated measures) [8].
years (6 days 1 tab/day than 2 months 2 tabs/weekly, and then 1 tab/weekly till 2 years) has proven its ability in control vaginal pH during the long-lasting treatment time [8] (Figure 3).

**CONCLUSIONS**

The results obtained with Lactobacillus rhamnosus BMX 54 vaginal application seems to support Wilson’s assumption to combine all aspects of vaginal interrelation [6]. Treating the overgrowth of anaerobic bacteria with metronidazole together with replacing vaginal lactobacilli with Lactobacillus rhamnosus BMX 54 vaginal application significantly improve the recurrence rate of B.V. The long term use of Lactobacillus rhamnosus BMX 54 vaginal application with a specific treatment schedule seems to be useful and mutually supported the traditional antimicrobial therapy recommended for B.V.

**REFERENCES**


The Antiprogestin Telapristone Shrinks Fibroids When Used Orally or as a Vaginal Suppository

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SUMMARY

Fibroids (leiomyomas) are a common benign tumor of the myometrium of the uterus that nevertheless represents a concern for women for their attendant bulk symptoms and non-menstrual vaginal bleeding episodes. Previously, anti-progestins including Telapristone (aka Telapristone Acetate, Proellex, Progenta, and CDB-4124) have demonstrated the ability to reduce bleeding and shrink fibroids in women when given orally. In the case of Telapristone, treatment resulted in anovulation and suppression of estrogen and a near total ablation of progesterone. Forty women were enrolled in a Phase 2 Clinical Trial (ZPV-200) that compared four doses of Telapristone (3 mg, 6 mg, 12 mg, and 24 mg) in the form of a vaginal suppository, a gel capsule. Bleeding was the primary outcome and assessed by the Pictorial Blood Loss Assessment (PBAC) score. Secondary endpoints were changes in tumor volume by MRI and well-being through the Uterine Fibroid Symptom Quality of Life (UFSQoL) score. The vaginal use of Telapristone at 12 mg per day was associated with similar favorable outcomes for women with fibroids as compared to the higher oral dose. Although results were similar in the extent of shrinkage, the effects required a longer dosing period. The 24 mg dose of the suppository was ineffective probably due to the melting characteristics of the gel capsule.

Keywords: leiomyoma, PRMs, therapy, clinical trial, non-oral dosing, MRI, PBAC.
INTRODUCTION

Antiprogestins trail antiestrogens in preclinical exploratory studies as models for the treatment of diseases in women. At the same time, many antiestrogens or antiestrogen therapies can have pernicious effects that can greatly reduce their acceptability. Tamoxifen has deleterious effect on the endometrium and GnRH agonists and antagonist can have negative effects on bone because they reduce estrogen so drastically. In our experience, antiprogestins (PRMs) lead to anovulation in young otherwise normal women and lower estrogen to mid-follicular levels but completely ablate luteal progesterone. This appears to be a central effect at the level of the hypothalamus through altered LH and FSH release [1]. We feel that in such a low progesterone environment, an antiprogestin can work optimally in tissues at the level of the progesterone receptor [2]. Effects are often mediated by changes in proliferation and apoptosis [3, 4]. Fibroids are benign tumors of the myometrium of the uterus that lead to significant non-menstrual blood loss plus bulk symptoms. Both effects, when severe, can interrupt daily routines and affect a women’s quality of life, sometimes leaving them home-bound when they desire a normal active life. We have found that the PRM Telapristone leads to strong effects on women that include both anovulation and amenorrhea [5] which result in a greatly reduced vaginal bleeding and increased quality of life. Women with fibroids experience greatly reduce vaginal bleeding (Figure 1). A frank loss of fibroid tumor volume is another result of treatment in many women [6] (Figure 2). At an effective dose of telapristone, ovulation does not occur and progesterone levels are very low. In an unreported study (ZP-204), oral doses as low as 3 mg induced amenorrhea whereas the 1 mg oral dose did not. In order to avoid liver toxicity we found at high oral dose, we made use of non-oral delivery using a vaginal suppository of telapristone in a proprietary capsule that melts at body temperature.

MATERIALS AND METHODS

The vaginal study reported here (ZPV-200) was a phase II, 5-arm-study with 16 weeks of active dosing in arms 1 and 5 and 12 weeks for arms 2-4. The study was conducted in 3 stages. In the first stage, the 6 subjects were enrolled into Arm 1 and were treated at the 12 mg dose level. In addition to the other required study assessments these subjects were also monitored with a 24-hour PK assessment on Day 14, as well as daily drug trough assessments for the first 14 days. This was to assess whether any
**Fig. 1. Effect of Oral Telapristone on Vaginal Bleeding.** Women with fibroids were assessed in a Phase 2/3 Clinical Trial. Subjects were treated for 3 months with placebo (black bars), or oral telapristone at 12.5 mg per day (vertical striped bars) or 25 mg per day (stippled bars). Assessment was by the PBAC. A normal PBAC value for bleeding is 80. Both treatment groups demonstrated significantly less bleeding by PBAC after treatment ($P < 0.001$, T-test).

**Fig. 2. Effect of Oral Telapristone on Fibroid Size.** In a 4-women preliminary study of fibroids by MRI, individuals were treated with 50 mg oral Telapristone per day for 4 months. Given are results for one woman (05058) with three individual fibroids. Shown is the size of each fibroid by volume before and after treatment. Reductions are 72%, 72% and 52%, respectively. Overall, the four study women demonstrated a reduction of about 60% in volume and reductions in 11 out of 12 fibroids.
subject exceeded the mean serum drug level for the highest safe oral dose administered in the ZP-204 study (12 mg). In the second stage, further subjects were randomized to arms 2, 3 or 4 and received a dose of 3, 6 or 12 mg (single-blind). In the third stage, after arms 2, 3 and 4 had been fully enrolled; an additional 12 subjects received single-blind treatment at 24 mg (arm 5).

For all subjects in arms 2, 3, 4, and 5 there was a run in period of up to 6 weeks, to establish baseline parameters (bleeding, size, and quality of life) followed by treatment at one of four single-blind Proellex doses (3, 6, 12 or 24 mg daily, administered vaginally in capsule form). The primary efficacy endpoint was bleeding assessed using the PBAC after 16 weeks of treatment for arm 5 (12 weeks for arms 2-4). The secondary endpoints were changes in size of fibroids assessed by MRI and improvement in quality of life assessed using the UFSQoL. For subjects enrolled in Arms 2, 3, 4 and 5, PK was assessed after the first and last doses and trough levels every 2 weeks. Forty women were enrolled. ZPV-200Ext was extended to a second cycle.

RESULTS

In study ZPV-200, vaginal administration of telapristone resulted in very low systemic exposure at all doses tested. Remarkably the 12 mg vaginal dose achieved a statistically significant and clinically relevant improvement in bleeding as assessed by PBAC and in tumor size as assessed by MRI. The 12 mg vaginal dose yielded exposures a mere fraction of the oral counterpart (< 1/6 AUC of a 12 mg oral dose and Cmax < 1 mg oral dose). The low exposure of the vaginal formulation is particularly important when one considers that the target organ of telapristone toxicity is the liver.

The other doses tested also exhibited varying degrees of efficacy depending on the assessment performed but only the 12 mg dose, in addition to achieving reduction in PBAC bleeding scores and UFSQoL symptom severity scores at 16 weeks (Table 1), also reduced fibroid volume after the initial and second 4-month treatments (Figure 3).

<table>
<thead>
<tr>
<th></th>
<th>3 mg</th>
<th>6 mg</th>
<th>12 mg</th>
<th>24 mg</th>
</tr>
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<tbody>
<tr>
<td>PBAC</td>
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<td>-76</td>
<td>-98</td>
<td>-100</td>
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<tr>
<td>UFSQoL</td>
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<td>-8.7</td>
<td>-50</td>
<td>-100</td>
</tr>
</tbody>
</table>

Tab. 1. Effects of Vaginal Telapristone on PBAC and UFSQoL. Median % change from Baseline.
The 12 mg dose also exhibited a significant increase in drug levels as compared to the three other doses. This comparison includes the 24 mg dose which exhibited less consistent exposure. The sponsor believes this is due to the current formulation. The 12 mg Proellex-V formulation consists of a solution of hydrophobic telapristone in PEG-1000 at a concentration of 2.19% w/w (includes 0.02% BHT as an anti-oxidant). The 24 mg dose is at a concentration of 3% w/w. Volume of the final products were intentionally kept low in order to minimize vaginal leakage. We believe the 24 mg dose may have precipitated in the vagina due to exudates produced either during insertion or the normal moisture produced in a non-stimulated state.

**CONCLUSIONS**

As in previous clinical trials of oral telapristone, the results of this study once again show the significant efficacy signal for this antiprogestin intended for the treatment of uterine fibroids. At 12 mg consistent efficacy signals are achieved for all important assessments. Combined with the low but more consistent exposure achieved at the 12 mg dose and avoidance of the anticipated
potential for breakthrough uterine bleeding, the sponsor believes this dose warrants further study in a placebo-controlled Phase 3 study.

REFERENCES


Preoperative Volume Reduction Plan in Large Uterine Laparoscopically Assisted Vaginal Hysterectomy

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SUMMARY

Sixty-eight patients who had undergone laparoscopically assisted vaginal hysterectomy (LAVH) indicated a huge uterus were selected. In 34 patients (group I), the dissected uteri were vaginally removed by a randomized small piece wedge resection and in group II (34 patients), the uteri were planned reduction by bisection technique. The operating time and the duration of uterine removal were significantly better in group II by statistical analysis. In the moderate enlarged sub-group, the statistically significant differences were also found in the operating times, median 132.5 min (range 95-185) in group I vs 85 min (range 70-100) in group II and in the duration of uterine removal, median 21 min (range 15-35) in group I vs 3 min (range 1.5-15) in group II. In the very huge uterine sub-group, the comparison were also presented in the same results.

Keywords: Laparoscopically assisted vaginal hysterectomy, Uterine reducing method.

INTRODUCTION

LAVH is becoming increasingly popular. Uteri larger than 300 g were hard to pull out through the vagina without any incisions [1-4]. Many uterine removal techniques, including uterine bisection, cervical amputation with rotational
bivalve, intramyometrial coring, wedge resection, and morcellation, were recommended [5, 6]. However these techniques have been still unpredictable in the difficulty of the processes. This study was to compare the operating time and the duration of uterine removal between the our bisection techniques which was preoperatively planned using preoperative ultrasound image and the unplanned small wedge resection.

**MATERIAL AND METHODS**

The preoperative uterine classifications using ultrasonographic uterine image were described as follows: A) Transverse and AP diameters were less than 8 cm which was estimated to be less than 12 weeks of gravid uterus or less than 200 g of uterine weight. B) One of the transverse or AP diameters was less than 10 cm and the other was between 8-14 cm which was estimated to be similar to 12-14 weeks of the gravid uterus or > 200-500 g of uterine weight. C) Both of the transverse and AP diameters were between 10-14 cm which was estimated to > 14-18 weeks of gravid uterus or > 500-800 g of uterine weight. D) Transverse diameter or AP diameter was more than 14 cm which was estimated to more than 18 weeks of gravid uterus size or more than 800 g of uterine weight.

In Category B, the uterus was planned for volume reduction by manual bisection method (Fig. 1). The procedures were as follows: 1) After freeing the uterus from the vessels and supporting ligaments, it was pulled vaginally with downward traction by two single-tooth tenaculums. 2) A knife was used to bisect the cervix and extended upward until approximately 7 cm or more in depth. 3) The right bisected uterine portion was then transversely cut at the deepest bisected point until completely separated from the uterine body and then was removed through the vagina. 4) With a single-tooth tenaculum, downward traction was continued on the left cervical portion until the uterine body was fixed to the pelvic cavity and then the traction-direction was changed to the right. 5) The second single-tooth tenaculum was then used to grasp on the surface of the left side of uterine corpus close to the left cornu. 6) By traction on the second tenaculum, the left uterine portion was rotated clockwise until the transverse dimension of the uterine corpus was rotated in its direction to longitudinal and then was easily pulled through the vagina. 7) Occasionally, an additional transverse bisection of the left portion might be necessary in some difficult cases.

In category C, the complete uterine bisection by a novel saw-containing device was recommended (Fig. 2). In detail, the following steps were done: 1) The procedures were started after freeing the uterus from vessels and supporting
ligaments and then, the lower uterine bisection of approximately 7 cm or more in depth, had been performed. 2) The laparoscopic scissors with monopolar electrocoagulator was used to bisect at the upper uterine portion for approximately a 10 cm in length until entering into the uterine cavity. 3) A saw-containing device was then inserted though the posterior cul-de-sac into the abdominal cavity posterior to the uterus. 7) A small, long-handled hook or grasper was passed through the cervix, uterine cavity and then deeply into the abdominal cavity at the bisected upper portion. 8) By using the laparoscopic grasping forceps to manipulate to the inner-end saw-loop which was located near the tip of the device, the saw-loop was then hooked and pulled downward to outside the vagina. 9) The posterior middle portion of the uterus was then slowly sawn until complete separation. 12) The anterior middle portion was then sown by the same processes. 13) Each portion was then removed vaginally outside the abdominal cavity. 14) Occasionally, the bisected portion was still too large and a combined manual bisection was also needed.

Fig. 1. The left figure presents a bisected uterine specimen which was performed in the large uterus. The right figures are showed the clockwise rotation in the removal processes.
RESULTS

Sixty-eight patients who had undergone LAVH from November 2007 through February 2011, were selected according to preoperatively determined ultrasonographic uterine classifications into a huge uterus (category B, C and D). In 34 patients who were operated before December 2008, the dissected uteri were vaginally removed by a randomized small piece wedge resection (group I) and, in contrast, in the other 34 patients who were operated after that, the uteri were planned reduction by bisection (group II). In comparison between each group, the operating time and the duration of uterine removal were significantly better in the group II, median 155 min (range 95-365) vs 90 min (range 70-155) and median 37.5 min (range 15-180) vs 4.5 min (range 0.5-30). In sub-group analysis, 17 patients in group I and 18 in group II who were classified into large
Continuous variables with normal distribution are given in mean ± SD and compared by Student’s t-test.

† Continuous variables with non-normal distribution are given in median (range) and compare by Mann-Whitney U test.

‡ Nominal variables are given in N (%) and compared by Chi-square or Fisher’s exact test.

Significance is set at 2-tailed p<.05 for all tests.

### Tab. 1. Demographic characteristics and ultrasonographic results.

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<th>N 34</th>
<th>Group II</th>
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Continuous variables with normal distribution are given in mean ± SD and compared by Student’s t-test.

† Continuous variables with non-normal distribution are given in median (range) and compare by Mann-Whitney U test.

‡ Nominal variables are given in N (%) and compared by Chi-square or Fisher’s exact test.

Significance is set at 2-tailed p<.05 for all tests.

Uterine size (category B), the statistically significant differences were also found in the operating times, median 132.5 min (range 95-185) in group I vs 85 min (range 70-100) in group II and in the duration of uterine removal, median 21 min (range 15-35) in group I vs 3 min (range 1.5-15) in group II. In the very
Continuous variables with non-normal distribution are given in median (range) and compare by the Mann-Whitney U test. Significance is set at 2-tailed p<.05 for all tests.

large uterus (category C), 14 patients in group I and 15 patients in group II were presented in the same results with a median of the operating times 185 min (range 175-210) in group I vs 130 min (range 100-155) in group II and median of the duration of uterine removal was 57.5 min (range 40-90) in group I vs 29 min (range 25-30) in group II.

**CONCLUSIONS**

The prolonged operating time is associated with the technical ability required for large uterine volume reduction and removal procedures [1, 5, 6, 8-10]. The bisection planning in the large and very large uteri have been introduced and classified by our group since 2008. The 250-500 g uteri were defined as large uteri and more than 500 g were very large uteri [1, 11, 12]. Preoperative pelvic ultrasonography, provides an accurate uterine size and the positions of myomas [7], has helped to assess the appropriate uterine volume reduction and removal method.
The patients with uteri larger than 250 g had a significant increase in duration of surgery compared with uteri less than 250 g (median 135 vs 97 min, range 45-345 vs 29-330 min) [11]. Lin [1] reported a helical incision technique for extraction of uteri between 300 and 500 g in which the operating time was 73 ± 19 min. With our manual bisection technique, the uteri could be completely removed through the vaginal canal in the 3 min (range 1.5-15 min).

Chen et al. [12] reported laparoscopic in situ morcellation (LISM) in LAVH for a very large uterus. A comparison study was performed between 69 patients with uteri weighing 500 to 749 g. The operating time was significantly improve. Wong et al. [5] recommended their novel “paper roll” vaginal morcellated technique, in very large uteri (> 500 g) in which the mean operating time was 95 ± 37 min. The bisection technique used in this present study with saw-containing device had significantly less operating times compared to the unplanned wedge resection, median 130 min (range 100-155 min) vs 185 min (range 175-210 min). Although it took more time than Wong reported but was close to the time in Chen’s report, it is hoped that operating time will be reduced after a learning period. This procedure would be considered as an alternative method in very large uterine volume reductions (500-800 g).

**REFERENCES**


Abnormal Uterine Bleeding in Pre and Postmenopausal Women: Hysteroscopic and Histological Findings

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Gynecology Department, Faro’s Hospital, Faro, Portugal

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SUMMARY

Abnormal Uterine Bleeding (AUB) is one of the most frequent gynecologic complaints. A retrospective study including 220 women submitted to histeroscopy for AUB was conducted in Faro’s hospital and 2 groups were analyzed: Pre and Postmenopausal women. Endometrial thickening in the ultrasound, hysteroscopic finding of polyps and endometrial hypertrophy and histological results of premalignant or malignant endometrial tumors in postmenopausal group revealed statistical significance regarding premenopausal group, concluding that there is a higher risk of endometrial disease and malignancy after menopause.

Keywords: Menorrhagia, Metrorrhagia, Menometrorrhagia, Endometrial polyps, Endometrial cancer, Histeroscopy, Menopause.

INTRODUCTION

Abnormal Uterine Bleeding (AUB) represents 1/3 of all gynecological appointments [1] and is defined as an abnormal frequency, duration or volume of expected uterine bleeding [2]. It can be caused by structural uterine pathology, anovulation, pregnancy, neoplasia, systemic diseases, among others. Knowing the underlying cause is extremely important to exclude malignancy and correctly guide the treatment. The aim of this study is to evaluate the main differences between AUB in pre and postmenopausal women.
MATERIAL AND METHODS

A retrospective study was conducted in Faro’s Hospital including 220 women submitted to hysteroscopy for abnormal uterine bleeding, between 2009 and 2011. Data were obtained by consulting clinical processes.

Two groups (pre and postmenopausal women) were analyzed for: Age, concomitant diseases (hypertension, diabetes, obesity, hypercholesterolemia, history of breast cancer treated with tamoxifen), endometrial thickening on ultrasound, hysteroscopic findings, need for surgery and histological results of biopsies or surgical specimens.

Descriptive and comparative statistical analysis were done using Statistical Package for Social Sciences (SPSS) 20.0 and nonparametric tests. Quantitative data are presented as mean ± standard deviation (STD) and qualitative data are presented as absolute (n) and percentage (%). For a significance level \( \alpha = 0.05 \), we consider that there is statistical significance when \( p < 0.05 \).

RESULTS

220 women were included in this study: 137 (62.7 %) were postmenopausal and 83 (32.3 %) were premenopausal.

On the premenopausal group, age ranged between 28 and 55 years (average age 44 ± 6.364). 76 (91.6 %) were healthy, 5 (6.0 %) had hypertension, 2 (2.4 %) history of breast cancer treated with tamoxifen and 1 (1.2 %) type 2 diabetes. 54 (65.1 %) revealed endometrial thickening on the ultrasound. Endometrial polyps were found in 32 (38.6 %) women and were the most frequent hysteroscopic finding followed by submucosal myoma (11 women – 13.3 %) and 22 (28.0 %) revealed no pathological findings in hysteroscopy (Table 1). 39 (47.0 %) were referred for surgery (resectoscopy – 82.1 % or hysterectomy – 17.9 %) but 3 (3.6 %) of them refused. From 38 (45.7 %) women who went to surgery/biopsy, histological results revealed submucosal myoma on 18 (47.4 %), endometrial polyps on 13 (34.2 %) and no changes on 12 (31.6 %) (Table 2).

On postmenopausal group, age ranged between 45 and 87 years (average age 61 ± 7.660) and the mean time since menopause was 10.7 years ± 8.694. 46 (33.6 %) had hypertension, 18 (13.1 %) hypercholesterolemia, 17 (12.4 %) type 2 diabetes, 4 (2.9 %) had history of breast cancer treated with tamoxifen and 79 (57.7%) were healthy. 130 (94.9%) revealed endometrial thickening on ultrasound. Polyps were the most frequent hysteroscopic finding (73 women – 53.3 %) followed by endometrial hypertrophy in 29 (21.2%) and 20 (14.6 %) revealed no pathological findings in hysteroscopy (Table 1). 81 women (59.1 %) were referred for surgery (resectoscopy – 86.4% or hysterectomy – 13.6 %).
and 1 (0.7 %) has refused. From 102 women who went to surgery/biopsy, histological results revealed endometrial polyps on 42 (41.2 %), simple hyperplasia without atypia on 12 (11.8 %), complex or atypical hyperplasia/malignancy on 12 (11.8 %) and no changes on 24 (23.5 %) (Table 2). Complex or atypical hyperplasia and malignancy represented 8.8 % of women in postmenopausal group.

The difference between the 2 groups revealed statistical significance regarding: endometrial thickening (p < 0.01), presence of hypertension or diabetes (p < 0.01), hysteroscopic findings of polyps (p < 0.05) and endometrial hyperplasia (p < 0.01) and histological results of endometrial polyps (p < 0.05) and

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<th>Histeroscopic Findings</th>
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<th>Postmenopausal (n=137)</th>
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<tr>
<td>Frequency (n)</td>
<td>Percent (%)</td>
<td>Frequency (n)</td>
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<tr>
<td>Endometrial Polyps</td>
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<tr>
<td>Uterine submucosal Fibroids</td>
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<td>13.3</td>
</tr>
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<td>Endometrial Hypertrophy</td>
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<tr>
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<td>3.6</td>
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<tr>
<td>No Pathological Findings *</td>
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<td>26.5</td>
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* Endometrial atrophy wasn’t considered as a pathological finding.

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<th>Histological Results of biopsies or surgical specimens in Pre and Postmenopausal Women.</th>
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<td>Premenopausal (n=38)</td>
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<td>Frequency (n)</td>
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<td>Normal histology</td>
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premalignant or malignant endometrial tumors ($p < 0.01$) in the postmenopausal group, such as for the presence of normal hysteroscopic findings ($p < 0.05$) and histological results of myoma in the premenopausal group (Table 3).

### Tab. 3. Comparative Results for Pre and Postmenopausal Group.

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<td>94.5</td>
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<td>38.6</td>
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<td>0.038</td>
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<td>7.2</td>
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<tr>
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<td>10.8</td>
<td>7.3</td>
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<td>37.2</td>
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<tr>
<td>- Endocervical Polyps</td>
<td>3.6</td>
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<tr>
<td>- No Pathologic Findings</td>
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<td>14.6</td>
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<td>47.9</td>
<td>59.1</td>
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<td>30.7</td>
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<td>1.000</td>
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<td>1.2</td>
<td>8.8</td>
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Hysteroscopic finding of endometrial hypertrophy was found on 35 women (16.0 %) with AUB and it was related with premalignant or malignant histological results in 6 of these women (17.1 %) comparatively to 4 (2.2 %) of 185 women who didn’t have endometrial hypertrophy ($p < 0.01$).
CONCLUSIONS

Abnormal uterine bleeding has multiple causes and their frequency varies depending on the woman’s age and hormonal status (pre or postmenopausal). The results revealed a higher prevalence of endometrial thickening found in ultrasound and endometrial hypertrophy and polyps in hysteroscopy as well as a lower prevalence of normal histeroscopic findings compared to premenopausal women. These results allow us to conclude that the risk of endometrial pathology as cause of AUB is higher in older women, which includes malignant and premalignant pathology [3,4]. As expected, the prevalence of associated disease was higher in those women too, which may constitute risk factors for endometrial disease as described in literature [5]. We haven’t found any data on clinical files regarding obesity, which is known to be a risk factor for endometrial cancer [5]. This shows that obesity and its potentially harmful effects may be being underestimated and missing on clinical records.

Ultrasound and hysteroscopy play an important role in the study of the endometrium and the main goal is to exclude malignancy. Evaluation of endometrial thickness on ultrasound is considered a reliable method in screening for endometrial pathology on postmenopausal women and an endometrial line under 5mm almost excludes pathology, becoming unnecessary the use of invasive techniques such as sampling or hysteroscopy. The same doesn’t happen for premenopausal women [6], but ultrasound is essential to look for structural lesions [7]. Endometrial hypertrophy found on hysteroscopy, despite being a subjective sign, seems to be associated with a higher risk of malignancy. Hysteroscopy, apart from allowing the complete visualization of the uterine cavity, allows to perform targeted biopsies, but must, for risks and costs, be reserved for when there is a based suspicion of structural abnormalities [6,8].

In the premenopausal group, mean age was 44 years, which explains a higher rate of normal hysteroscopy, probably associated with dysfunctional bleeding.

REFERENCES

2. Fraser IS, Critchley HO, Munro MG, et al. A process designed to lead to international agreement on terminologies and definitions used to describe abnormalities of menstrual bleeding. Fertil Steril, 87, 466 (2007).
Menopausal Metabolic Disorders and Endometrial State

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SUMMARY

Perimenopausal period is conducive to android obesity (AO) which predisposes to derangement of cell division and apoptosis. Relationship between metabolic disorders and predilection for neoplastic endometrial transformation was studied in order to improve management of perimenopausal-age women (PW) with endometrial hyperplasia (EH). 149 PW were allotted respective of AO and endometrial state. The targets were serum insulin, lipoproteins, apoptosis markers (sFas, Fas-ligand, TNF-α). Endometrial samples underwent immunohistochemical investigation including to Ki-67 and p16INK4a. Then women with AO were administered metformin. The lowest sFas was in group without AO and EH (Ia). It was almost the same in the cases of combination of AO with quiescent endometrium (Ib) and simple EH without AO (II). In III group with AO and non-atypical EH more significant sFas was noticed with vast range and two distinctive peaks. Considering that group was divided: IIIa – range 8-11 ng/ml, IIIb – 13-17 ng/ml. The highest sFas was in IV group of atypical EH (73% women with AO). Immunohistochemical investigation confirmed incremental frequency of apoptosis abnormalities depending on EH, AO, especially atypical EH. That pattern was consistent with sFas fluctuations. Follow-up showed trend towards improvement in Ib, IIIa and IIIb with regard to weight loss, insulin susceptibility, lipoprotein spectrum, except sFas: in IIIb it was unyielding and went with worse clinical outcomes. The study elicited trend towards high incidence of prospective atypical EH if sFas >13 ng/ml irrespective of metabolic treatment.
Keywords: endometrial hyperplasia, android obesity, apoptosis markers.

INTRODUCTION

The expansion of the metabolic syndrome prevalence among perimenopausal women of the developed countries has become cumbersome for modern health service taking into consideration increase of mean female life expectation and supervening changes in age structure of the population [1, 3]. AO is acknowledged to be the corner stone of other signs of the syndrome. Alterations inherent to the natural perimenopausal period are conducive to AO which is an unfavourable background distinctive for predisposition to derangement of cell division and apoptosis as a result of both proliferative insulin stimuli and metabolic cell immune oppression. Shift of the balance between inductors and inhibitors of cell division at favour of formers can underlie in possible accumulation of cells bearing local mutation. The loci responsible for production of apoptosis factors are the most vulnerable towards mutagen influences and it can eventually be the cause of that pool slipping out of control [9]. Hyperplastic endometrium is subjected towards abovementioned events and can be affected easily but only 6-8% of non-atypical hyperplasia causing recurrent climacteric uterine bleeding undergo malignant transformation and it remains being challenge to choose appropriate management of women with combination of AO and benign EH [5, 6, 7, 8]. We started our research in order to improve the management of PW with AO and different types of EH by revealing relationship between metabolic perimenopausal disorders and predilection for neoplastic endometrial transformation and on these grounds to work out predictive score for high risk groups.

MATERIAL AND METHODS

Research comprised 149 PW subdivided respective of AO and endometrial state. 90 ones with variable types of EH, and 60 women the same age without endometrial pathology involved as control group: 28 of them without signs of AO constitute Ia subgroup, and 32 ones with recognized AO were allocated at Ib subgroup. II and III groups consisted of the patients with non-atypical variants of endometrial hyperplasia: 33 ones without AO were allocated at II group, and 32 ones with AO constituted III group. IV was formed by 25 patients with atypical EH, 76% of whom suffered from AO. The targets for evaluation were serum value of insulin, apoptosis markers (sFas, Fas-ligand, TNF-α), lipoprotein range. Endometrial samples obtained by curettage underwent immunohistochemical investigation including by MCA to protein Ki-67 and p^{16INK4a}. The
follow-up of women with AO was maintained and the results were estimated finally after the year of their treatment aimed at gradual weight loss and alleviating of insulin resistance [4]. They had been administered mandatory metformin, one of statins, and tribestan – herbal complex drug used usually for improvement of male and female sexual function, but in this study chosen for alleviating signs of rapidly precipitating estrogen deficiency due to menopause.

RESULTS

The lowest sFas level was revealed in group without AO and EH (Ia) - 0,86±0,31 ng/ml. It was almost the same in two different group: Ib – AO with quiescent endometrium 4,08±0,37, II – simple EH without ao 4,14±0,39. Much more significant sFas was noticed in group with combined AO and non-atypical EH (III) 13,78±1,27. The highest sFas was in group of atypical EH (IV), where 73% women suffered from AO -19,86±1,92. Peculiarities of sFas pattern were revealed in III group: vast range and multimodal distribution with two distinctive peaks. Considering sFas level group was divided into two subgroups: IIIa – moderate 8-11 ng/ml and IIIb – high 13-17 ng/ml. Lipoprotein pattern of all groups with AO was distinguished by increased low density fraction and reduced high density one. Those groups baseline insulin value surpassed Ia and II group level a bit but after glucose challenge test that difference became proven, although insulin value of the Ia group was less than III and IV groups one that reflected more pronounced extent of insulin resistance in latter groups. Pattern of leptin and tumor necrosis factor serum values were very similar, the extent of Ib, III and IV groups preponderance above Ia and II ones was less pronounced though. That proves insulin resistance in former groups from one side, but also it might be the evidence of increasing TNF-α gene expression as a respond to lack of FAS-ligand. Reactions with antibodies towards proliferative protein Ki-67 and oncoprotein p16INK4a additionally showed some peculiarities consistent with sFas fluctuations in IIIa and IIIb subgroups. This concurrence confirms pertinent division III group according to sFas level and its possible predictive value.

All women of IV group underwent hysterectomy [5, 6, 8]. Follow-up surveillance showed that there had been a trend towards improvement in all three subgroups (Ib, IIIa and IIIb) with regard to weight loss, restoring of tissue susceptibility to insulin, shift in lipoprotein spectrum towards normal value except sFas: in Ib and IIIa it appeared to be reduced to almost Ia level, contrariwise sFas in IIIb was unyielding and that went with worse clinical outcomes: whilst there was no cases of endometrial hyperplasia recurrence in IIIa subgroup, 70% women of IIIb subgroup presented with recurrent uterine bleeding,
underwent curettage after which two thirds of them were treated with radical surgery, and there were two cases of confirmed atypical hyperplasia, thus frequency of subsequent hysterectomies in that subgroup amounted to 60%.

There is proven predilection for development of AO during perimenopausal linked with relative predominance of active testosterone on the background of oestrogen deficiency. Visceral fat cells easily release free fat acids in bloodstream because of their weak responsiveness towards insulin that is why susceptibility of the liver cell and striated muscle towards insulin is inhibited. Insulin resistance entails hyperinsulinaemia which has proliferative influence on the target-tissue. Growth of cortisole, leptin and tumour necrosis factor production which is inherent to android obesity aggravates insulin resistance bringing up vicious circle and also facilitates the development of metabolic immunodepression. Impairment of T-lymphocytes may give rise to aberration of the apoptosis factor Fas-ligand manufacture and occurrence of sFas which is the product of pathological splicing. It is not capable to evoke appropriate apoptosis but competing with Fas-ligand for target-sites sFas oppresses Fas-ligand-induced apoptosis [2, 9]. However we should suggest that tribestan administered at the age of perimenopause mitigated signs of metabolic changes due to estrogen deficiency and it was not related with relapse and aggravation of the endometrial pathology.

CONCLUSIONS

There is distinctive trend towards high incidence of prospective atypical endometrial hyperplasia in the case of high sFas more than 13 ng/ml irrespective of metabolic treatment. sFas level reflects worst background for treatment and can be offered as predictive factor for choice of management of perimenopausal-aged women with non-atypical endometrial hyperplasia and android obesity. sFas level more than 13 ng/ml should be claimed as recommendation to treat such women with radical surgery.

REFERENCES

3. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults


Milton Mc Call Suspension (MCS) Versus Leanza Axial Vault Suspension (AVS)

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SUMMARY

Objective: to compare two methods of vaginal vault suspension: Milton Mc Call Suspension (MCS) Versus Leanza Axial Vault Suspension (AVS).

Materials and Methods: 590 women with uterine prolapse (first, second or initial third Half Way System grades) were randomly alternatively allocated to two treatment groups depending on the modality of post-hysterectomy apical suspension of vagina. 18 refused the operation (590-18=572). All the patients recruited underwent total hysterectomy with pelvic floor reconstruction. 286 (A-group) were treated with Milton Mc Call Suspension (MCS) and 286 (B-group) by means of Leanza Axial Vault Suspension (AVS). There were 38 dropouts from follow-up, among them 16 (286-16=270) in the former and 22 (286-22=264) in the latter.

Results: Mean follow up was 87 months (range 12-110). SUI was solved in 88% of A-group and in 90% of B-group. 199/270 (73.7%) among MCS and 216/264 (81.8%) among AVS patients (χ²=0.54; p=0.463) were cured. 4 (1.5%) patients of MCS and 1 (0.4%) of AVS group complained of dyspareunia. Blood transfusion was necessary in two cases of both groups. MCS vs AVS showed a variation mean of POP-Q «C» point: -6 vs -8 (P<0001; t-student: 9.63 and TVL) 7 vs 10 (P<0001, t-student: 14.44).

Conclusions: AVS appears more effective in preventing central recurrences owing to the suitable use of six ligaments.
Keywords: Prolapse; Leanza AVS; Mc Call procedure, incontinence, life quality.

INTRODUCTION

Post hysterectomy suspension of vaginal apex is the most challenging feature to avoid central compartment recurrences\(^{(1)}\). This principle is equally suitable for both abdominal and vaginal routes\(^{(2-3)}\). The standard surgical procedure to maintain in site vaginal walls following uterus removal is the Milton Mc Call suspension (MCS) described in 1957\(^{(4)}\). This technique has been considered excellent as it reattaches the vaginal cuff to both uterosacral ligaments, obliterates the cul-de-sac and, contemporarily, suspends proximal vaginal wall. One should consider this procedure at the time of every vaginal hysterectomy. This technique can be used prophylactically or for first, second and initial third degree of prolapse. An alternative technique, used for the same indications was reported by Vito Leanza et al. (2003) and it is called axial vaginal suspension (AVS)\(^{(5)}\). According to AVS, vaginal apex anchorage is assured by using six ligaments (uterosacral, cardinal and either proximal adnexial pedicle when adnexa are preserved or round ligaments when Salpingoophorectomy is carried out). Ligamentous apparatus is sutured to the incised vagina by means of contact between two continuous wound solutions. Aim of this study is to compare two methods of vaginal cuff suspension: Milton Mc Call Suspension (MCS) Versus Leanza Axial Vault Suspension (AVS)

MATERIALS AND METHODS

590 women with uterine prolapse (first, second or initial third Half Way System grades) were randomly alternatively allocated to two treatment groups depending on the modality of post-hysterectomy apical suspension of vagina. 18 refused the operation (590-18=572). All the patients recruited underwent total hysterectomy with pelvic floor reconstruction. 286 (A-group) were treated with MCS and 286 (B-group) by means of AVS. There were 38 dropouts from follow-up, among them 16 (286-16=270) in the former and 22 (286-22=264) in the latter. Various defects of anterior and posterior compartment were associated too, excluding fourth grade. Mean age was 60 years (range 43-84). 518/590 (87.8%) patients referred vaginal births only, the others both vaginal and either one or more caesarean sections. Mean parity was 3.6 (range 1-10). Patients were followed at least after 6 weeks, 6 months and annually. Before the operation multichannel urodynamics was done to identify functional troubles as for as
urinary incontinence (UI) and drive the suitable surgical correction\(^{(6)}\). Stress urinary incontinence (SUI) was found among 151/534 (28.3%) patients. The various defects of other compartments were solved during surgery. All the points Aa, Ba, C, Ap, Bp, D, TVL and VH, according to Pelvic Organ Prolapse-Quantification, were evaluated. Comparisons of group means were performed with “t-student” test for independent samples. Proportions were compared with chi-square test \((\chi^2)\). A logistic regression analysis was performed to control for covariates that differed in our two groups despite randomization. Questionnaires was used for Life Quality\(^{(7-8)}\).

**RESULTS**

Mean follow up was 87 months (range 12-110). Among MSC patients, 75 (27.8\%) underwent antincontinence procedure and 195 (72.2\%) anterior repair. Among AVS patients, 76 (28.8\%) underwent antincontinence procedure and 188 (71.2\%) anterior repair\(^{(9,10)}\). Posterior repair was carried out for all the patients. 199/270 (73.7\%) among MCS and 216/264 (81.8\%) among AVS \((\chi^2=0.54; p=0.463)\) were cured for apical prolapse, respectively. SUI was solved in 88\% of A-group and in 90\% of B-group. 4 patients (1.5\%) of MCS and 1 (0.4\%) of AVS group complained of dyspareunia.

No cases of rectal trauma, nerve injury, ischiorectal abscess, postoperative haematoma were observed. Blood transfusion was necessary in two case of both groups. MCS vs AVS showed a variation mean of POP-Q «C» point: -6 vs -8 \((P<0.001; \text{t-student: 9.63 and TVL})\) 7 vs 10 \((P<0.001; \text{t-student:14.44})\). Significant differences in VAS scores and in the majority of the main domains of Questionnaires regarding preoperative and postoperative data \((p<0.01)\) were found. Satisfaction was statistically significant in both procedures (93.2\% versus 95.7\%).

**CONCLUSION**

Many procedures have been used for surgical apical support\(^{(12-14)}\). The success of every operation interferes with Life Quality\(^{(15)}\). In our report, both procedures are eligible for post-hysterectomy vaginal suspension, furthermore AVS appears more effective in preventing central recurrences owing to the suitable use of the whole ligamentous apparatus. In AVS procedure, since six ligaments are sutured to incised vagina by means of contact between two continuous wound solutions, distal revascularization of pedicles takes place allowing an efficient and stable apical support.
ACKNOWLEDGEMENT

Special thanks to the English language Professor Salvatore Rino Sciarreta.

REFERENCES

HPV
Cervical Screening Program in Alentejo: Our Experience

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SUMMARY

Cervical cancer is an important public health problem that can be prevented and diagnosed early by screening programs. Since 2008, one of these screening programs for cervical cancer was made available in Alentejo (a south-central region in Portugal), supported by a specified hospital team for guidance and follow-up of abnormal cytologies.

Our goal is to explain some aspects of this screening program as well as our work within the scope of a specific cervical pathology consultation.

Keywords: Cervical cancer, screening, human papillomavirus (HPV), cervical intraepithelial neoplasia (CIN), cytology.

INTRODUCTION

In Portugal, cervical cancer is the third most common neoplasy in women and the second leading cause of cancer death in this population [1]. Being an important public health problem it is, however, preventable and early diagnosis is possible by implementing screening programs [2]. In January 2008, a screening program for cervical cancer was implemented in Alentejo (with 759190 residents [3]) and a specific consultation was created at our hospital for guidance and follow-up of abnormal cytology in women living in Central Alentejo (with 166802 residents [3]).
In this program, all women aged between 30-65 years (since August 2011 women aged over 25 were also included) are convened for a Pap test (using Thinprep®). Women with symptoms related with cervical cancer/precancerous lesions or with a previous treatment for cervical cancer are excluded from this cervical cancer screening program. The first two Pap tests are annual and, if both are normal, the following cytology is preformed every three years. If any changes are detected (except for atypical cells of undetermined significance (ASC-US) negative for HPV), women are referred to our hospital.

**MATERIAL AND METHODS**

This study included all women referred to our hospital who attended a specific consultation service in cervical pathology between January 2008 and March 2012 with abnormal cytology (n=253) detected by cervical screening (with a total of 282 abnormal cytologies in that period). Data were analyzed by using SPSS statistics v.20.

**RESULTS**

Most women were referred due to low-grade intraepithelial lesions (LSIL) (Graphic 1). Among high risk HPV, there was a prevalence of HPV 16 (Graphic

![Graph 1](image-url)

**Graph. 1** Abnormal cytology (atypical squamous cells of indetermined significance [ASC-US], low grade squamous intraepithelial lesion [LSIL], high grade squamous intraepithelial lesion [HSIL], atypical squamous cells – cannot exclude HSIL [ASC-H], atypical glandular cells [AGC], adenocarcinoma in situ [AIS]).
2). 46% women were aged between 35 and 49 years. In terms of risk behaviors for HPV infection, women were inquired about their age at first sexual intercourse and number of sexual partners, type of contraception and tobacco consumption (Table 1). A colposcopy was performed on 250 women and 66% were considered abnormal. In those cases, 143 women underwent a biopsy, on 8 women we performed an endocervical curettage and in 26 cases an endocervical curettage and a biopsy. The histological study has revealed 5 squamous cell carcinomas, 37 cases of CIN III, 15 cases of CIN II and 17 CIN I.

**CONCLUSIONS**

Although only 1% of all cervical cytologies performed under the scope of this cervical screening program was abnormal, the high incidence of cervical cancer along with its great morbidity rate when lately diagnosed justify the need to continue the cervical screening programs and a specific team for early guidance on precancerous lesions, since the establishment of an effective screening program is more cost-effective and operational than the development of good therapies for cervical cancer [4].
**REFERENCES**

Human Papillomavirus Vaccine and the Ovary: the Need for Research

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SUMMARY

New onset of menstrual disturbance and oligomenorrhoea commencing four months after quadrivalent human papillomavirus vaccine (HPV4) and proceeding to premature ovarian failure over the next twenty four months occurred in a well 16-year-old girl. Exclusion of metabolic, other endocrine, genetic and overt autoimmune causes left unknown causation as it does in 90% of cases. Enquiry of vaccine animal testing found no research reports were available of ovarian histology or of ongoing ovarian function in vaccine tested rats. Histology reports were available of vaccine tested rat testes and epididymides. Pre-clinical studies did not consider the duration or capacity of the reproductive life-span. Subsequent phase II and phase III clinical studies before vaccine licensing have lacked the capacity to attest to ovarian function due to weaknesses in study design and hormonal contraceptive usage. Studies since licensing lack capacity to evaluate ovarian function due to focus on emergency department presentations, and definitional limitations. Vaccine adverse event notifications of amenorrhoea are poorly investigated and followed up. Other documented published cases of premature menopause following HPV4 vaccination indicate the need for further research of the ovary after HPV4 vaccination. In the interests of women’s reproductive health and egg-bearing capacity, this issue needs to be resolved prior to the implementation of universal vaccination programmes.

Keywords: HPV4 (Gardasil™) oligomenorrhoea periods ovarian failure menopause.
A 16-year-old girl presented with secondary amenorrhea following 12 months of oligomenorrhea after HPV4 vaccinations, completed in August 2008. Menarche had occurred at age 13 and a regular monthly pattern had established. Menstruation became irregular in early 2009 and scant and infrequent by 2010. Menstruation ceased in January 2011 and hot flushes commenced. The oral contraceptive pill (OCP) was prescribed, which she declined, preferring further assessment for continuing amenorrhea at which premature ovarian failure (POF) was diagnosed [1]. Amenorrhea and POF after HPV4 in young teens have been notified as vaccine adverse events to safety surveillance programmes such as the Vaccine Adverse Event Reporting System (VAERS) [2] in the USA. Cases of secondary amenorrhea in very young teens following HPV4 administration and subsequently diagnosed as premature ovarian failure have recently been published [3]. The incidence of POF between ages 15 to 29 years has been reported as 10/100,000 person-years [4] but the prevalence of idiopathic POF in very early to mid-teen age groups is uncertain. Research on ovarian health and safety in early teens after HPV4 was sought.

**MATERIAL AND METHODS**

Pre-clinical toxicology studies, clinical pre-licensing studies and post-licensing research and surveillance were reviewed. Request was made to the Therapeutic Goods Administration (TGA) for a histology report of the vaccine tested rodent ovary and for data concerning tested rats’ subsequent litters and numbers of pups therein. Clinical studies which had been identified by the Vaccine and Related Biological Products Advisory Committee [5] (VRBPAC) as HPV4 pre-licensing safety studies were reviewed for evidence of ongoing ovarian safety after HPV4. Post-licensing studies were reviewed for their capacity to detect safety signals for ovarian malfunction. VAERS database case histories (August 10th 2013) were searched for notifications indicating possible deterioration in ovarian function following HPV4.

**RESULTS**

*Pre-clinical studies*

The TGA agreed to a ‘freedom of information’ request (FOI 001-1112) for a histology report of the vaccine-tested rat ovary; and numbers of subsequent pups and litters produced by vaccine-tested rats. No histology report of vaccine-
tested rat ovaries was available. No research was available concerning ongoing rat reproductive function and subsequent fecundity. Tested rats conceived once at the onset of sexual maturity and evaluation of the ovary at post-weaning euthanasia recorded only a numbering of corpora lutea present. No record of ovarian cellular integrity was available [6]. A histology report of testes and epididymides is included in The TGA Public Assessment Report for HPV Vaccine February 2011 [7]. Pre-clinical studies to evaluate the safety profile and biological activities of vaccines inform subsequent clinical trials.

**Clinical Pre-licensing Safety Studies**

Pre-licensing studies of HPV4 which were identified by the VRBPAC [5] to the Food and Drug Administration as safety studies were Phase II study protocols V501: 007 [8], 016 [9], and 018 [10] and phase III protocols 013 (‘Future I’) [11] and 015 (‘Future II’) [12]. Of these safety studies, only phase II protocols 016 and 018 studied adolescents under 16 years. A vaccine report card recorded temperatures and adverse events occurring within 2 weeks of each vaccination and prompted for recording of local site reactions.

Protocol 016 [9] studied 506 healthy girls aged 10 to 15 years. Only 240 girls, 47.4%, completed the planned 12 month follow-up. The VRBPAC describes it as a ‘Phase II study of the safety and immunogenicity of Gardasil when administered to approximately 2500 healthy children’. The unexplained loss of the majority of participants to 12 month follow-up and small numbers of those remaining who had reached menarche, precludes this study from competence to evaluate ongoing ovarian function. One participant experienced vaginal haemorrhages 26 and 42 days after 2nd and 3rd vaccinations respectively. This event was initially deemed vaccine related.

Protocol 018 [10] fully vaccinated 492 girls aged 9 to 15: mean study age 11.9. The proportion post menarche is not clear. Protocol 018 Vaccine report card (VRC) prompted for injection site reactions and also prompted for reporting of headaches, rashes, muscle/joint pain and diarrhea that occurred within 14 days of each vaccination, but not menstrual aberration. Follow-up interviews to 18 months assessed general safety and all serious adverse events [13]: hospitalization, life threatening illness, disability, death, illness requiring surgical/medical correction. Health interviews within 18 months of 1st vaccination may not have the ability to detect menstrual abnormalities in very young teens at an undetermined interval post menarche when cycle patterns are still establishing. Investigators judged which events were vaccine related, and deemed that none, including dysfunctional uterine bleeding causing anaemia, were related.

In Protocol 007, 256 older women 16 to 23 years received 3x HPV4 Gardasil™. Participants ‘were required to use effective contraception throughout the
trial’ [14]. 57.9% used hormonal contraception and 13.2% who commenced vaccination did not complete the 3 year trial (a further 771 completed vaccination with other vaccine formulations or differing aluminium placebos).

Phase III ‘Future 1’ [11] and ‘Future 2’ [12] studies enrolled mostly older subjects 16 to 23 years. 58% to 60% of these phase III participants used hormonal contraception, potentially masking ovarian dysfunction. All phase III subjects were ‘required to use effective contraception day 1 through month 7’. A subsequent HPV4 safety studies review [15] of 21,480 females in licensing trials states ‘new medical conditions were not considered adverse events if they occurred post month 7 or were not considered by the investigators to be vaccine/placebo- or procedure-related’ [15]. These studies were inadequate for assessing reproductive safety. The TGA licensing body classifies an association between HPV4 and female fertility as not biologically plausible [16].

Selected placebo controls for safety trials comprised aluminium adjuvant or a combination [10] of polysorbate 80, borate, sodium chloride and yeast in young teen study 018. Each control has components implicated in ovarian pathology [3] [17].

Post-licensing Safety Studies

The major post-licensing study of HPV4 safety [18] reviewed 189,629 vaccinated females including 44,000 who had received three doses. Selected outcome measures were subjects’ hospitalizations and emergency department visits following vaccination. 11 to 12 year olds who received 3 doses comprised 4.3% of the overall study population; 9 to 15 year olds comprised 12.9%. The consultation context for seeking medical management of oligomenorrhoea or amenorrhoea is not normally the emergency department and will not require hospitalization. This study had no capacity to evaluate ongoing ovarian health or to monitor ovarian safety.

The Protocol 018 group of 577 girls who completed vaccinations became the sentinel study for long term safety of HPV4 in adolescents [19]. Surveillance comprised: annual physical examination and serum collection to age 16, then twice yearly collection of a sexual history and genital clinical specimens. Serious adverse experiences deemed by the investigator to be vaccine related, pregnancy outcomes and deaths are monitored. Protocol 018 reiterates ‘the relationship between adverse experiences and vaccine was reported by the investigator according to his/her best judgment, based on exposure, time course, likely cause and probability with vaccine profile’ [10, 15]. However, this vaccine’s reproductive safety profile in 2005-2007 and since has not yet been established.

The VAERS [2] notes 104 cases of new amenorrhoea post HPV4 of whom less than 9% reported a return of menses at follow-up. Only one subject out of
105 notifications had an FSH level recorded, and it was ‘elevated at 72’ (no units specified). In 62% of ongoing amenorrhea notifications to VAERS, no further information was obtained. No anti-Mullerian hormone levels were recorded. Four teens with POF following HPV4 are recorded under ‘amenorrhoea’, ‘ovarian failure’ and ‘premature menopause’. Other published cases [3] describe onset of declining menstrual function at ages of 13, 14 and 20 after HPV4 preceding diagnoses of premature ovarian failure.

Diminished menstrual patterns do not signal as ‘Serious Adverse Events’ in surveillance and are invisible with OCP; ongoing Vaccine Safety Datalink surveillance of conditions arising post HPV4 does not include menstrual abnormalities in its focus [20]. Other surveillance methods rely on known background prevalence and controls [21].

**CONCLUSION**

Pre-clinical, clinical and post-licensure safety studies of HPV4 were unable to evaluate ovarian safety. This matter needs to be resolved, since a potential compromise of future ovarian function could have serious implications for population health and fecundity.

**ACKNOWLEDGMENTS**

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**REFERENCES**

1. BMJ Case Reports 2012; doi:10.1136/bcr-2012-006879.
6. Extract Study no.TT#03-703-0(CTD) Module 4, volumes 1-3) summary for non-clinical study report ‘Intramuscular developmental toxicity and immunogenicity study in rats with postweaning evaluation’.
7. Wise DL, Jayanthi JW, Caplanski CV et al. Lack of effects on fertility and developmental
Oncology
Proteomic Studies to Identify Key Proteins Associated with Platinum Resistance in Ovarian Cancer

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SUMMARY

Whereas platinum resistance is associated with increased expression of anti-apoptotic factors and pathways such as NF-κB and AKT, a number of phytochemicals serve to dampen their expressions so that they may act synergistically in combination towards the cell kill. In this study, combinations of platinums and selected tumour active phytochemicals including colchicine, curcumin, EGCG, andrographolide, thymoquinone, resveratrol, lupeol, genistein, betulinic acid and ursolic acid administered to human ovarian A2780, A2780cisR and A2780ZD0473R cancer cell lines are found to produce sequence- and concentration dependent synergism. Generally the degree of synergism is greater when the drugs are administered with 2 to 4 h time gap than as a bolus. Proteomic studies have identified over thirty proteins that are differentially expressed in the resistant A2780cisR cell line as compared to the parent A2780 cell line. The proteins that are restored back to normally due to treatments with synergistic combinations, belong to the following six major groups: i. cytoskeletal proteins involved in invasion and metastasis, ii. molecular chaperone and stress related proteins, iii. proteins involved in detoxification and drug resistance, iv. proteins involved in metabolic processes, v. mRNA processing proteins and vi. others. The results relating to combined drug action, cellular accumulation of platinum, the level
of platinum-DNA binding, changes in protein expression after treatment with drug combinations all provide support to the idea that the presence and nature of the phytochemicals and that of the platinum drug as well as the sequence of administration are all important factors influencing the combined drug action.

Keywords: Ovarian cancer; cisplatin; phytochemicals; drug combination; synergism; proteomics.

INTRODUCTION

Tumour active phytochemicals exert their antitumour activity through regulation of cell signalling pathways different from those of platinum drugs so that they can act synergistically in combination with platinum drugs. Such combinations may also reduce systemic toxicity caused by chemo- and radiotherapies because of lower doses required [1]. The major factors involved in the development of platinum resistance are multidrug resistance gene, nuclear factor-κB (NF-κB), and serine/threonine protein kinase Akt; whereas about 15% of all solid tumours are driven by NF-κB as a player, most cancer preventive agents are believed to be NF-κB inhibitors [2]. As a part of our continued studies to

Fig. 1. Structures of platinum drugs cisplatin, carboplatin and oxaliplatin, and a number of phytochemicals used in combination studies.
overcome drug resistance, we applied sequenced combinations of targeted therapy and selected phytochemicals including EGCG, capsaicin, genistein, curcumin, quercetin, resveratrol and thymoquinone (TQ) that are well-known antioxidants and display a variety of biological activities including chemoprevention and inhibition of tumour growth [2]. Quercetin exerts antitumour activity by inhibiting the activation of NF-κB [3], resveratrol by down-regulation of NF-κB, STAT3, anti-apoptotic and cell survival gene products [4] and COX expression [5] and up-regulation of the tumour suppressor p53 and cytokine (MIC-1) [6-9], thymoquinone through modulation of apoptosis signalling, inhibition of angiogenesis, and cell cycle arrest [5], curcumin by targeting pro-apoptotic proteins including p53 and bax, transcription factors including NF-κB, Akt, p38 MAPK, cytokines including growth factors such as EGF and PDGF. In this study we report on studies on the combination between platinum drugs and phytochemicals (Figure 1) applied to ovarian tumour models.

METHODS

A number of techniques including cell culture, MTT reduction assay, methods to determine cellular accumulation of platinum, levels of Pt-DNA binding, proteomics and glutathione assay to monitor oxidative stress have been used in the study.

RESULTS AND DISCUSSION

Figure 2 gives combinations indices (CIs), figure 3 gives cellular accumulation of platinum and levels of Pt-DNA binding as applied to the sequenced combinations of cisplatin (Cis) and oxaliplatin (Oxa) with selected phytochemicals. The results indicate that generally administrations with 2 h time gap are synergistic whereas administration as a bolus is additive to antagonistic. Also, generally synergistic combinations are associated with higher Pt accumulation as well as higher levels of Pt-DNA binding than the bolus. Proteomic studies show that a number of proteins undergo differential expressions in the resistant A2780cisR cell line as compared to parent A2780 cell line (table 1) but are restored back to normalcy after treatment with synergistic combinations highlighting that cell death is actually brought about by the proteins as would be the escape from apoptosis. Specifically synergistic combinations were found to down-regulate the expressions of proteins that are known to play key roles in tumour development and progression including inhibition of apoptosis, angiogenesis and cell invasion.
Fig. 2. Combination indices at median effect dose applying to the combinations of cisplatin (Cs) and oxaliplatin (Oxa) with a number of phytochemicals in A2780 and A2780 cisR cell lines, indicating that generally administrations with 2 h time gap are synergistic whereas bolus addition is additive to antagonistic: CI > 1: synergistic; = 1: additive and >1: antagonistic.
Fig. 3. Cellular accumulations of platinum and the levels of Pt-DNA binding indicating that administrations with 2 h time gap result into higher values for both.
Tab. 1. Proteins found to undergo changes in expression in A2780cisR cell line as compared to A2780 cell line.

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Finally, if confirmed in vivo, the results of the study support the idea that synergistic combinations of targeted therapy and tumour active phytochemicals can provide a means of overcoming drug resistance in ovarian cancer.

ACKNOWLDEGMENTS

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REFERENCES

Combinations of Platinum Drugs with Bortezomib in Ovarian Cancer Cell Lines

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SUMMARY

Platinum drugs such as cisplatin (CS), carboplatin (CB) and oxaliplatin (OX) are believed to enter cells by both passive diffusion and use of carriers such as copper transporter 1 (CTR1). However, the drugs can induce internalization followed by proteasomal degradation of CTR1 thus decreasing their own uptake and consequently the level of binding to DNA. Bortezomib (BORT) plays a protective role against platinum-induced proteasomal degradation of CTR1 so that in presence of BORT cell kill can be enhanced due to increase in the cellular platinum accumulation and consequently the level of platinum-DNA binding. Proteomic studies have identified 72 proteins that are differentially expressed in the ovarian cancer A2780 and A2780cisR cell lines; at least 30 of them are restored back to normalcy due to treatment with synergistic drug combinations of BORT and platinum drugs, indicating their relevance in synergistic drug action.

Keywords: Bortezomib, carboplatin, cisplatin, copper transporter 1, drug combination, resistance, oxaliplatin, proteasomal degradation.
INTRODUCTION

The management of ovarian cancer remains an ongoing challenge as the disease is usually diagnosed at a late stage where it is likely to have metastasized. Currently, chemotherapy based on platinum drugs such as cisplatin is the primary treatment for the disease. However, the development of drug resistance is a major hurdle in the advancement of platinum chemotherapy. One of the multiple factors responsible for platinum resistance is the reduced drug uptake. CS can cross the cell membrane by both passive diffusion and use of carriers such as CTR1 [1, 2]. However, CS is found to trigger the down-regulation and proteasomal degradation of CTR1 in human ovarian cancer [3]. A proteasome inhibitor known as bortezomib has been reported to block cisplatin-induced down-regulation of CTR1 so that in the presence of bortezomib the cellular uptake of cisplatin may be increased and consequently the level of its binding with the DNA may also be increased [4]. This suggests that cisplatin and bortezomib may act synergistically in combination.

MATERIAL AND METHODS

In this study, efficacy from the sequenced combinations of CS, CB and OX with BORT in the human ovarian A2780, A2780\textsuperscript{cisR}, A2780\textsuperscript{ZD0473R} and SKOV-3 cancer cell lines was evaluated. The levels of cellular platinum accumulation and platinum-DNA binding as well as the levels of total and oxidized glutathione were determined. Finally, changes in expression of key proteins associated with platinum resistance were determined using proteomics.

![Graphs showing Combination Index (CI) values for different combinations.](image)

**Fig. 1.** Combination Index (CI) values applying to the sequenced combinations of BORT with a) CS, b) CB and c) OX, administered to the ovarian cancer A2780, A2780\textsuperscript{cisR}, A2780\textsuperscript{ZD0473R} and SKOV-3 cell lines. Drugs were added at their equipotent ratios and CI values were calculated following 72 h treatments. CI values of <1, =1 and >1 indicate respectively synergism, additivity and antagonism in combined drug action.
Fig. 2. Cellular platinum accumulation in ovarian cancer A2780 and A2780\textsuperscript{cisR}, cell lines as applied to CS, CB and OX and their selected combinations with BORT. Cells were treated with the drugs for 24 h followed by collection, lysis and finally the detection of Pt was using AAS. Data were statistically analyzed using the paired Student’s t test: * $p < 0.05$ indicates significant difference from control. Error bars represent the standard deviation.

Fig. 3. Platinum DNA binding levels in the ovarian cancer A2780 and A2780\textsuperscript{cisR} cell lines following treatment with CS, CB and OX and their combinations with BORT. Cells were treated with the drugs for 24 h followed by collection, DNA extraction and finally detection of Pt using AAS. Data were statistically analyzed using the paired Student’s t test: * $p < 0.05$ indicates significant difference from control. Error bars represent the standard deviation.
RESULTS

The sequenced combinations of platinum drugs with BORT are found to enhance cell kill in ovarian cancer A2780, A2780\textsuperscript{cisR}, A2780\textsuperscript{(cisD0473R)} and SKOV-3 cell lines (Fig. 1). Combination of CS, CB and OX with BORT is also found to enhance cellular accumulation of platinum (Fig. 2) and the level of Platinum-DNA binding (Fig. 3) in line with the idea that bortezomib plays a protective role against cisplatin-induced CTR1 degradation. The presence of BORT was also found to induce oxidative stress (Fig. 4). The expression of over thirty proteins associated with drug resistance is found to be altered by the selected combinations. Proteomic studies have shown that of the 72 proteins found to be differentially expressed in A2780 and A2780\textsuperscript{cisR} cell lines, at least 30 of them are restored back to normalcy as a result of treatment with synergistic drug combinations (Fig. 5), indicating their relevance in synergistic drug action.

CONCLUSIONS

Combinations of platinum drugs with BORT are found to increase cellular accumulation of platinum and the level of platinum-DNA binding, increase oxidative stress, and alter expressions of key proteins associated with drug resistance [5]. The results of the studies may be significant both from basic scientific and clinical view points.
ACKNOWLEDGMENTS

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REFERENCES

Detection of Testicular Cancer in a Physical Examination in Patients Receiving Assisted Reproductive Technology


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SUMMARY

Male infertility is not commonly caused by a testicular tumor. Testicular tumors are typically discovered by palpation when patients visit urologists because of testicular enlargement, an elastic firm mass in the testis, or testicular pain. Progress in assisted reproduction technology (ART) has reduced the number of testicular biopsies.

However, ART has also resulted in increased incidental detection of testicular tumors upon performance of testicular sperm extraction (TESE), but these tumors may still be overlooked in the first physical examination. The objective of the study was to evaluate the utility of physical examination as a means of detecting testicular tumors in patients undergoing fertility treatment.

Keywords: assisted reproductive technology (ART), testicular tumor, physical examination.

INTRODUCTION

Spermatogenesis in patients with a concomitant testicular tumor often occurs before diagnosis of the tumor. Thus, Ho et al. [1] found impaired spermatoge-
nosis at the time of tumor diagnosis in more than 50% of patients. Testicular tumors are typically discovered by palpation when patients visit urologists because of testicular enlargement, an elastic firm mass in the testis, or testicular pain. Progress in ART has increased the rate of successful pregnancy, but current infertility evaluation tends to downplay the conditions that lead to male infertility, including the occurrence of testicular tumor.

Moreover, it has reduced the number of testicular biopsies. However, ART has also resulted in increased incidental detection of testicular tumors upon performance of TESE, but these tumors may still be overlooked in the first physical examination. The findings from these cases suggest that patients with male infertility should be examined by careful palpation and ultrasonography to rule out concomitant diseases complicating infertility.

**PATIENTS AND METHODS**

Among 653 infertile patients who visited our clinic for testicular sperm extraction (TESE) for assisted reproduction technology (ART) over an eight-year period, only 2 were found to have testicular tumors. The rate of detection at this stage in our institution was 2 of 653 infertile patients (0.31%). The first case was a 39-year-old male who presented initially for ART with a 6-year history of primary infertility with oligoasthenozoospermia and he was referred to our male infertility department for preparation for intracytoplasmic sperm injection (ICSI). On physical examination, a thumbtip-sized elastic firm, irregular, painless mass was palpable at the head of the right testis.

The second case was a 41-year-old azoospermic man who was referred to our Department with a 16-year history of primary infertility, swelling of the left testicle and pain. He was diagnosed with epididymitis and treated for several days by his neighborhood physician. His previous history was orchiopexy for bilateral cryptochism. A 6-g atrophic left testis and a walnut-sized elastic, firm, painful, nodular mass in the right testis were palpable on physical examination.

**RESULTS**

In both cases, endocrinological findings were unremarkable except for elevated serum hCG, hCGβ and AFP. In the second case, blood biochemical analysis revealed a positive CRP response. In case 1, CT and MRI demonstrated no metastatic foci. Right high orchiectomy was performed. Tumor showed of 3.5 × 4 cm on the affected side. The initial diagnosis was embryonal carcinoma, but further investigation was performed because of elevated hCG and AFP. This
revealed embryonal carcinoma intermixed with multinucleated giant cells, and these features were taken to indicate embryonal carcinoma with syncytiotrophoblastic giant cells (STGCs). Teratoma was also suspected based on the presence of mature cartilage, mature cellular fibrous tissues and mature smooth muscles in a few small areas, and immature tissues (cartilage) in others. hCG staining was positive for giant cells, but some cytotrophoblast clusters and glandular structures were also stained, in addition to trophoblastic giant cells (Figure 1). Additional staining revealed AFP-positive cells, indicating embryonal carcinoma based on the cell structure. The final diagnosis was embryonal carcinoma and seminoma with STGCs accompanied by mature and immature teratoma.

Fig. 1. Histopathological findings for the right testicular tumor in Case 1. Hematoxylin and eosin (HE) stained section showing embryonal carcinoma + seminoma with STGCs + teratoma, mature and immature, hCG(+), AFT(+) germ cell tumors of more than one histological type. A. Embryonal carcinoma with STGCs. B. Embryonal carcinoma with seminoma. C: Teratoma with embryonal carcinoma and mature cellular fibrous tissues. D. Mature cartilage (arrow).
(Figure 2). After three courses of bleomycin, etoposide and cisplatin (BEP) as postoperative adjuvant therapy, tumor markers normalized. The patient has been closely followed-up for 8 years without recurrence. ICSI has been successfully performed.

In the second case, diagnostic CT and ultrasonographic imaging failed to confirm a diagnosis, but biopsy eventually led to the diagnosis of seminoma of the right testis. Radical orchietomy was performed. Tumor showed of 4.5 × 6 cm comprising large cells with clear endoplasmic reticula. The case was diagnosed as Stage I anaplastic seminoma (Figure 3). Attempts at ICSI were not continued for this patient.

**CONCLUSIONS**

Testicular tumor is the most common malignant tumor in men aged 15-35 years old and has a curability of less than 10% [2]. Its incidence in Japan is 0.7-1.4 per 100,000, which is lower than that in Europe and the USA. In 1983, Skakkebeck [3, 4] found that male infertility patients with atypical germ cells in testicular biopsy later developed embryonal carcinoma, and suggested that these cases should be classified as CIS of the testis. Pasqualotto et al. reported 7 infertility patients diagnosed with subfertility with testicular tumors [5]. Honing
et al. [2] described cases of male infertility caused by pathohistological factors, including 10 with malignant tumors that included 6 testicular tumors. It is noteworthy that two of the cases of testicular tumor were diagnosed with normozoospermia upon semen analysis. This emphasizes that not every case of male infertility gives abnormal findings in semen analysis, although such results are common in both male infertility and cases of testicular tumor. Diagnosis of testicular cancer is often based on clinical clues during routine history taking and physical examination. A testicular tumor, which may impair spermatogenesis or cause male infertility under different pathological conditions, can be diagnosed easily by careful palpation and is curable if discovered early. Zapzalka et al. [6] reported that oncologists estimated that 27% of their patients chose to cryopreserve sperm.

However, only 26% of the oncologists were aware of ICSI. The cancers that were mainly perceived to warrant cryopreservation were lymphomas, leukemias, and testicular carcinomas, whereas the treatment modalities perceived to warrant cryopreservation included various chemotherapy and radiation regimens.

The prevalence of testicular tumor in Japan has remained unchanged in the
past 15 years, but is likely to rise with the progress of ART. This study here suggest that male infertility should be regarded as a symptom that can be associated with various underlying pathological conditions. Pretreatment cryopreservation of semen may be useful psychologically in patients with newly diagnosed testicular cancer, but has doubtful clinical significance. These findings indicate an important problem in ART that can only be resolved through cooperation among specialists from different fields.

REFERENCES

Silibinin Induces Cell Death Through ROS-Dependent Down-Regulation of Notch-1/ERK/Akt Signaling in Human Breast Cancer Cells

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SUMMARY

The present study was undertaken to determine underlying mechanism of silibinin-induced cell death in human breast cancer cell lines MCF7 and MDA-MB-231. Silibinin induced cell death was attenuated by antioxidants, N-acetyl-cysteine (NAC) and Trolox, suggesting that the effect of silibinin was dependent on generation of reactive oxygen species (ROS). Western blot analysis showed that silibinin induced down-regulation of ERK and Akt. In conclusion, ROS generation and Notch-1 signaling act upstream of the ERK and Akt pathway in the silibinin-induced breast cancer cell death. Silibinin induced cell death through an AIF-dependent mechanism in MCF7 cells and a caspase-3-dependent mechanism in MDA-MB-231 cells. These data suggest that silibinin may serve as a potential agent for induction of apoptosis in human breast cancer cells.

Keywords: Silibinin, breast cancer, Notch-1/ERK/Akt.

INTRODUCTION

Breast cancer is the most common cancer in Korean women. Although, ef-
Effective treatment for breast cancer includes surgery, radiation and chemotherapy, breast cancer frequently show resistance to these therapies. The aim of the present study is to determine the molecular mechanisms of the silibinin-induced cell death in MCF7 and MDA-MB-231 breast cancer cell lines. Our data demonstrated that silibinin induced human breast cancer cell death through the ROS/Notch-1/ERK/Akt pathway, followed by the nuclear translocation of AIF in MCF7 cells, and the caspase-3 activation in MDA-MB-231 cells.

MATERIALS AND METHODS

Cell viability was evaluated using a MTT assay and apoptotic cells were identified by condensation and fragmentation of nuclei. The intracellular generation of ROS was measured using DCFH-DA. AIF nuclear translocation was measured. Cells were cultured on cover glasses and treated with silibinin. Cells were viewed under a fluorescent microscope. Silencing of AIF expression was achieved by the small interfering (siRNA) technique.

RESULTS

Silibinin significantly decreased cell viability in both cell lines (Figure 1). The cells treated with silibinin exhibited nucleus morphology with DNA fragmentation, which is the typical feature of apoptosis. Silibinin induced the increase of ROS generation, and these effects were blocked by an antioxidant, NAC. ROS generation increases to 12 hr after silibinin treatment in a time-dependent manner (Figure 2). These results suggest that the silibinin-induced cell death is associated with ROS generation in breast cancer cells.

Silibinin induced down-regulation of phospho-ERK and phospho-Akt in a time dependent manner. Expression of both kinases was increased compared with cells transfected with the empty vector (EV). The data show that silibinin-induced cell death was prevented by caMEK and caAkt. The decrease of phospho-ERK and phospho-Akt by silibinin was prevented by NAC in both breast cancer cells. These data suggest that downregulation of ERK and Akt depends on ROS generation and play a critical role in silibinin-induced cell death. Silibinin induced down-regulation of Notch-1 mRNA expression. Likewise, it decreased Notch-1 protein synthesis time-dependently in both breast cancer cells. Both breast cancer cells overexpressing Notch-1 exhibited resistance to silibinin-induced cell death, indicating that the silibinin-induced cell death is associated with downregulation of Notch-1 signaling pathway in both MCF7 and MDA-MB-231 cells. Results suggest that silibinin-induced inhibition of pho-
spho-ERK and phospho-Akt was due to down-regulation of Notch-1 signaling in both breast cancer cells. The effect of silibinin on the AIF nuclear translocation was measured by Western blot analysis. Silibinin increased the translocation of AIF into the nucleus in a time-dependent manner in MCF7 cells, whereas only a small amount of AIF was translocated into the nucleus in MDA-MB-231 cells as compared with MCF7 cells.

**DISCUSSION**

Silibinin has been shown to have the anti-tumor activity and the anti-inflammation effect in various cancer models. Silibinin is a dietary ingredient in which we can take easily from foods such as fruits, vegetables, grains, and tea. It has a wide range of pharmacological effects such as the inhibition of DNA synthesis, cell proliferation, cell cycle progression, and apoptosis in various cancer models.
cell lines including breast cancer cells [1]. However, molecular mechanism of silibinin-induced cell death has not been clearly defined.

In the present study, silibinin induced ROS generation and the anti-oxidant NAC prevented the silibinin-induced cell death. These results indicate that the silibinin-induced breast cancer cell death is associated with ROS generation. These results are consistent with those reported in MCF7 cells [2]. Silibinin has been shown to induce cell death through down-regulation of ERK1/2 and Akt activity in various cancer cells [3]. Therefore, ERK and Akt expression level may be critical in silibinin-induced cell death. Silibinin-induced inhibition of ERK and Akt was blocked by the antioxidant NAC, suggesting that silibinin induces down-regulation of ERK and Akt through a ROS-dependent mechanism.

Notch signaling promotes cell growth migration, invasion, and apoptosis in various cancer cells. It has been reported that cell survival kinases such as ERK and Akt are involved in Notch signaling pathway [4]. Silibinin decreased Notch-1 mRNA and protein level. In addition, silibinin-nduced cell death was prevented by overexpression of Notch-1 in both cell lines, indicating involvement of Notch1 signaling in the silibinin-induced cell death in breast cancer cells.

![Fig. 2. Role of ROS generation in silibinin-induced cell death. Cells were loaded with DCFH-DA for 1 hr and treated with 30 µM silibinin at indicated various times. Data are mean ± SEM of three independent experiments performed in duplicate. * p<0.05 compared with silibinin alone. C, control.](image-url)
AIF is released from mitochondria, and translocate from the cytosol to the nucleus. In nucleus, it induces oligonucleosomal DNA fragmentation, a hallmark of caspase-independent apoptosis [5]. Our data show that silibinin increased nuclear translocation of AIF in the both breast cancer cells as demonstrated by the Western blot analysis and immunohistochemistry.

In conclusion, silibinin induces ROS-dependent down-regulation of Notch/ERK/Akt signaling, which leads to the cell death through a caspase-3-dependent mechanism in MDAMB-231 cells and an AIF-dependent mechanism in MCF7 cells. The data suggest that silibinin induces cell death through different mechanisms between MCF7 and MDA-MB-231 cells (figure 3).

Figure 3. Proposed model of silibinin-induced cell death in human breast cancer cell lines. Silibinin increased ROS generation which inhibited Notch-1 signaling pathway. These signal transductions lead to down-regulation of phosphorylation of ERK and Akt. Down-regulation of ERK and Akt induced cell death through a caspase-3-dependent mechanism in MDA-MB-231 cell and an AIF-dependent mechanism in MCF7 cell.
ACKNOWLEDGEMENTS

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REFERENCES

Stage 1 Ovarian Carcinoma: Clinico-Pathological Correlations

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SUMMARY

Ovarian Carcinomas (OC) are the most common and lethal gynecological tumors due to their late stage diagnosis, being rarely detected as Stage I when the 5 year survival is 90%, compared to 35% for all stages. In our study histologic slides and clinical files of 99 patients diagnosed with Stage I OC were reviewed and correlated with clinical presentation. Serous and non-serous OC (OSC and NSOC) were analyzed showing a different histologic pattern distribution in Stage I, as compared to all-stage OC. Serous OC, the vast majority of OC in all stages of the disease, represented about 27% of cases, while the otherwise uncommon NSOC predominated as 73%. OSC are notorious for being asymptomatic and were detected in Stage I fortuitously in many patients with histories of breast cancer due to frequent pelvic examinations. NSOC were often discovered in Stage I because of their associated symptomatic pathological lesions: endometriotic painful masses and uterine pathology, manifested as abnormal vaginal bleeding. The patients had also different clinical profiles, those with NSOC being younger, hyperestrogenic and harboring slower growing tumors. The clinical-pathologic correlations revealed a shift in OC distribution with potential impact for early diagnosis. Molecular and genetic studies confirm the existence of two different carcinogenic processes in OC. Patients with endometriosis, endometrial pathology, history of breast cancer should be considered at high risk for OC and are diagnosed earlier because of the associated symptomatic pathology.
**INTRODUCTION**

Ovarian Carcinomas (OC) are the most lethal gynecologic cancer, more frequently lethal than all other gynecologic cancers combined. OC are most commonly diagnosed in late stages, when spread beyond the ovaries to other pelvic and/or abdominal locations. Early diagnosis of OC, when the tumor is confined to the ovary(ies) is a major challenge, given the fact that the five year survival is 80-90% for patients diagnosed in Stage I versus 19-32% for those diagnosed in Stages III-IV. The reason for the late diagnosis is the relative paucity of symptoms (now challenged) and lack of reliably specific and sensitive tumor markers.

A small percentage of OC however are diagnosed in Stage I. We have analyzed 99 such cases and tried to identify clinical-pathologic correlations that can have an impact on early diagnosis. The vast majority of OC (68-87%) including the most lethal tumors are Epithelial Ovarian Carcinomas (EOC), arising in ovarian, fallopian tube fimbrial and possibly peritoneal lining cells.

**MATERIALS AND METHODS**

Clinical files and histologic slides from 99 patients diagnosed with Stage I OC were reviewed and classified, according to the World Health Organization (WHO) into serous (OSC) and non-serous (NSOC) carcinomas: endometrioid (OEC), Clear Cell (CCC), mixed OEC and CCC, and mucinous (OMC) carcinomas (see Table 1). Associated lesions such as ovarian and pelvic endometriosis, ovarian adenofibroma, endometrial polyps and hyperplasia, and endometrial carcinoma were included in the study. The histo-pathologic findings were correlated with patients’ age, history of breast cancer and presenting symptoms: pelvic masses, symptomatic or incidentally discovered (asymptomatic), vaginal bleeding, ascites, gastrointestinal symptoms. The histologic slides were reviewed independently by two pathologists (LD, AM). Ovarian tumors of borderline malignancy were excluded from the review.

Only confirmed primary invasive mucinous carcinomas were included, not associated with gastrointestinal tumors. In cases of synchronous endometrial and ovarian tumors, their independent primary origin was determined based on accepted criteria, including histological dissimilarity, no or superficial myometrial invasion, absence of lympho-vascular invasion and no evidence of tumor spread elsewhere.
RESULTS

OSC, by far the most common OC, were diagnosed in 27 of 99 patients (27.27%), while NSOC represented 72.7%, the majority of all Stage I OC studied. Most OSC patients presented with asymptomatic, bilateral pelvic masses (Fig. 1); only 18% had symptomatic pelvic masses. A history of breast carcinoma was present in 11 cases (40.7%), in patients who were under close medical surveillance; «silent» pelvic masses were discovered incidentally due to frequent gynecologic examinations. One patient had a synchronous primary endometrial carcinoma after Tamoxifen therapy for breast cancer. Four patients presented with gastrointestinal symptoms and three with ascites. The average age of the patients was 61 years.

NSOC representing over two thirds of the patients in our study, were in their majority OEC, few were OEC mixed with CCC, followed by pure CCC and OMC (see Table 1). In all stages CCC are uncommon tumors, representing about 6% of EOC; in our material there were 14 CCC cases (over 14%). In 9 cases asymptomatic Stage I OC was identified during hysterectomies for endometrial pathology.

Clinically, 47 patients with NSOC presented with symptomatic unilateral pelvic masses and 23 with vaginal bleeding due to endometrial pathology (Fig 2). Twenty one patients with NSOC were diagnosed with synchronous primary endometrial carcinoma. Ovarian endometriosis was present in 31 cases, often

<table>
<thead>
<tr>
<th>Clinical and pathological data</th>
<th>Serous carcinoma (n=27)</th>
<th>Non-serous carcinomas (NSOC) (n=72)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average age</td>
<td>61.4</td>
<td>55</td>
</tr>
<tr>
<td>Asymptomatic pelvic mass</td>
<td>16 (59.2%)</td>
<td>3 (6.12%)</td>
</tr>
<tr>
<td>Symptomatic pelvic mass</td>
<td>5 (18.5%)</td>
<td>25 (31.02%)</td>
</tr>
<tr>
<td>Vaginal bleeding</td>
<td>1 (3.7%)</td>
<td>18 (26.73%)</td>
</tr>
<tr>
<td>History of breast cancer</td>
<td>11 (40.74%)</td>
<td>0</td>
</tr>
<tr>
<td>Ascites</td>
<td>3 (11.11%)</td>
<td>0</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>4 (14.81%)</td>
<td>0</td>
</tr>
<tr>
<td>OC diagnosed at hysterectomy</td>
<td>0</td>
<td>4 (8.16%)</td>
</tr>
<tr>
<td>Endometrial adenocarcinoma</td>
<td>1 (3.7%)</td>
<td>20 (40.81%)</td>
</tr>
<tr>
<td>Ovarian endometriosis</td>
<td>2 (7.40%)</td>
<td>31 (63.26%)</td>
</tr>
<tr>
<td>Ovarian adenofibroma</td>
<td>1 (3.7%)</td>
<td>4 (8.16%)</td>
</tr>
<tr>
<td>Endometrial polyp/hyperplasia</td>
<td>4 (14.81%)</td>
<td>14 (36.16%)</td>
</tr>
</tbody>
</table>

Tab. 1. Clinico-pathological correlations in ovarian carcinomas.
associated with OEC, CCC and OMC. Endometrial polyps were over twice more frequent in patients with NSOC compared to OSC. The average age of the patients with NSOC was 55 years.

**CONCLUSIONS**

When is OC diagnosed in Stage I? According to some data, in only 1.25% of high grade OSC [1]. A spectacular decline in the morbidity and mortality of cervical and endometrial cancer, as well as of many other malignant tumors, took place over the past 5 decades due to the diagnosis of early stages and identification of precancerous lesions. Unfortunately, this was not the case with OC the morbidity and mortality of which has not changed much over this period.

Despite annual screening with serum marker CA125 and transvaginal ultrasound, over 70% of patients are presenting in late stages, not different from the unscreened population. The routinely used CA125 is elevated in <50% of stage...
I ovarian cancers; furthermore, its specificity is poor due to falsely elevated values in a large number of benign and malignant conditions (such as pregnancy, endometriosis, pelvic inflammatory disease, lung and colon cancers). For that reason, the traditional methods for early diagnosis (serum CA125, transvaginal ultrasound and magnetic resonance imaging), with high false-negative rates, as well as lower sensitivity and specificity for clinical needs, have not demonstrated the capacity to reduce morbidity and/or mortality of this neoplasm.

Stage I OC is still an uncommon diagnosis but it is possible to envision progress based on identification of associated clinical and histological factors, as well as understanding the tumors’ molecular background. In their latest article, Dr. RJ Kurman and colleagues describe the earliest molecular alterations that seemingly cause ovarian neoplasia, alterations that begin in the secretory cells of the fallopian tube, so called SCOUTs. The same research team speaks about a subset of normal-appearing secretory cells that overexpress p53 and about the recent sequencing of the entire genome of ~ 400 ovarian high grade serous carcinomas that showed $TP53$ mutations in more than 96% of cases [2].

Fig. 2. Primary ovarian non-serous carcinoma with concomitant primary uterine adenocarcinoma.
Other recently published studies have revealed that aberrant DNA methylation, which usually occurs before patients develop clinical manifestations and radiographic evidence, provides a new molecular approach for the early diagnosis of cancer. Zhang et al. identified seven candidate genes (APC, RASSF1A, CDH1, RUNX3, TFPI2, SFRP5 and OPCML) with a high frequency of methylation and established a multiplex methylation-specific PCR assay to improve the early detection of ovarian cancer, via identification of the methylation status of cell-free serum DNA [3, 4, 5].

The association between vascular endothelial growth factor (VEGF) overexpression and survival outcomes in ovarian cancer patients was studied extensively by different research teams; most studies showed that overexpression of VEGF in primary ovarian tumors and serum is associated with poor progression-free survival and overall survival for patients with ovarian cancer. While the association between high tissue VEGF level and poor prognosis exists in early stage patients, it does not exist in advanced stage patients [6].

A research group from Sweden investigated ovarian cyst fluid as a source for discovering biomarkers for use in the diagnosis of epithelial-derived ovarian adenocarcinoma, identified 87 differentially expressed proteins and validated two candidates to verify the quantitative mass spectrometry, iTRAQ method [7].

Our study showed that OSC, by far the most common and most lethal ovarian cancer, is rarely diagnosed in Stage I (in less than one third of cases) while less common ovarian cancers, NSOC including the rare clear cell carcinoma, are predominant. The OSC were diagnosed in women in whom asymptomatic masses were discovered fortuitously because of frequent gynecologic check-ups due to histories of breast cancer. The concomitant presence of endometriosis was rare in this group (7.40%); there was one case of endometrial carcinoma in a patient with a history of breast cancer treated with Tamoxifen.

The group of patients diagnosed with NSOC represented the majority of Stage I OC. They had a different clinical profile: younger age, symptomatic pathology such as painful pelvic masses (endometriotic cysts), abnormal vaginal bleeding due to endometrial hyperplasia and neoplasia and occasional «mute» ovarian carcinomas discovered at hysterectomy for uterine pathology. There were 22 cases of coexisting primary endometrial carcinoma among the 72 NSOC, and in 9 cases a Stage I OC was discovered during hysterectomy for endometrial pathology.

Molecular and genetic studies revealed substantial differences between low-grade and high-grade OC, the two categories analogous to our OSC and NSOC. A reliable tumor marker for early OC however, is still elusive. Patients with endometriosis, endometrial pathology, history of breast cancer should be considered at high risk for OC and can be diagnosed earlier due to the associated symptomatic pathology.
REFERENCES


Molecular Prognostic Factors in Patients with Cervical Cancer Treated with Radiochemotherapy

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SUMMARY

The aim of the presented study was to evaluate the prognostic and predictive significance of selected molecular factors. The analysis was performed on retrospective data of 131 patients with squamous cell carcinoma, FIGO stages IB2-IIIB and treated with radiochemotherapy and brachytherapy between 2003 and 2008. The following groups of factors were assessed: population-based (age), clinical (haemoglobin level before and during treatment, SCC-Ag level after treatment, FIGO stage, overall treatment time), microscopic (grade, mitotic index, presence of atypical mitoses), immunohistochemical (P53, P16, Bcl2, EGFR, Bax, Cox-2, MVD expression, Ki-67 index), cytofluorometric (ploidy, S phase fraction (SP), S+G2M phase fraction (proliferative index PI), percentage of aneuploidy cells) and fluorescence in situ hybridisation to assess the EGFR1 gene amplification and chromosome 7 ploidy. Significantly better 5-year overall survival (OS) was observed in patients characterized by: Hb level during the treatment above 11g/dl, not elevated after treatment SCC-Ag level, expression of the BP epitope of P53 protein < 12, Ki-67 index < 52, mitotic index < 40, SP < 11%, PI < 20%, and diploid type of tumor. The following factors affected the disease-free survival (DFS): Ki-67 index > 52, atypical mitoses, mitotic index > 40, FIGO stage above IIA, SP > 11%, PI > 20%, SCC-Ag level. In the multivariate Cox analysis only SCC-Ag level was an independent prognostic
factor for OS and DFS. The best change for complete clinical regression after treatment have the patients with SP $\leq 11\%$ and Ki-67 index $< 52$.

**Keywords:** prognosis, predictive factors, irradiation, cisplatin, gynecologic cancer.

**INTRODUCTION**

Standard treatment of cervical cancer stages IB2-IIIB is cisplatin given during teleradiotherapy and brachytherapy, resulting in a 5-year survival of 66% (1). Further outcome improvement depends on treatment based on the molecular characteristics of the tumor, new irradiation techniques and drugs more effective than standard chemotherapeutics, including molecular targeted compounds. In clinical practice clinicopathologic factors, including stage and tumor histology are the most important prognostic factors in cervical cancer treated with radiochemotherapy (2). For over two decades many biological markers involved in tumorigenesis, tumor progression, apoptosis, angiogenesis, and cell growth have been studied in relation to survival and response to treatment. Conclusions are contradictory and they are not recommended for standard diagnostic work-up and treatment decision (3-8). The aim of this study was to assess prognostic and predictive importance of the selected molecular factors in patients with cervical cancer treated with radiochemotherapy.

**MATERIAL AND METHODS**

This retrospective analysis involved data of 131 patients with squamous cell carcinoma, FIGO stages IB2-IIIB, treated between 2003 and 2008 with radiochemotherapy and brachytherapy. The age ranged from 29 to 80 (mean 53.5). Eighty nine patients (67.9%) were in stages IIB-IIIB and 42 (32.1%) in stages IB2-IIA. All patients received 3D conformal external beam radiotherapy with concurrent, weekly cisplatin in the dose of 40 mg/m$^2$ and high-dose-rate brachytherapy with Ir-192. External beam radiotherapy was performed with 6-12 MV photon beams. Dose per fraction 2 Gy was given five times per week to a total dose of 50 Gy calculated to PTV (Planning Target Volume) and using 4 field box technique. Brachytherapy was delivered once a week in four fractions, dose per single application calculated at point A was 7 Gy. Evaluation of radiochemotherapy results was made based on clinical assessment and gynecological examination performed 6 to 12 weeks after completion of oncological treatment.
Pathology re-evaluation included such parameters as: histological type, histological grade, presence of atypical mitoses, mitotic index.

In immunohistochemistry staining, antibodies detecting the following antigens were used: COX 2, P16, bax, bcl 2, P53 BP, P53 1801, CD34, EGFR and Ki67. Expression of bcl-2, bax, COX-2, EGFR, and P16 was assessed based on reaction intensity scored according to a 4-point scale (0 = no reaction, 1 = poor, 2 = moderate, and 3 = strong reaction) as well as the proportion of stained cells. Immunohistochemistry reaction with anti-P53 antibody was assessed according to the Remmel and Stegner scale (the product of staining intensity score and proportion of positive cells). In assessment of expression of the Ki-67 proliferation antigen, proportion of positively stained cell nuclei per 100 cells was counted. Mean number of blood microvessels (MVD) was counted across 5 vision fields with 100x magnification.

Fluorescence microscope was used for the FISH test (Fluorescence in Situ Hybridization). Using green spectrum and orange spectrum filters, the number of signals from the centromeric probe (green colour) and the number of signals from gene EGFR1-complementary probe (red colour) were counted in 60 cancer cells for each case examined. Gene EGFR1 amplification and chromosome 7 ploidy were assessed. Particular cases were defined as cancers with disomy, low and high trisomy or low and high polisomy.

On cytofluorometry, aneuploidy grade was defined by DNA index (fluorescence intensity for phase G0/G1 cells in the studied population to fluorescence intensity for phase G0/G1 cells in normal population ratio). For a diploid population, the DNA index is 1.00. Percentage of cancer cells in phases S and S + G2M were also determined. The 5-year OS and DFS were used as the end point for this analysis. Survival probability was estimated with Kaplan-Meier method. Differences were tested by the log-rank test; p values < 0.05 were considered significant. Factors, statistically significant in the univariate analysis were included into the multivariate analysis of the prognostic factors, which was done using the Cox proportional hazards regression model. Using the logistic regression method, an attempt was done to indicate predictive factors for complete clinical regression after radiochemotherapy.

**RESULTS**

In a univariate analysis the following factors had significant effect on the DFS:
In a univariate analysis the following factors had significant effect on the OS:

<table>
<thead>
<tr>
<th>Tested parameter</th>
<th>P value (log-rank test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FIGO &gt; IIA</td>
<td>0.0480</td>
</tr>
<tr>
<td>MI &gt; 40</td>
<td>0.0367</td>
</tr>
<tr>
<td>Atypical mitoses</td>
<td>0.0377</td>
</tr>
<tr>
<td>Ki-67 ≥ 52</td>
<td>0.0044</td>
</tr>
<tr>
<td>↑ SCC-Ag level</td>
<td>0.0012</td>
</tr>
<tr>
<td>SP &gt;11</td>
<td>0.0329</td>
</tr>
<tr>
<td>Píe ≥ 20</td>
<td>0.0329</td>
</tr>
<tr>
<td>Overall treatment time</td>
<td>0.0121</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tested parameter</th>
<th>P value (log-rank test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb ≤ 11g/dl</td>
<td>0.0063</td>
</tr>
<tr>
<td>MI &gt; 40</td>
<td>0.0384</td>
</tr>
<tr>
<td>Epitop BP P53 = 12</td>
<td>0.0311</td>
</tr>
<tr>
<td>↑ SCC-Ag level</td>
<td>0.0001</td>
</tr>
<tr>
<td>% of SP &gt; 11</td>
<td>0.0269</td>
</tr>
<tr>
<td>% PI ≥ 20</td>
<td>0.0124</td>
</tr>
<tr>
<td>Aneuploidy vs diploidy</td>
<td>0.0479</td>
</tr>
</tbody>
</table>

In the multivariate Cox analysis only SCC-Ag level was an independent prognostic factor for OS and DFS. Using logistic regression method, a model was constructed that included the factors significant for achievement of complete remission after treatment completion. A decreased chance (by about 60%) to achieve complete remission have the patients whose tumours show a proportion of cells in the S phase exceeding 11% and Ki-67 value higher than 52. Logistic regression showed also unfavourable significance of persistent elevated SCC level after radiochemotherapy.

**CONCLUSIONS**

The analysis that was performed confirmed significance of classic prognostic factors: FIGO stage, total treatment time and Hb level. The following tumour
proliferation markers had significant effect on the OS: MI, SP, PI, as well as other markers: ploidy type, P53 protein expression and SCC-Ag level. The same proliferation markers had significant effect on the DFS, as well as Ki-67. Atypical mitoses, one of the histopathological feature of tumor and SCC-Ag were also important. Two tumour proliferation markers: Ki-67 and S fraction, as well as SCC antigen are also predictive factors for radiochemotherapy.

ACKNOWLEDGEMENTS

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REFERENCES

Intensity Modulated Radiotherapy (IMRT) in Cervix Cancer Complicated by a Singular Pelvic Kidney

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SUMMARY

We report a case of a 62 years old female with squamous cell carcinoma of the cervix, stage IIB (FIGO), complicated with single pelvic kidney, who underwent chemoradiotherapy. Transposition of the kidney was not considered feasible. She had exclusive radiotherapy with IMRT, full tumor dose of 70.4 Gy. Technically it was not possible to perform HDR brachytherapy. Kidney: V20 (63%), V15 (72%) and mean dose (27.5 Gy). During CRT there was acute gastro-intestinal toxicity, grade 2 (RTOG). Renal function unchanged. With a follow up of 24 months, there is regular kidney function and complete response (CR), without GI or GU toxicity. In these case IMRT was a valid technique preventing kidney from radiation damage and with good locoregional control and toxicity.

Keywords: IMRT, Single Pelvic kidney, Cervix carcinoma.
INTRODUCTION

Concurrent chemoradiation (CRT) is the standard of care for patients with bulky cervical cancer.

Treatment of cervical cancer in a patient with a single congenital pelvic kidney is a rare clinical case. The incidence of unilateral renal ectopia in the population was estimated at 1 in 2100-3000 [1], while the overall incidence of invasive cervical cancer is 8.5 per 100,000 [2].

There are five cases described in literature reporting the simultaneous occurrence of these diseases [3-7].

The presence of both these pathologies further complicated the pelvic radiation treatment due to the kidney being located in a standard area of radiation.

High dose irradiation to the kidney is associated with malignant hypertension in clinical studies [8, 9] which may result in therapeutic nephrectomy and in case of single kidney to renal disfunction.

However, Intensity Modulated Radiation Therapy (IMRT) uses a high gradient radiation dose which allows us to treat the target volume while protecting normal tissues in an attempt to reduce the incidence and severity of side effects.

MATERIAL AND METHODS

Retrospective analysis of clinical case and evaluation of response to treatment according to RECIST, dosimetry ICRU 83 and RTOG toxicity.

RESULTS

A 62 years old patient, female sex, Caucasian ethnicity, was referred to the service of Radiotherapy IPOFGL-EPE, with the diagnosis of cervical cancer, FIGO stage IIB. In February 2010 started with complaints of metrorrhagia, and had a biopsy that revealed invasive squamous cell carcinoma of cervix. The clinical observation showed cervix tumor with 4 cm, with invasion of the pouch of Douglas, compromising the third upper vagina. Abdomen-pelvic CT showed a tumor of the cervix uterus with about 2.3 cm and a single congenital kidney produced by fusion cross of ectopic kidneys. Furthermore, an MRI describes the cervical tumor has 4.5 cm in greatest diameter, with invasion of parametrium and upper third of the vagina. Due to the existence of a single pelvic kidney, the hypothesis of transposition of the kidney was placed, but this was not considered feasible by the Urologist of Hospital Curry Cabral (HCC), it was considered of high risk of lost of the kidney and high risk of renal disfunction.
The patient was classified as FIGO IIB and received 6 cycles of Gemcitabine with concurrent IMRT.

The patient was treated with IMRT dynamic (sliding window), clinac 2100 CD linear accelerator of 6 MV photon energy in reverse planning.

The clinical treatment volume (CTV) incorporated the gross disease, parametrium, uterosacral ligaments, vaginal margin from gross disease of 3cm, presacral nodes, and other nodal volumes risk, (entire external iliac, internal iliac, obturator and comom iliacs), see figure 1.

The prescribed dose to cover 95% of the target volume was 50.4 Gy at 1.8 Gy per fraction (ICRU83).

Fig. 1. Isodose curves.
Technically it was not possible to perform boost of tumor with HDR brachytherapy.

So, we made a second CTV that incorporated the gross disease and prescribed to the target volume a boost of 20 Gy at 2 Gy per fraction.

She had exclusive radiotherapy with IMRT, full tumor dose of 70.4 Gy. Kidney V20 was 63%, V15 was 72% and the mean dose 27.5 Gy.

The analysis of the dose volume (DVH) is shown in figure 2.

During treatment the patient had gastro-intestinal toxicity (grade 2 RTOG), and the treatment progressed uneventfully. Renal function remained unchanged throughout the RT treatment. When seen for the last follow-up appointment – 24 months after the treatment – the patient was disease free, with no change in renal function, had a satisfactory aspiration cytology, with complete response according to RECIST without GI or GU toxicity.

Fig. 2. HDV.
CONCLUSIONS

Patients with a pelvic kidney should not receive RT unless it is a mainstream in the treatment of that type of tumor.

There are few studies regarding the treatment of patients with pelvic kidneys. It is important to have a larger number of cases to establish the need for, and benefits of RT for patients with this condition.

Rosenshein et al. in 1980, suggested nephrectomy or translocation to the upper abdomen of a normal ectopic kidney. Alternatively, the radiation oncologist can delete the rim of the irradiation field, but with conventional techniques, there can decrease the high risk volume coverage. [3]

In the presented case, a surgical approach was not feasible due to the high risk of lost of renal function, in an attempt to prevent hemodialysis.

There is no scientific evidence that defines the optimal treatment for the patients with pelvic kidneys undergoing radiotherapy.

Castilho et al. reported in 2006 for the first time the use of IMRT for a patient with cancer of the endometrium with a congenital pelvic kidney. [10]. Ectopic kidney was centrally located in the pelvic region to the level of the sacroiliac joints. Two thirds of ectopic kidney received 21 Gy, and one third received 31 Gy. The patient had no recurrence or side effects of both kidneys at 18 months.

The case here reported demonstrates the applicability of pelvic IMRT in a patient with a solitary kidney pelvic, different from the one reported by Castilho et al.

Firstly, the patient’s kidney here presented was located down in the iliac fossa, which is a more common clinical presentation. Secondly, the area of recurrence occurs upwards of the location of the pelvic kidney. Thirdly, our patient has only one kidney, so lower functional reserve.

To evaluate renal function of a single pelvic kidney, we performed a blood test evaluating: creatinine, ionogram and urea. Neither showed changes during or after treatment.

The tolerance for the kidney radiation dose highly depends on the volume irradiated. Dose tolerance for a probable 5% delayed adverse effects within 5 years is estimated to be 50 Gy to one third of the kidney, two-thirds of 30 Gy and 23 Gy to the whole kidney [11]. This risk increases to 50% for late toxicity, if two thirds are irradiated with doses of 40 Gy, or a third for doses of 28 Gy. As noted in the DVH (fig. 2) these parameters were respected.

We reported a rare case, managed with IMRT in order to spare a single pelvic kidney without compromising the field of pelvic irradiation.

IMRT was a good alternative in this complex case, as it allowed us to prevent a patient from undergoing kidney transplantation or hemodialysis. However should not be used as a routine alternative to braquitherapy.
In these case IMRT was valid technique preventing kidney from radiation damage and with good locorregional control and toxicity.

REFERENCES

2. Federação das Sociedades Portuguesas de Obstetrícia e Ginecologia (FSPOG).
Multi-Metronomic Bevacizumab Regimens for Resistant Ovarian Cancer

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SUMMARY

Problem: The use of Bevacizumab for refractory ovarian cancer has been impeded by adverse events, length of therapy (long maintenance) and tumor rebound. Eligibility limits and ideal time(s) and length of application remain unknowns, and cost benefit remains problematic.

Methods: Bevacizumab 10 mg/kg day 1, Cyclophosphamide 250-150 mg/m² day 1-day 2 were combined with GFLIC(D): Gemcitabine 500mg/m², Fluorouracil 1500mg/m², Irinotecan 80mg/m², day1 +/-Docetaxel 25mg/m² and Carboplatin AUC 2, day 2. (doses reduced 20% on addition of docetaxel and then increased). Docetaxel was added at complete response, plateau of response or any Bevacizumab stop. When Bevacizumab was resumed Docetaxel was continued. GFLIC(D) was dose and drug adjusted but never stopped. Exclusions: Helsinki criteria, urgent surgery or four weeks survival.

Results: 30 patients had no severe «limiting» adverse events (AEs), Bevacizumab, when held, was safely resumed all but twice. RECIST response rates were ~ 82% and additional 12% stable disease for multi, median 5 (3-8) line resistant, and 5/ 5 complete responses for second line, platinum and paclitaxel, resistant tumors. For greater than 4cm tumors, 60% were reduced to less than 2 cm, 40% to less than 0.5cm. No rebound was observed. Resumption of docetaxel was possible and for half improved responses. Progression free survival,
for performance status 0-2 patients was not reached at 1 year and was 6 months for performance status 3 patients.

**Conclusions:** It is feasible to use Bevacizumab: a second time after adverse events and also after it's earlier outright failure, defined by active progression; as a neoadjuvant; with less toxic, low-intermediate dose chemotherapy (rarely tested doses for Gemzar, Irinotecan, docetaxel and Carboplatin). It is feasible to replace treatment (treatment) to progression with neoadjuvant, novel investigational and stop-go treatments. Multi-metronomic treatment can create new high rates of ideal patients for further investigation and treatment. Docetaxel added to cores can improve responses. CyGFLIC can expand eligibility to performance score 3 patients but it is not advisable to delay treatment. It is feasible and safer to use 5-6 drugs at metronomic dose levels than to use many 1-2 drug standard dose regimens.

**INTRODUCTION**

Cores consisting of Gemcitabine (G), fluorouracil (F), leucovorin (L), and cisplatin (P) or Oxaliplatin (O), with each drug at 1/2-1/4 it’s standard dosage, can provide empirical treatments (treatments) for patients with multiline drug resistant tumors. In ten applications the failed drug (Irinotecan (I), Docetaxel (D), Mitomycin-C or Bevacizumab (Bev) outperformed expectations when added to a previously used and no longer effective core. [1-4]. Drugs produce simultaneous geometric biochemical modulation of 4-5 of the core drugs, and (vice versa) all the core drugs can modulate the drug added to the cores. Sequential core treatments improved overall survival (OS) and cumulative response rates (RRs) [1-4]. Core drugs were reusable 3 and 4 times over years. Addition of Bev or D to GFLIC enabled neoadjuvant (neoA) treatments; they produced complete (CRs) and pathologic (pCRs) for patients with multiline resistant pancreatic (PC) and gastric (GC) as well as neoA partial (PRs) for cholangiocarcinoma (CCA) [1-4]. Independent trials have since confirmed the efficacy of both the moderate doses and added drugs.

For patients with resistant ovarian cancer (ROC), Bev previously produced CRs, PRs and SD. The addition of metronomic low dose Cyclophosphamide (Cy) improved these Rs. Both Bev and Cy can interact favorably with each of the core drugs. In vitro, one or more cytotoxins remain potent and can produce synergistic drug pairs for some 95% of ROCs.

In order to produce high RR, a treatment must inhibit many critical metabolic pathways, simultaneously, or produce an extracellular mechanism of tumor inhibition, because there is no common target in ROCs. MZB laboratory tests found cores of 4-5 drugs that «best» produced empirical synergism at «low-moderate»
concentrations, and geometrically produce 6-10 concurrent drug pairs. This provided reason to combine the drugs as ACy GFLIC D. (Table 1)

An algorithm was devised to: 1. replace open-ended maintenance treatments which have poor safety and are not cost effective; 2. prevent tumor rebound; complete discontinuation of Bev will otherwise allow the tumor to relapse quickly.

MATERIALS AND METHODS

ACy GFLIC-/+D was used in sequence to produce Rs which can facilitate NeoA- like treatments and to evaluate the safety, feasibility, optimum time and efficacy of the sequential treatments. Eligibility (E): Failed treatment with Paclitaxel-Carboplatin (PC): high grade papillary serous ROC; Helsinki and IRB E; ECOG PS 0-2; grade 0-1 organ function and «expectation» of safe treatment. Patients were not E if: not E for Bev-Cy or C and CisP; organ function; not recovered, ANC<1500/mcl, plts <120,000/mmol, creatinine > 2 mg/dL, bilirubin > 2 mg/dL, poorly controlled infection, other urgent co-conditions, severe allergy to a test drug, ECOG PS 4; or 3 in need of hospital care; CNS metastases or no measurable tumor. E was expanded to include patients with either «early» or repeated «failure» of PC to produce CR (defined as plateau with disease) or classic progression (PG) during treatment. E for multiline ROC included all the above: and urgent need for response (R); failed some or all core drugs; size > 2cm; active tumor growth; +/- extra peritoneal disease, +/- suitable for neoA treatment following a R. Analyses: Pre registration captured all patients with

Tab. 1. Schema 1: Bev-Cy GFLIC-D Q 2 WKS.

<table>
<thead>
<tr>
<th>Drug, Day 1 below</th>
<th>Dose mg</th>
<th>Add D mg</th>
<th>Time min</th>
<th>Rate min</th>
<th>AE, Drug Modified</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bevacizumab</td>
<td>10 kg</td>
<td>10 kg</td>
<td>00</td>
<td>90</td>
<td>GI, oral, renal, HTN</td>
<td>30</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>250 M²</td>
<td>250 M²</td>
<td>100</td>
<td>30</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Gemcitabine</td>
<td>500 M²</td>
<td>400 M²</td>
<td>140</td>
<td>Fixed rate</td>
<td>10ug/M²/min</td>
<td>Plt, WBC, GI, skin, renal*, hepatic*</td>
</tr>
<tr>
<td>Leucovorin</td>
<td>200 M²</td>
<td>200 M²</td>
<td>190</td>
<td>39</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Irinotecan</td>
<td>60 M²</td>
<td>60 M²</td>
<td>240</td>
<td>90 &gt; 60 &gt; 30</td>
<td>GI</td>
<td>25</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>1200 M²</td>
<td>1200 M²</td>
<td>240</td>
<td>24 hour CI</td>
<td>GI, oral, skin</td>
<td>20</td>
</tr>
</tbody>
</table>

Titration METHOD REQUIRED. See text. CI continuous infusion 24 hr. Add D: reduce other drugs %. proportion of current dose, as shown G, I; FU, C one step. As tolerated, re-escalate to full dose in half steps at third and forth 2 week cycles. For prior AE, after a well tolerated dose reduction, Re-escalate in 1/2 steps q 4 wks.* Escalations not applicable to renal R or hepatic (H AEs).
ovarian, tubal and primary peritoneal cancer. Intent to treatment analyses examined: safety; RR, patient characteristics, absolute PFS and OS; ability to downsize tumors, to enable neoA cytoreduction S and intraperitoneal IP treatment. RR was compared to RRs, set at 50% for the multiline PS 0-2 patient and 33% chance of second line CRs. Series size was open ended.

Monitoring included: CT scan at 6, 12 and then every 16 wks, and CA-125 every 3 wks. Tests included: CBC wkly, liver and renal function, K, Mg and Ca wkly x 4 then every 2 wks and urine, RBC- protein every 4 wks and baseline PT, PTT, anemia and thyroid profile. Records employed in NCI toxicity scale. R and PG, (signal lesions only) was based on RECIST criteria. Treatment: The sequence included: 1) Acy GFLIC (schema 1) given to «best» R (no evaluable disease or plateau, time of best R) q 2 wks, 2) At best R, add D, begin GFLIC-D (schema 1) and continue treatment to best R or 4 cycles past CR, 3) If best size (0-5mm) permits, begin percutaneous hyperthermic IP treatment x 2-4 times, 3b) Size 6-20 mm proceed to S followed by HIPEC. 4) Resume Cy-GBdM-D x 6 and then +/- immuno-vaccine treatment. Bev was stopped 3 wks before IP and 6 wks before S. Whenever Bev was stopped, D was added as Cy GFLIC D and continued in order to provide a bridge treatment, to prevent rebound, and also to improve and consolidate Rs (Table 1).

Dose Modification (DM) Schema 1: Dosages were titrated to produce brief, grade Gr 3-4 CBC nadirs. Escalation was in half steps to achieve a best R and on recovery from adverse events to resume initial dose levels of G, I, C and D and produce Gr 3 borderline Gr 4, uncomplicated, WBC and plt nadirs. Return to toxic doses was not allowed unless both treatment delay, and Gr 4 adverse events were prevented by two 300 mcg doses of granulocyte colony stimulating factor (G-CSF). Drug(s) responsible for treatment delays, Schema I, were reduced two steps, and for adverse events with no treatment delays, one step.

For Bev, support included prospective mouth and nose care, ulcer medication and low roughage diet. For any Gr2 gastroenteritis, Bev and Irinotecan were withheld, until recovery, and other drugs reduced as shown. BEV was titrated or briefly withheld In R section for mild epistaxis, moderate nephrotoxicity and severe HTN. Drugs responsible for any 14 day delay were reduced 50%, and after two treatments, re-escalated in half steps, to Gr 2 borderline Gr 3 nadirs. Treatments resumed 24hrs after use of G-CSF, immediately on schedule or on recovery. Future CBCs and G-CSF were timed, to precede prior nadirs by 2-3 days.

RESULTS

Safety: Treatments produced uncomplicated nadirs which sometimes requi-
red 1-2 days of G-CSF (twice for the lead patient). G-CSF x 2 was rapidly (24-48 hr) effective. Adverse events included RBC transfusion for ~ 17% of patients without severe symptoms and with resumption of D as ACy GFLIC D, limiting neuropathy 8% or fatigue. After recovery from mild epistaxis or HTN Bev was safely resumed ~ 83%; ~17% required a second (safe) stop. Rebound did not follow brief stop or complete end of therapy with Bev.

Patient Characteristics: (incomplete review-interim denominator**); Lines resistant: As Second line. N.5 (also 1 in treatment, and 1 neoA) Multiline N** 24; Tumor size: Evaluable small: 40/0%; 2-4.cm: 20/25%; 4-8 cm: 20 and 1 in Rx/42%; 8cm: 20 (and 1 neoA)/33%.

Grade: High: All*/All. *1 neoA & 1 multiline had mixed high gr and endometrioid elements.

Prior lines: primary: 5 (+1 neoA)/0%; 2-3: -/-17%; Four: -/-29%; Five or more: -/-54% Bev: -/-25%.

Performance ECOG zero: 60/17%; one: 1 (+1 neoA), 20/33%; two: 1.20/ 33%; three: -/-17%.

Weight loss, 10% 1 (+1 neoA)/50% Pathologic Fluid: - (+1 neoA)/ 33% bowel partial obstruction: 1/17%; BRCA positive: 4, ~25% Other* familial (*BRCA negative) ~17%.

Age: median: 68 range: 32-78.

RESPONSE (several responses too early in treatment to score «best» response):

Second line: P=1/2. 0.03 to 5th. 0.003. Primary NeoA: clinical CR, one remaining small nodule resected, Ro, followed by IP treatment. Multiline: 21/ 24 ~ 83% RR and 3 brief Rs /SD 12.5% One, PS 3, failed. First Seven consecutive PS O-2 patients all responded p=0 .008. First Seven consecutive 4-6 cm downsized to less than 2 cm, 3 CR, p=0.008.

Overall > 4 cm tumors: 60% to less than 2 cm, 40% to less than 0.5 cm. Docetaxel contributed to Rs, improved benefit for half, and produced some CRs. Among ECOG 3: half had six months of benefit: PS; small bowel obstruction, drainage dependence and fluid retention were corrected, with one exception. Progression and Survival: (Absolute) ECOG 0-2 patients were one year without progression except one. PS 3 patients had a median PFS of six months. Overall survival: The 2 lead patients have reached greater than three years.

CONCLUSIONS

ACyGFLIC +/- D is test worthy for (urgent) niche treatment needs, many patients are otherwise ineligible for (safe) treatment. Sequential treatment, improves acute PS, and (expands) E for treatments with Bev and D; both can
produce CRs and downsize ROC. Test worthy neoA or research treatments can follow best R. These are controversial new comparators for treatments and place a new emphasis on the replacement of maintenance treatment, «to failure», with an opportunistic effort not to waste a good response. ACy GFLIC D creates two chances, early and late, to downsize half, some 300 new events a day, of all current patients with ROC. Downsizing improves patients for treatment and trials. However because of the low doses ACy GFLIC D should not be used alone when there are safe standard curative treatments nor should the low doses be used alone or for a drug pair without a rigorous review process. Prior research is noteworthy both for the dismal failure of dose intensification for primary ovarian and breast ca and the general absence of either tests of dose reduction, free of selection bias, (the sick get less) or such tests of modern drugs.

Some view these multi-drug regimens as use of standard drugs, in standard or near dose intense schedules, as another step in a personalized, integration of safe evidence based methods. Early results satisfy phase II efficacy threshold criteria for reuse of the drugs. However, it would be desirable to have more information regarding the mechanism(s), optimum dosages, ideal simultaneous drugs, to obtain more OS follow up and, difficult to generate for niche patients, randomized trials. The MZB effort has prioritized «low hanging fruit», laboratory identified targeted treatments and cytotoxic substitutes or additions to the current core drugs.

REFERENCES

Cervical Intraepithelial Neoplasia in the Ob-Gyn Department of the Arad County Hospital During the 1998-2012 Period

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³ «Vasile Goldiș» Western University of Arad, Faculty of Medicine, Internal Medicine Department, Romania
⁴ «Vasile Goldiș» Western University of Arad, Faculty of Medicine, Anatomy Department, Romania
⁵ Clinical County Emergency Hospital, Anatomopathology Department, Arad, Romania

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SUMMARY

Cervical Intraepithelial Neoplasia (CIN) lesions are of high importance because of their relatively high frequency, potential evolution towards cervical cancer and relatively easy diagnosis.

Our study concerning the incidence of Cervical Intraepithelial Neoplasia (CIN) covers the 1998-2012 time span, the data being collected from the Histopathology Exams (HPE) registers.

CIN lesions were discovered in 1722 cases (95.93%) and Cervical Intraglandular Dysplasia (CIGD) in 73 cases (4.07%). CIN I, CIN II and CIN III lesions represented 64.52% (1111 cases), 20.67% (356 cases), and 14.81% (255 cases) of the total CIN cases, respectively. The mean patients’ age was 44.62 ± 9.86
years for all cervical dysplasia cases, 44.55 ± 9.81 years for all CIN cases, 43.89 ± 9.40 years, 45.78 ± 9.84, and 45.70 ± 11.18 years for CIN I, CIN II, and CIN III, respectively. The mean patients’ age for the CIG case was 46.36 ± 10.87 years.

There were 19 cases (1.06% of all cervical dysplasia cases, 1.10% of all CIN cases, 1.86% of the 1020 cervical cancer cases diagnosed during the same period) of microinvasive carcinomas combined with CIN III lesions, ten cases of invasive carcinoma combined with CIN lesions (0.56% of all cervical dysplasia cases, 0.58% of all CIN cases, 0.98% of the cervical cancer cases), and two cases of CIN III combined with in situ carcinoma (0.11% of all cervical dysplasia cases, 0.12% of all CIN cases, 0.20% of the cervical cancer cases). Early detection of CIN lesions through adequate clinical and paraclinical exams is of utmost importance for preventing cervical cancer, which remains a serious and frequent health problem in Romania.

**Keywords:** cervical intraepithelial neoplasia, cervical intraglandular neoplasia, CIN, LSIL, HSIL, human papilloma virus, cervical cancer, mean age, Student’s t-test, statistical significance.

**INTRODUCTION**

Squamous cell carcinomas of the ectocervix are preceded by cervical intraepithelial neoplasia (CIN), and are usually related to infection with human papilloma viruses (HPV) (1, 2).

Experience with the CIN terminology led to further reclassification of the terminology for reporting cytologic abnormalities consistent with preinvasive disease (3, 4), the CIN grading being very subjective, (3-6); separating CIN 2 from CIN 3 is often not reproducible (7). A continuous range of morphologic abnormalities exists among these lesions (8).

The rubric CIN 3 includes not only severe dysplasia, but also carcinoma in situ of the cervix (they cannot be separated objectively). Intraepithelial lesions also may be graded in a binary system as low-grade squamous intraepithelial lesions (LSIL) or high-grade squamous intraepithelial lesions (HSIL) (9). The 3-tiered system of grading is currently the most widely used: CIN 1 (mild squamous dysplasia), CIN 2 (moderate squamous dysplasia), and CIN 3 (severe squamous dysplasia/carcinoma in situ) (10, 11). CIN 3 is a genuine surrogate marker of subsequent cancer risk (12, 13). CIN 1 is viewed as an insensitive histologic marker of HPV infection. (14). Standardized for positivity for a given high-risk HPV type, a diagnosis of CIN 1 does not predict a meaningfully higher risk of CIN 3 than does a negative biopsy (12).
Histologically confirmed CIN 1 lesions confer a lower risk of developing cervical cancer than does a Pap smear report of LSIL (12, 13). CIN 2 can be produced by noncarcinogenic HPV types and is equivocal in cancer potential (13). The stepwise progression of increasingly severe CIN to invasive cancer, implicit in the CIN continuum, remains an important histopathological concept to assist clinical management (14).

HPV infection is a broad transition state between normal and precancer stages (16). CIN 3, particularly full thickness carcinoma in situ, shares the same HPV-type spectrum and cofactor profile as invasive cancer; at this time there is no reliable predictor of CIN 3 lesions likely to progress to cancer and as such all are managed as definite precancer (16). CIN 2 demonstrates greater heterogeneity in biology and definition (14), being often caused by low-risk HPV types rarely found in cancer and with a greater regression potential. Although of equivocal malignant potential, in the absence of reliable predictors of risk of progression, CIN 2 lesions tend to be managed as precancer to provide a further safety margin against development of cancer (14).

A histological diagnosis of LSIL (HPV infection/CIN 1) is increasingly viewed as not representing precancer, while persistence of oncogenic HPV types is strongly linked to precancer. Only a fraction of precancers arise from HPV infection in the absence of mild or equivocal microscopic abnormalities (18, 19, 20).

High-grade lesions are commonly found within a broader field of low-grade disease, suggesting that CIN 3 may develop in high-risk HPV-infected epithelium independent of and within a CIN 1 lesion, rather than as a classical stepwise progression (1). Positive margins and glandular involvement by CIN II or CIN III are independent predictors of residual or recurrent disease (21, 22, 23), but that may not be the case for low-grade CIN (CIN I) (24).

**OBJECTIVES**

The purpose of this study is to analyze the type and age distribution of cervical dysplasia cases in our hospital over a ten year period and to statistically compare the mean ages of each dysplasia type with all other types and with the mean age of the patients with cervical cancer.

**MATERIALS AND METHODS**

Our study concerning the incidence of Cervical Intraepithelial Neoplasia (CIN) covers the 1998-2012 time-span, the data being collected from the Histopathology Exams (HPE) registers.
RESULTS

During the 1998-2012 period, 1795 cases of cervical dysplasia cases were discovered: 1722 CIN cases (95.93%) and 73 CIGD (cervical intraglandular dysplasia) cases (4.07%).

There were 1111 cases of CIN I (64.52% of all CIN cases, 61.89% of all dysplasia cases), 356 cases of CIN II (20.67% of all CIN cases, 19.83% of all dysplasia cases), and 255 cases of CIN III (14.81% of all CIN cases, 14.21% of all dysplasia cases).

The mean patients’ age was 44.62 ± 9.86 years for all cervical dysplasia cases, 44.55 ± 9.81 years for all CIN cases, 43.89 ± 9.40 years, 45.78 ± 9.84, and 45.70 ± 11.18 years for CIN I, CIN II, and CIN III, respectively. The mean patients’ age for the CIG case was 46.36 ± 10.87 years.

Table 1 and figures 1 and 2 show the age distribution for the cervical dysplasia cases.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>20</th>
<th>21-30</th>
<th>31-40</th>
<th>41-50</th>
<th>51-60</th>
<th>61-70</th>
<th>71-80</th>
<th>81-90</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIN I</td>
<td>0.36</td>
<td>6.76</td>
<td>26.09</td>
<td>47.22</td>
<td>13.22</td>
<td>3.50</td>
<td>0.72</td>
<td>0.12</td>
</tr>
<tr>
<td>CIN II</td>
<td>0.00</td>
<td>6.48</td>
<td>16.19</td>
<td>49.80</td>
<td>18.62</td>
<td>7.29</td>
<td>1.21</td>
<td>0.40</td>
</tr>
<tr>
<td>CIN III</td>
<td>0.00</td>
<td>7.73</td>
<td>27.07</td>
<td>34.25</td>
<td>21.55</td>
<td>6.63</td>
<td>2.76</td>
<td>0.00</td>
</tr>
<tr>
<td>All CIN</td>
<td>0.24</td>
<td>6.85</td>
<td>24.28</td>
<td>41.86</td>
<td>16.80</td>
<td>4.70</td>
<td>1.11</td>
<td>0.16</td>
</tr>
<tr>
<td>CIGD</td>
<td>0.00</td>
<td>7.55</td>
<td>18.87</td>
<td>47.17</td>
<td>13.09</td>
<td>9.43</td>
<td>0.00</td>
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</tr>
<tr>
<td>All CIN</td>
<td>0.23</td>
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<td>24.06</td>
<td>44.91</td>
<td>16.73</td>
<td>4.89</td>
<td>1.07</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Fig. 1. Distribution of cervical dysplasia age groups.
There were 19 cases (1.06% of all cervical dysplasia cases, 1.10% of all CIN cases, 1.86% of the 1020 cervical cancer cases diagnosed during the same ten year period) of microinvasive carcinomas combined with CIN III lesions, ten cases of invasive carcinoma combined with CIN lesions (seven CIN I, one CIN II, and 2 CIN III; 0.56% of all cervical dysplasia cases, 0.58% of all CIN cases, 0.98% of the cervical cancer cases), and two cases of CIN III combined with in situ carcinoma (0.11% of all cervical dysplasia cases, 0.12% of all CIN cases, 0.20% of the cervical cancer cases).

Table 2 shows the statistically significant differences between the mean ages of different groups and between dysplasia cases and the 1020 cervical cancer cases (mean age 53.21 ± 13.21 years) by using Student’s t-test.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>All vs CIN I</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>All vs CIN II</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CIGD vs CIN I</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CIN I vs CIN II</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CIN I vs CIN III</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>CIN I vs CIN III</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cervical cancer vs CIN I</td>
<td>&lt;0.00000001</td>
</tr>
<tr>
<td>Cervical cancer vs CIN II</td>
<td>&lt;0.00000001</td>
</tr>
<tr>
<td>Cervical cancer vs CIN III</td>
<td>&lt;0.00000001</td>
</tr>
<tr>
<td>Cervical cancer vs CIGD</td>
<td>&lt;0.0006</td>
</tr>
</tbody>
</table>

**DISCUSSIONS, CONCLUSIONS**

Early detection of CIN lesions through adequate clinical and paraclinical exams is of utmost importance for preventing cervical cancer, which remains a
serious and frequent health problem in Romania, as there are, besides the clinical exam, several paraclinical methods which can achieve this goal.

If the mean ages of each type of cervical dysplasia are statistically more or less different among them in the present study, all of them are highly different from the mean age of the patients with cervical cancer, thus proving that the evolution from CIN lesions to invasive cervical cancer takes several years, leaving enough time for detection and adequate treatment.

The mean patients’ age in a paper concerning the same pathology over a ten year period (2000-2009) was 44.65 ± 9.83 years for all cervical dysplasia cases, 44.58 ± 9.75 years for all CIN cases, 43.81 ± 9.22, 46.50 ± 10.17, and 45.46 ± 11.05 years for CIN I, CIN II, and CIN III, respectively, and 46.45 ± 11.63 years for CIGD, all these values being close to the ones presented above (25).

REFERENCES

7. Wright Jr TC. Pathology of HPV infection at the cytologic and histologic levels: basis for a 2-tiered morphologic classification system. *Int J Gynaecol Obstet* 2006; 94:S22-S31.


Statistical Comparisons of Gynecologic Cancer Age Groups in the Ob-Gyn Department of the Arad County Hospital during the 1998-2012 Period

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SUMMARY

The purpose of this study is to statistically compare the mean ages of the patients with gynecologic cancer in our hospital during the 1998-2012 interval. The data was collected from the Histopathology Exams (HPE) registers.

Gynecologic cancer was discovered in 1754 cases; there were 1020 cases of cervical cancer, 556 cases of uterine cancer, 118 cases of ovarian cancer, 51 cases of vulvar cancer and nine cases of vaginal cancer.

The mean ages were 53.21 ± 13.21 years for cervical cancer (age range 22-87 years), 61.92 ± 9.55 years for uterine cancer (age range 32-88 years), 51.66 ± 14.22 years for ovarian cancer (age range 18-77 years), and 69.24 ± 9.29 years for vulvar cancer (age range 39-84 years); vaginal cancer was not consi-
dered because of the non-normal distribution of the age groups. The age groups with the most patients were: 41-50 years for cervical cancer (293 cases or 28.73%), 51-60 years for uterine cancer (207 cases or 37.23%), 51-60 years for ovarian cancer (34 cases or 28.81%), and 71-80 years for vulvar cancer (24 cases or 47.06%).

After performing Student’s test, the statistically significant differences were: cervical vs uterine (p<0.0001), cervical vs vulvar (<0.0001), uterine vs ovarian (<0.0001), uterine vs vulvar (<0.0001), and ovarian vs vulvar (p<0.0001). Cervical and ovarian cancer mean ages were not significantly different (p=0.23).

The mean ages of different cancer types in our study are similar to those in literature.

**Keywords**: cervical cancer, uterine cancer, vulvar cancer, ovarian cancer, vaginal cancer, statistical significance, Student’s t-test.

**INTRODUCTION**

The mean age for cervical cancer is 51.4 years, with the number of patients evenly divided between the age groups 30 to 39 and 60 to 69 years [1]; another retrospective study involving 36,122 patients diagnosed with cervical cancer during a 15 year period (1991-2005) revealed, over three five-year intervals, a mean age of 53.9 ± 13.3 years from 1991 to 1995, 55.0 ± 14.9 years from 1996 to 2000, and 56.7 ± 14.7 years from 2001 to 2005 [2].

The average age at diagnosis of endometrial carcinoma is approximately 60 years according to one study and approximately 5% of women are diagnosed younger than age 40, with up to one quarter present before menopause [3]. Other authors have found that, in case of cancer of the uterine corpus, the average age of patients with endometrioid cancer is approximately 63 years, while the average age of patients with nonendometrioid cancer is approximately 67 years [4].

The peak incidence of invasive epithelial ovarian cancer is situated between 56 and 60 years [5]. Approximately 80 to 90% of ovarian cancers, including borderline forms, occur after age of 40 years, whereas 30% to 40% of malignancies occur after the age of 65. The median age at diagnosis for sporadic disease is 60 years, although patients with a genetic predisposition may develop this tumor earlier, often in their fifth decade [6]. Mean ages for ovarian metastases from other cancer sites in two studies were, according to two studies, 55 years [7] and 51 years [8].

The mean age of patients with vaginal cancer is 68 years for invasive cancer and 58 years for in situ neoplasia [9]. As for vaginal cancer, about 80% of patients are older than 50 years [10]; squamous cell carcinoma is found most
often in women aged 60 or older, while adenocarcinoma is found most often in women aged 30 or younger [11]. Primary invasive carcinoma of the vagina is predominantly a disease of elderly women; 70% to 80% of cases are diagnosed in women older than 60 years [12].

Patients with vulvar cancer have a mean age of 62 years in one study [13] and between 65 and 70 years in other studies [14, 15].

Data from SEER [16] shows the following for the 2004-2008 period:

- the median age at diagnosis for cancer of the cervix uteri was 48 years of age, with the following age distribution: 0.2% were diagnosed under age 20; 14.3% between 20 and 34; 25.8% between 35 and 44; 23.9% between 45 and 54; 16.4% between 55 and 64; 10.6% between 65 and 74; 6.4% between 75 and 84; and 2.5% 85+ years of age [17].
- the median age at diagnosis for cancer of the corpus and uterus, NOS, was 61 years of age, with the following age distribution: 0.0% were diagnosed under age 20; 1.6% between 20 and 34; 6.1% between 35 and 44; 19.2% between 45 and 54; 31.8% between 55 and 64; 22.1% between 65 and 74; 14.2% between 75 and 84; and 4.8% 85+ years of age [18].
- the median age at diagnosis for cancer of the ovary was 63 years of age, with the following age distribution: 1.2% were diagnosed under age 20; 3.5% between 20 and 34; 7.3% between 35 and 44; 19.1% between 45 and 54; 23.1% between 55 and 64; 19.7% between 65 and 74; 18.2% between 75 and 84; and 8.0% 85+ years of age [19].
- the median age at diagnosis for cancer of the vagina was 68 years of age.
- the median age at diagnosis for cancer of the vulva was 68 years of age, with the following age distribution: 0.1% were diagnosed under age 20; 2.0% between 20 and 34; 7.3% between 35 and 44; 15.9% between 45 and 54; 18.3% between 55 and 64; 17.5% between 65 and 74; 24.3% between 75 and 84; and 14.6% 85+ years of age [20].

A study conducted by us for the 2000-2009 interval found that the mean ages were 52.94 ± 12.96 years for cervical cancer (age range 22-87 years), 61.71 ± 9.06 years for uterine cancer (age range 38-85 years), 51.46 ± 14.28 years for ovarian cancer (age range 18-77 years), and 65.90 ± 9.65 years for vulvar cancer (age range 39-81 years) [21].

**MATERIALS AND METHOD**

Data regarding the cases of gynecological cancers diagnosed during the 1998-2012 period was collected from the anatomopathology department of the hospital and statistically analyzed.
RESULTS

During the fifteen year period, a number of 1754 gynecological cancers were diagnosed in our hospital by the anatomopathology department: 1020 cervical cancers (58.15%), 556 uterine cancers (31.70%), 118 ovarian cancers (6.73%), 51 vulvar cancers (2.91%), and nine vaginal cancers (0.51%); the histological specimens were obtained from biopsies and/or surgery.

The mean ages were 53.21 ± 13.21 years for cervical cancer (age range 22-87 years), 61.92 ± 9.55 years for uterine cancer (age range 32-88 years), 51.66 ± 14.22 years for ovarian cancer (age range 18-77 years), and 69.24 ± 9.29 years for vulvar cancer (age range 39-84 years); vaginal cancer was not considered because of the non-normal distribution of the age groups. The age groups with the most patients were: 41-50 years for cervical cancer (293 cases or 28.73%), 51-60 years for uterine cancer (207 cases or 37.23%), 51-60 years for ovarian cancer (34 cases or 28.81%), and 71-80 years for vulvar cancer (24 cases or 47.06%).

We compared the mean ages of the cancer types with normal distribution of the cases by using Student’s t-test; the results are shown in table 1.

![Fig. 1. Gynaecological cancer age groups.](image)

<table>
<thead>
<tr>
<th>Type</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cervical vs. uterine</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Cervical vs. ovarian</td>
<td>0.33</td>
</tr>
<tr>
<td>Cervical vs. vulvar</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Uterine vs. ovarian</td>
<td>&lt;0.000001</td>
</tr>
<tr>
<td>Uterine vs. vulvar</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ovarian vs. vulvar</td>
<td>0.000001</td>
</tr>
</tbody>
</table>

Tab. 1. Gynaecological cancer age comparisons.
DISCUSSIONS, CONCLUSIONS

The mean ages of the patients from the groups with five gynaecological cancer types from our study are similar to those in literature:

- 53.21 ± 1.31 years for cervical cancer (1020 cases) versus 51.4 years [1], 53.9 ± 13.3 years, 55.0 ± 14.9, 56.7 ± 14.7 [2], 48 years [17], and 52.94 ± 12.96 years [21];
- 61.9 ± 9.55 years for uterine cancer (556 cases) versus 60 years [3], 61 years [18], and 61.71 ± 9.06 years [21];
- 51.66 ± 14.22 years for ovarian cancer (118 cases) versus 60 [6], 63 years [19], and 51.46 ± 14.28 years [21];
- 69.24 ± 9.29 years for vulvar cancer (51 cases) versus 68 [20] and 65.90 ± 9.65 years [21];
- 67.11 years for vaginal cancer (nine cases) versus 68 [9] and 66.25 years [21].

Unfortunately, there are still many cases of such cancers, with many cases in advanced and often incurable stages due to several factors, including lack of screening, lack of patient information and patients neglecting signs and symptoms or being afraid of going to the physician.

Paradoxically, more than half of the cases diagnosed in our hospital are cervical cancers, although premalignant cervical conditions are the easiest to diagnose both clinically and by laboratory methods, especially the Pap smear.

REFERENCES


Primary Neuroendocrine Carcinoma of the Breast


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SUMMARY

Neuroendocrine carcinoma of the breast constitutes less than 0.1% of breast cancers and less than 1% of neuroendocrine tumours. It expresses neuroendocrine markers in more than 50% of tumour cells; it’s best treatment is still unknown and various modes of management have been employed in treating this disease.

Methods: This study is a retrospective analyses of 4 neuroendocrine breast cancer collected in the department of radiotherapy oncology in CHU IBNROCHD of CASABLANCA-Morocco.

Results: The mean age is 51 years old, the mean size of the tumour is 3.3 cm. histologically, it was a neuroendocrine tumoral proliferation, immunohistochemical study confirmed the neuroendocrine nature showing expression of chromogranine in 3 cases synaptophysine in 2 cases and CD56 in one case. All cases were grade II SBR, hormonal receptors were positive in 3 cases; Her2 neu negative in the four cases, one tumour was triple negative. Tumours were clas- sed T1 in 2 cases and T2 in the two others. Nodes involvement was found in 2 cases. The treatment consisted in radical surgery followed by adjuvant che- motherapy, we used sequential protocol with 3FEC100-3Dcetaxel. Radiotherapy was used in 2 cases and hormonotherapy for the 3 patients with positive recep- tor. After a mean follow up of 2, 6 years a complete remission is obtained.

Conclusion: Neuroendocrine breast cancer are rare, the diagnosis is confir- med by immunohistochemical study. The primary treatment is surgery, the indication of chemotherapy and radiotherapy are the same as for adenocarcinoma. This entity of tumours needs larger series so as to clarify its histological, ther- apeutic and evolutive profile.
Keywords: Immunohistochemistry, neuroendocrine, breast carcinoma.

INTRODUCTION

Neuroendocrine breast carcinoma (NEBC) is a subtype of invasive mammary carcinoma in which 50% of the tumour cells expresses neuroendocrine markers. The reported incidence is 2%-5%, accounting for 0.1% of all breast cancers and 1% of all neuroendocrine tumours. We report 4 cases of primary NEBC, describing the histopathological features of NEBC, and giving some future challenges of this entity.

METHODS

This study is a retrospective analysis of 4 neuroendocrine breast cancer...

Fig. 1. Immunohistochemical stains for chromogranin.
collected in the department of radiotherapy oncology in CHU IBNROCHD of CASABLANCA-Morocco.

A review of the clinical files with immunohistochemistry study (hormonal receptor, synaptophysine, chromogranine, and Her-2/neu (C-erbB-2)) was carried out for all the cases.

**RESULTS**

The mean age is 51 years old, two patients had familiar history of mastopathy, the average time of diagnosis is 7 months, and the four patients presented a palpable mass in the breast.

The mean size of the tumour is 3, 3 cm, histologically, it was a neuroendocrine tumoral proliferation, immunohistochemical study confirmed the neuroendocrine nature showing expression of chromogranine in 3 cases (Figure 1), synaptophysine in 2 cases (Figure 2) and CD56 in one case. All cases were grade

![Fig. 2. Immunohistochemical stains for synaptophysin.](attachment:image)
II SBR, hormonal receptors were positive in 3 cases; Her2 neu negative in the four cases, one tumour was triple negative. Tumours were classed T1 in 2 cases and T2 in the two others. Nodes involvement was found in 2 cases. The treatment consisted in radical surgery followed by adjuvant chemotherapy; we used sequential protocol with 3FEC100-3Dcetaxel. Radiotherapy was used in 2 cases and hormonotherapy for the 3 patient with positive receptor. After a mean follow up of 2, 6 years a complete remission is obtained.

DISCUSSION

Neuroendocrine tumours of the breast (NEBC) are rare; their prevalence is about 0.5% in a series of 1368 histopathologically proven cancers and they account less than 1% of neuroendocrine tumours.

NEBC are most commonly seen in the 6th to 7th decade of life, and there is no specific clinical or imaging features [1, 2]. There is no reference about any predisposition for this type of tumours.

Their histogenesis is thought to arise from endocrine differentiation of a breast carcinoma rather than endocrine cells of the mammary tissue [3] NEBC include solid neuroendocrine (NE) carcinoma, large-cell neuroendocrine carcinoma, and small-cell carcinoma [4, 5].

The most helpful features characterizing the neuroendocrine neoplasm are cellular monotonity, nuclear palisading, and pseudorosette formation, as described by Tsang et al [6].

Positivity of neuroendocrine markers is required for the diagnosis. Immunohistochemical staining include chromogranin, synaptophysin, and neurospecific enolase. Chromogranin and synaptophysin have been widely accepted as specific markers.

To diagnose a primary neuroendocrine carcinoma of the breast, a nonmammary site has to be excluded clinically, and/or an in situ component should be demonstrated histologically. Chest, abdominal and pelvic computed tomography (CT) scan, bone scan, and positron emission tomography (PET) scanning are used to diagnose another primary tumours or distant metastases [7, 8] the presence of an in situ carcinoma is highly suggestive of a primary breast rather than a metastatic tumour [9].

There is no standard treatment protocol for NEBC. Surgery, radiotherapy, and/or chemotherapy, based on the clinical stage and presence of metastasis, seems to be the treatment of choice [10], a large variety of chemotherapy protocols have been employed in treating this disease. Systemic therapy principals have been derived from small retrospective case reviews of primary neuroendocrine breast carcinoma and extrapolated from studies of non breast neuroendo-
crine carcinoma since the clinical behaviour and histology are similar.

In small cell NEBC, all data suggest that chemotherapy should be similar to their pulmonary counterpart [9] and chemotherapy regimen is VP16-CDDP. Our patients have been treated as for adenocarcinoma 3FEC100-3docetaxel and a complete remission is obtained.

There is no consensus if NEBC have better or worse prognosis than the other breast tumours. Wei et al have demonstrated that NEBC is more aggressive with a higher tendency for local and distant recurrence and poorer overall survival [11]. moreover, they have demonstrated that neuroendocrine differentiation is an adverse prognostic factor independent of estrogen and progesterone receptor (ER/PR) status and nuclear grade [12].

In our experience, we cannot conclude to a different prognosis in NEBC, but all the 4 patients who underwent surgery are still alive, and have a complete remission.

CONCLUSION

Neuroendocrine breast cancer are rare, The lack of specific clinical or imaging features involves that the diagnosis often depends on the recognition of its histological growth pattern, and of the immunohistochemical staining for neuroendocrine markers. The primary treatment is surgery, the indication of chemotherapy and radiotherapy are the same as for adenocarcinoma. This entity of tumours needs larger series so as to give the recommendations for the correct handling, treatment, and surveillance as well as to clarify its histological, and clinical outcome.

REFERENCES

6. W. Tsang, J. Chan. Endocrine ductal carcinoma in situ (E-DCIS) of the breast. Form of low-


Invasive Lobular Carcinoma of the Breast Clinical and Evolutive Profile


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SUMMARY

Invasive lobular cancers (ILC) account for 5%-15% of all invasive breast tumours. The aim of this study is to determine their clinical and evolutive features. Our study is a retrospective analysis of 87 patients with ILC diagnosed from 2002-2010 in the department of radiotherapy oncology of CHU Ibn Roch of Casablanca. The median age is 47 years (28-75), 25 women (30%) have postmenopausal status and 12 cases (14%) have a relative breast cancer. The average consultation time was 11 months (1-36). The majority of women presented an ill-defined palpable mass (88%). 36 (43%) had right-sided breast cancer and 42 cases (51%) had a left-sided breast cancer whereas bilateral cancer was found in five cases (6%). The multifocality was found in four patients. The majority of cases (63%) were of lower histologic grade (grade 2) and were associated with 25% of vascular invasion. More than 69% (of the tumours) are oestrogen and progesterone receptor positive while Her 2 neu is negative in 73 cases (88%). Fifteen of our patients (18%) had a metastatic disease: the bone (60%), liver (40%) and lung (20%). The treatment consisted on neoadjuvant chemotherapy in 24 cases (29%), adjuvant chemotherapy in 43 cases (52%) follow up by radiotherapy, however Hormonal therapy was given to 57 patients (67%) and target therapy to 10 cases (12%). The 2-year survival rate is 72% according to Kaplan Meyer. ILC are a heterogeneous group of tumours. The choice of therapies depends on the individual characteristics of each patient and of the biological features of each tumour.

Keywords: breast cancer, invasive lobular carcinoma.
INTRODUCTION

Invasive lobular carcinoma of the breast accounts for 5 ± 15% of all invasive breast cancers, making it the most common histologic subtype after invasive ductal carcinoma (IDC). The clinical and radiological diagnosis is difficult to make. Its progression is slower than that of ductal cancer, and the prognostic factors are more favourable. The pattern of metastatic spread differs significantly from IDC. Both types of carcinoma are usually managed using the same clinical strategy. This study investigated the clinical and pathological features of ILC.

MATERIALS AND METHODS

Our study is a retrospective analyses of all patients with ILC diagnosed from 2002-2010 in the department of radiotherapy oncology of CHU IbnRoch of Casablanca. During this period a total of 4261 breast cancers were registered from which 87 patients were identified as ILC.

The clinical data (age, circumstances of discovery, mammographic appearance, treatment, evolution) were collected by analyzing all medical records. For each case, we determined the size of the tumour, number of lymph when metastatic axillary dissection was performed, the expression of hormone estrogen and progesterone receptors and the evaluation of HER-2/neu by immunohistochemistry and hybridization. The survival curve was calculated by the Kaplan-Meier method.

RESULTS

The annual incidence is 13.8 cases per year. 25 women were postmenopausal. The age of the patients ranged from 28 to 75 years old (mean: 47 years). The average consultation time was 11 months (1-36), the circumstances of discovery were a clinically palpable mass in 73 cases (88%), Mastodynia in 9 cases (11%), Inflammatory breast in 5 cases (6%), Nipple changes in 16 cases (20%) and change in the size of the breast in 9 cases (11%).

The right side was affected in 36 cases (43%), the left side in 42 (51%) while the disease was bilateral in 5 cases (6%). Histologically, the most common stage at presentation is stage II in 37 cases (43%). Multifocality was found in 4 patients. 63% of patients had grade II with 25% of vascular invasion. Lymph node involvement was noted in 1/3 of cases. More than 69% (of the tumours) are oestrogen and progesterone receptor positive while Her 2 neu is negative in
73 cases (88%). 5 patients presented with metastases. The treatment consisted on surgery in all cases: patey in 62 cases and conservative surgery in 25 patients. 5 cases with positives margins underwent a mastectomy in a second time. 27% has received neoadjuvant chemotherapy, 50% has received adjuvant chemotherapy followed by radiotherapy. Hormone therapy was prescribed in 57 patients (65%) and target therapy was performed in 10 patients (12%) cases.

After a mean follow up of 30 months, the evolution of our patients is indicated in table 1: a complete remission is obtained in 70 patients, 80% of local recurrence was observed in 4 patients whereas 24 patients developed metastases. The metastatic sites most commonly affected are the bone (60%), liver (40%) and lung (20%). The 2-year survival rate is 72% according to Kaplan Meyer (Fig. 1).

| Tab. 1. Evolution of patients after a mean follow up of 30 months. |
|----------------------------------|-----------------|-----------------|
| Complete remission               | Local           | Metastasis      |
| Recurrence                       | 4               | 24              |
| Complete remission               | 46              | 70%             |

DISCUSSION

Firstly described by Foote et al. in 1941 [1], ILC represents 5-15% of all breast cancer in Europe and the United States, respectively [2]. In our department the incidence was 6% in 2007.

The incidence of this type of breast cancer is increasing, especially among postmenopausal women [3, 4].

The clinical presentation of lobular carcinoma often has been described as an area of poorly defined thickening, in contrast to the typical finding of a discrete nodule in IDC. ILC tumours are often larger in size and more often multifocal, multicentric and bilateral [5].
Radiologically, the CLI is not accompanied by particular microcalcifications. The most frequent radiological presentations are asymmetric opacities ill-defined or speculated [6-8]. The rate of false-negative accounts in general in the literature is 8 and 19% [9 to 11]. In fact, it is often reported that mammographic abnormality is that of low density, lower or equal to the overall density [9, 11].

Mammographic size generally underestimates the histological size of these lesions [7, 12, and 13]. Sonography had a greater sensitivity than mammography for the detection of ILC and had the advantage of evaluating the presence of the axillary lymph node metastasis [4, 8, 15].

Enhanced magnetic resonance (MRI) imaging of the breast is extremely sensitive and is also useful to evaluate the extent of the disease [4]. It’s useful in detecting the multifocality and bilaterality of the lesions [4,8].

Histologically, the typical form consists of cells round isolated or arranged in single file. The cells are monomorphic round the nuclei [8]. The cytoplasm is acidophilic.
Compared to IDC, ILC is more often ER-positive and progesterone receptor (PR)-positive. And is generally HER2-negative [4,8]. In our study 69% and 66% are positive for ER and PR respectively, Her2 neu is negative in 88%. ILC is more often diploid, of lower histological grade. Nearly all ILC tumours are E-cadherin negative [19–22]; based on this, E-cadherin negativity is often used as a histological marker for confirming diagnoses of ILC. However, since it is a negative marker, it can be difficult to interpret, particularly in cases of low grade lesions and metastatic disease [6].

Because of the infiltrative growth pattern and frequent discontinuity (multicentric development) that is observed in ILC, there has been a tendency to treat patients with more aggressive surgery, including mastectomy and standard axillary lymph node dissection. The rate of local recurrence after conservative surgery and radiation therapy is high because of the frequent incidence of multicentricity and bilateral occurrence [6]. The rate of positive margin in the breast conservation therapy for ILC is reported to be over 50% and it is more frequent than that observed in IDC [6, 8]. In the present study, 20 patients underwent breast conservation therapy, but as five of them had positive margins they underwent a mastectomy in a second time. The response to primary chemotherapy for ILC is lower than IDC; therefore systemic therapy should be restricted to patients with inoperable or recurrent ILC [7]. It is necessary to consider a prospective randomized trial to evaluate the role of adjuvant chemotherapy versus hormonal therapy in ILC patients [7, 15].

Several studies have reported a high rate of multiple metastases. Metastatic ILC model differs from the IDC, a higher frequency of tumour extension concerns bone, gastrointestinal tract, uterus, meninges, ovary, and serous membranes [13]. In our study 60% of locations are represented by metastatic bone and 13% in the brain.

At an initial equal stage, there is no difference of prognosis between ILC and IDC [6, 8]. Silverstein and Toikkanen [8] found at the same stage a better prognosis of CLI compared to IDC.

CONCLUSION

In conclusion, there are several clinicopathological characteristics of ILC, however, its prognosis is not significantly different from other types of invasive breast carcinoma and therefore no differences in the therapeutic management are considered to be necessary. The choice of therapies depends on the individual characteristics of each patient and of the biological features of each tumour.
REFERENCES

An Open-Label Trial of SMK Treatment of Advanced Metastatic Cancer

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SUMMARY

The purpose of this study was to determine the safety, tolerability, and efficacy of SMK in patients with advanced metastatic breast cancer. SMK (Lumeria) is a unique therapy that creates alteration in defenses to oxidative stress and increases free radical exposure to the cancer cell. This was an IRB-approved study for metastatic cancer. No additional chemotherapy was allowed. The first 30 (14 with breast cancer) patients meeting entry criteria were consented. SMK was given orally and subcutaneously (SC), 5 days/week for 6 weeks (1 cycle). Subjects were allowed to continue with additional cycles based on their preferences. Before they entered the study, 4/14 (29%) had declined routine treatment, 10/14 (71%) had used many available treatments, and all were considered incurable in the end stage of progressive disease. The median number of cycles was 3 (range: 1-10). At the end of the study, 13/14 (93%) were alive with a median survival over 15 months; 11/14 (79%) had a 1 to 3 point improvement in ECOG rating; 10/14 (71%) had a 1 to 5 point improvement in EORTC (scale 1-7) rating. There was no treatment-associated toxicity except for cutaneous hyperpigmentation. In conclusion, SMK is a promising life extension treatment for metastatic breast cancer with no significant toxicity.
Keywords: Oxidative stress, free radical, cancer cell, tyrosine, rapamycin, phenytoin, melanin.

INTRODUCTION

Properties of the host micro-environment can significantly affect tumor morphology and growth dynamics, emphasizing the importance of understanding the tumor-host interaction. Cancer cells are anaerobic and have been designed or evolved to live in oxygen deprived conditions using hypoxia-inducible factor (HIF) protein [1].

HIF causes new blood vessels to grow around and into the tumor. The tumors continue to reproduce and replicate the anaerobic environment, thus, allowing for rapid reproduction of cancer cells. Current cancer drugs, for example, typically attempt to either limit the growth of tumors by cutting off the blood supply or attempt to limit the ability of the cancerous cell to replicate, but these therapies inevitably involve damaging healthy cells that surround the cancerous area, compromising the patient’s immune system and ability to heal, and resulting in a therapy that is highly toxic to the patient.

SMK Therapy is a unique therapy designed to kill cancer cells with oxidative stress techniques of forcing cancer to be an electron donor and potentiating a reaction with reactive oxygen. Cancer cells are anaerobic in nature, requiring a complex reductase synthesis process to deal with oxygen. A hypothesized toxicity is that by introducing oxygen directly into the cancer cell, oxygen acts as the invading electron scavenger. Oxidative stress follows and the cancer cell dies. SMK is a combination of agents that alter the defenses of cancerous cells to oxidative stress. One class of such therapies increases free radical availability to cancerous cells.

To understand the rationale for this combination drug therapy, it is necessary to consider cancer differently, i.e., as a purposeful tool in maintenance of homeostasis that becomes rogue. For example, many cancers originate in areas of the body that may be exposed to viruses. Cancer may be manufactured as a system to eliminate viruses, designed to be a sacrificial cell, easily killed by abundant oxygen. Cancer cells are sent to engulf an infected cell infected with a virus as a boundary between that cell and other needed cells. Upon viral exposure the cancer cell is to be killed by the body, taking all remnant of «active» virus with it. The «disease» cancer may be from one or more of the following subsequent errors: 1) The virus changes the perishable cancer cell to become more robust; 2) The body fails to identify the completion of the cancer cell’s mission and allows it to continue replication; 3) The body does not have the strength to apply the oxidative stress to kill the cancer cells.
The exact failure of the system may someday be diagnostically determined in *vivo*, but a method to address all of this variation may be achieved in a non-toxic combination therapy as follows: The first step is to weaken a cancer cell. The protective mucin layer surrounding cancer cells has a complex structure with recurring amino acids. Following well-understood uptake of such imaging agents as 3-123I-Iodo-L-methyltyrosine (3-IMT), we incorporate a mix of enantiomers as our tyrosine isomer component. The ability to enter the cell is confirmed by imaging (a benefit of melanin connection, described later) and facilitated by l-transfer modulation also disclosed. The exact mechanisms of utility within the cancer cell are not well understood, but the effect is reduced or incomplete mucin protection, allowing access of free radical based oxygen.

The availability of free radicals to react with the cancer is accomplished by a multi-stepped process. The origin of the free radicals is the cytochrome p450 site of the liver, and is up regulated with the 3A4 inducer, phenytoin. The steering of these dielectrically encased and preserved free radicals is accomplished naturally, as evidenced by free radicals abundant in cancer clusters’ periphery.

The next step is mutually advantageous to 2 parts of the process, and is achieved by use of C51H79NO13 rapamycin orally or bestatin, a leucine aminopeptidase inhibitor. This masking of leucine causes the liver to react as if a ketosis state is occurring, and recall leucine from systemic distribution. The leucine in part comes from the exterior surface of the cholesterol, releasing the still potentiated free radical contents. The absence of leucine has the dual function of greatly increasing l-transfer at the cancer cell.

At this point we have a mucin reduced cancer cell and directly contacted free radicals, requiring only a catalyst to compel a reaction. The melanin elevated in the body creates this electrical catalytic inducement. Melanin is elevated with oxsoralen (9-methoxy-7H-furo [3,2-g] [1] benzopyran-7-one methoxsalen) as oral melanin inducer or melanotan 2, (Ac-Nle-cyclo[Asp-His-D-Phe-Arg-Trp-Lys]-NH₂) in the suspension. Both of these materials encourage melanin creation in vivo. Additionally, a combination of melanin, either naturally occurring or artificially created, can be structurally bound to the tyrosine isomer, bringing melanin directly to the reaction point, and potentially creating residual electrolysis fed oxygen creation within the cancer cell. This yields a potential longer term treatment benefit [2, 3, 4, 5].

**MATERIAL AND METHODS**

This was an IRB approved, open-label, pilot study. SMK was administered orally as 3 capsules and injected as an SC suspension, 5 days per week for a
period of 6 weeks (1 cycle). Each dose was comprised of the following: Capsule 1: melanin 50 µg, tyrosine derivative 75 mg, Capsule 2: dilantin 30 mg, tyrosine derivative 150 mg, Capsule 3: 3-Amino-2-hydroxy-4-phenylbutyryl]-L-leucine 50 µg, tyrosine 75 mg and suspension for subcutaneous (SC) injection: 3-Amino-2-hydroxy-4-phenylbutyryl]-L-leucine 5 µg, melontan 10 µg, dilantin 2 mg tyrosine derivative 5mg, in NaCl bacteriostatic water.

Inclusion Criteria

Patients were age ≥ 18 years; provided written informed consent; had evidence of histologically confirmed, metastatic cancer; and an ECOG performance status of 0-2. Measurable disease was required by RECIST criteria 1.1 [6] as follows: (a) at least one target lesion, that had not previously been radiated, measurable in at least one dimension of greater than 2 cm by conventional CT or MRI, or at least 10 mm by spiral CT; or (b) palpable disease which was biopsy-proven to be metastatic (eg, skin nodule or lymphadenopathy), and that was superficial and measurable by caliper. All patients had adequate renal and hepatic function.

Exclusion Criteria

Patients with known leptomeningeal metastases or symptomatic brain metastases were excluded. Chemotherapy, radiotherapy (other than palliative radiotherapy to non-target lesions), biological, or investigational agents within 2 weeks of baseline disease assessments were not allowed. Any clinically significant gastrointestinal abnormalities that might have impaired intake, transit, or absorption of SMK were excluded.

Assessments

Easter Cooperative Oncology Group (ECOG) performance status was assessed at Screening, Baseline, every 2 weeks, and at the End of Treatment Visit. Laboratory studies (i.e., hematology [including WBC and differential], blood chemistry, urinalysis, and coagulation studies) were done up to 72 hours prior to treatment and were performed at Baseline, every 2 weeks, and at the End of Treatment Visit. Pregnancy tests for women of child bearing potential were done at Baseline within 72 hours of start of dosing and at the End of Treatment Visit if mandated by institutional policy.

Safety and adverse event (AE) assessments included recording of tumor-related, treatment-related, and unrelated signs and symptoms. Targeted questioning for AEs was done between Days 10 to 14 of the cycle, and on Day 28 and at End of Treatment Visit. Patients were followed for AEs at the Post-Treatment Follow-up visit at least 28 (and no more than 35) days after the end of treat-
Toxicity grading was by NCI Common Terminology Criteria for Adverse Events Version 3.0.1 (NCICTCAE 3.0.1.).

Patients completed the European Organization for Research and Treatment of Cancer Quality of Life Questionnaire (EORTC QLQ-C30), its lung cancer module (QLQ-LC13) [7], and the Dermatology Life Quality Index (DQLI) [8, 9] at Baseline, between Days 12 to 14, on Days 28 to 30, and at the End of Treatment Visit. Patients completed the questionnaire prior to being informed of their disease status.

At least 28 days, and no more than 35 days, after the last dose, patients underwent a physical exam including skin exam and recording of concomitant medications. Thereafter they were followed at least monthly for progression, treatment, and survival. For patients who came off treatment, follow-up data were collected either by follow-up visits with the investigator or designee, or telephone contact to the patient’s outside physician on a monthly basis to assess tumor status. For all patients, the following information was collected on a monthly basis: date of start of new treatment, type of systemic therapy administered, duration of second line systemic therapy; and survival status.

RESULTS

Breast tumor characteristics included: 12/14 (86%) ER(+), 10/14 (71%) ER/PR(+), 1/14 (7%) ER/PR/HER(-), 2/14 (14%) ER/HER(+); with metastasis to: 15% each: bone, lung, bone/lymph; 8% each: lung, lymph, bone/brain/lung, bone/brain/spine, bone/liver, bone/brain, liver/bones/lymph. The average age was 55(40-70) years; all were female; 93% Caucasian and 7% Asian.

Prior to study participation, 4/14 (29%) declined routine treatment, 10/14 (71%) had used all available treatment, and all were considered incurable in the end stage of progressive disease. All patients completed at least 1 cycle (1/14 [7%]), 4/14 (29%) completed 2 cycles, 3/14 (21%) 3 cycles, 3/14 (21%) 6 cycles, and 3/14 (21%) 10 cycles, depending on patient preference.

Eleven of 14 (79%) had a 1-3 point improvements in ECOG rating and 10/14 (71%) had a 1-5 point improvements in EORTC (scale 1-7) rating. Four of 14 gained weight (1-5 lbs), 6/14 (43%) remained the same, and 4/14 (28.5%) lost weight (1-2 lbs.). Eight of 14 (57%) had a reduction in pain levels (1-9 points on a scale of 1-10); 6/14 (43%) entered the study with no pain and maintained the same level; 6/14 (43%) entered the study on pain medication and 5 of those 6 (83%) no longer needed pain medication at the end of Cycle 1.

Three of 14 (21%) after Cycle 1 and an additional 2/14 (14%) after Cycle 2 had no detectable disease with routine exam, and imaging by RECIST1.1 criteria showed complete remission (CR) and no uptake on a PET scan. Five of
14 (36%) had significant reductions in quantity and/or size of the tumor, 2/14 (14%) had a reduction in quantity and/or size of the tumor, and 4/14 (29%) had no progression of disease. Two of 14 (14%) remain disease free, 4/14 (29%) remain stable with no additional treatment, 8/14 (57%) remain stable with additional treatment through August 2013.

Survival times are shown in Fig 1. Thirteen of 14 (93%) are alive with median survival over 15 months: 9/14 (64%) 15 months, 3/14 (21%) 16-17 months, and 1/14 (7%) 18 months. From the first day of treatment until August 2013, 1/14 (7%) died after 9 months. All subjects developed hyperpigmentation and tolerated the SMK compounds well. No other AEs have been reported related to the product. In all patients, tumor markers improved and liver function remained stable.

Fig. 1. Subject Survival in Days from First Treatment.
CONCLUSIONS

SMK is a promising treatment for metastatic breast cancer. It is a unique therapy with no significant toxicity except for cutaneous hyperpigmentation. It was well tolerated among 30 subjects with a variety of cancers, including 14 patients with breast cancer, and all patients either maintained the same ECOG rating (13.3%) or had improvements of 1 to 3 points; most patients also experienced improvements on the EORTC health rating and quality of life ratings. In part, these benefits are likely related to tumor response as documented by imaging. Equally important, the median survival as of August 2013 in the study sample is now over one year. Moreover, several of the individual patient case reports include dramatic radiographic responses such as, «No functional evidence of active neoplastic.»

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REFERENCES
