Dear Author,

Please check your proof carefully and mark all corrections at the appropriate place in the proof (e.g., by using on-screen annotation in the PDF file) or compile them in a separate list. Note: if you opt to annotate the file with software other than Adobe Reader then please also highlight the appropriate place in the PDF file. To ensure fast publication of your paper please return your corrections within 48 hours.

For correction or revision of any artwork, please consult http://www.elsevier.com/artworkinstructions.

Any queries or remarks that have arisen during the processing of your manuscript are listed below and highlighted by flags in the proof. Click on the ‘Q’ link to go to the location in the proof.

<table>
<thead>
<tr>
<th>Location in article</th>
<th>Query / Remark: click on the Q link to go</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>Please confirm that given names and surnames have been identified correctly.</td>
</tr>
<tr>
<td>Q2</td>
<td>The word “splenectomy” was changed to “splenectomy”. Please check here and in the subsequent occurrences if correct and amend if necessary.</td>
</tr>
</tbody>
</table>

Thank you for your assistance.
Ischemia modified albumin: An oxidative stress marker in β-thalassemia major

Samir M. Awadallah a,⁎, Manar F. Atoum b, Nisreen A. Nimer b, Suleiman A. Saleh b

a University of Sharjah, College of Health Sciences, Department of Medical Lab Sciences, Sharjah, United Arab Emirates
b The Hashemite University, Faculty of Allied Health Sciences, Department of Medical Lab Sciences, Zarqa, Jordan

► Levels of ischemia modified albumin (IMA) are increased in β-thalassemia major. ► IMA correlated significantly with ferritin and ferroxidase in thalassemic patients. ► Iron overload and oxidative stress induce the formation of IMA. ► IMA is suggested as an additional marker for oxidative stress in thalassemia.
Ischemia modified albumin: An oxidative stress marker in β-thalassemia major

Samir M. Awadallah a,⁎, Manar F. Atoum b, Nisreen A. Nimer b, Suleiman A. Saleh b

a University of Sharjah, College of Health Sciences, Department of Medical Lab Sciences, Sharjah, United Arab Emirates
b The Hashemite University, Faculty of Allied Health Sciences, Department of Medical Lab Sciences, Zarqa, Jordan

ARTICLE INFO

Article history:
Received 12 December 2011
Received in revised form 30 January 2012
Accepted 31 January 2012
Available online xxxx

Keywords:
Ischemia modified albumin
Thalassemia
Oxidative stress

ABSTRACT

Background: Ischemia modified albumin (IMA) is an altered type of serum albumin that forms under conditions of oxidative stress. This study reports on the levels and clinical significance of IMA in patients with β-thalassemia major.

Methods: Blood specimens were collected from 166 subjects (101 β-thalassemia major patients and 65 healthy controls). Serum levels of IMA, ferritin, malondialdehyde (MDA), ferroxidase, transaminases, total protein and albumin were determined using conventional methods.

Results: Serum levels of IMA (ABSU) were significantly higher in patients than in controls (0.725±0.155 vs 0.554±0.154, p=0.000). Similarly, higher levels were also observed for ferritin, MDA, ferroxidase, and transaminases. No significant differences were observed between patients and controls with respect to total protein and albumin. Spearman univariate analysis demonstrated significant correlation between IMA and ferritin, MDA, ferroxidase, and transaminases. Multiple linear regression analysis revealed significant association of IMA with ferritin and ferroxidase after adjusting for the other variables (r=0.343, p=0.002; r=0.228, p=0.029 respectively). MDA however, correlated significantly with ferritin only (r=0.654, p=0.000).

Conclusion: Our findings suggest that increased levels of IMA in thalassemic patients are likely to be a result of iron-induced oxidative stress and hence its potential significance as a new marker of oxidative stress in such patients.

© 2012 Published by Elsevier B.V.

1. Introduction

Thalassemia is a group of inherited disorders that generally affects people of the Mediterranean, Middle East, Africa, India, and South Asia [1]. It is characterized by chronic hemolytic anemia resulting from defective globin chain synthesis and ineffective erythropoiesis. β-thalassemia, the most common type, arises as a result of decreased synthesis or total absence of the β chain of hemoglobin. Consequently, excess free alpha chain accumulates and precipitates within erythrocytes leading to shortened life span and ineffective erythropoiesis [2,3]. Furthermore, patients with β-thalassemia major require continuous blood transfusion which leads to iron overload and subsequent organ and tissue damage. The status of iron overload and iron-induced oxidative stress has been repeatedly investigated in patients with thalassemia major [4–8].

Many studies reported increased blood levels of the redox active fractions of non-transferrin bound iron (NTBI) and labile plasma iron (LPI) in patients with β-thalassemia [3,5,9]. It has also been demonstrated that such patients experience decreased antioxidant capacity and increased products of peroxidative damage [6–8]. Collectively, these findings demonstrate that patients with β-thalassemia are under significant iron-driven oxidative stress.

The N-terminus residues of human serum albumin tend to bind with transition metals such as cobalt, copper and nickel [10,11] and alterations in this region of albumin hinder its binding capacity to such elements. Reactive oxygen species (ROS) resulting from conditions such as ischemia, hypoxia, acidosis, free radicals, and free iron can decrease the ability of the N-terminus to bind with transition metals [10–13]. Human serum albumin with a decreased binding capacity as a result of ischemic events is referred to as ischemia modified albumin (IMA) [12]. IMA as measured by the albumin cobalt binding test is currently used as an early marker for myocardial ischemia and acute coronary syndrome [14]. However, recent studies have reported increased levels of IMA in conditions other than ischemic heart diseases including diabetes mellitus, hyperlipidemia, chronic renal disease, obesity, and others [15–21]. Accordingly, it has been suggested that elevated levels of IMA may reflect a generalized rather than organ- or tissue-specific state of oxidative stress [21].

Oxidative stress, redox active forms of iron, and generation of ROS are well documented in patients with β-thalassemia major [3–9]. It is therefore possible that under such conditions, the structure of human serum albumin is modified in such a way that permits for excessive production of IMA. However, the relationship between β-
thalassemia major and IMA as a marker of oxidative stress has yet to be investigated.

2. Materials and methods

The study population consisted of 101 β-thalassemia major patients along with 65 age- and sex-matched healthy controls. In the patients group, the disease was detected at an early age of life (1–2y) using hemoglobin electrophoresis at alkaline pH (HPLC Labs, Beaumont, TX). None of the patients were classified as thalassemia intermedia and none of the controls were thalassemia minor. Subjects under 5 years of age were not included in the study. All patients were recruited from the Blood Bank and Transfusion Center of Zarqa, Jordan. Clinical history and other relevant data were collected from patients’ files with prior permission of attending physician. All patients received approximately 350 to 500 ml of packed red blood cells at each transfusion (3 to 4-week intervals) and were all on iron chelation therapy using either deferasirox (Exjade) or deferoxamine. Mean serum ferritin level measured over the last two years was used for evaluation. Clinical data relevant to cardiac complications, endocrine disorders, or growth retardation were not documented and not included in the study. The control population was selected from various outpatient clinics in the same area. None had any history of blood transfusion, anemia, liver disease, or active inflammatory conditions. All participants were informed of the purpose of the study and were asked to sign a written consent, which was previously approved by the local research ethics committee.

Blood was collected in plain and EDTA tubes; samples were collected from patients just prior to blood transfusion. Serum was separated by centrifugation and divided into several aliquots and kept at −80 °C until they were analyzed. Serum total protein, albumin, alanine transaminase (ALT), and aspartate transaminase (AST) were determined by automated chemistry analyzer (Hitachi 912, Roche, Germany). Serum ferritin levels were determined by immunoassay analyzer (Elecsys, Roche, Germany). Ferroxidase activity was determined by spectrophotometry using O-dianisidine dihydrochloride method [22]. Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thiobarbituric acid (TBA) method [22]. Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thiobarbituric acid (TBA) method [22]. Lipid peroxidation was measured by determining the malondialdehyde (MDA) production using thiobarbituric acid (TBA) method [22].

3. Results

Table 1 shows the general characteristics and lab findings for both patients and healthy controls. The levels of ALT and AST were significantly higher and the levels of HB were significantly lower in patients as compared with controls. No significant differences were observed between patients and healthy counterpart with regard to serum total protein and albumin. Data was also analyzed separately to test the bearing of HCV and splenectomy as potential sources of variation with respect to all measured parameters. No significant differences were observed between patients with or without HCV or splenectomy.

4. Discussion

Results of this study point to several significant findings. (i) The levels of IMA are significantly higher in thalassemic patients as compared with healthy controls. (ii) High levels of IMA in thalassemic patients significantly correlate with ferritin, MDA, and ferroxidase. (iii) While ferritin was found to be the only predictor of MDA status in thalassemic patients, both ferritin and ferroxidase were found to be the predictors of the IMA status in such patients.

Preclinical studies have demonstrated that overproduction of ROS resulting from conditions related to ischemia, hypoxia, acidosis, free radicals, and free iron plays a major role in the formation of IMA [10–14]. Several recent studies however have demonstrated that IMA elevates in conditions other than ischemic heart disease [15–21]. In that, increased levels of IMA were reported in conditions

Table 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Thalassemia (101)</th>
<th>Controls (65)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>12.8±7.0</td>
<td>15.8±5.8</td>
<td>NS</td>
</tr>
<tr>
<td>Male/Female</td>
<td>48/53</td>
<td>32/33</td>
<td>NS</td>
</tr>
<tr>
<td>Hepatitis C, n (%)</td>
<td>15 (14.9)</td>
<td>None</td>
<td>NS</td>
</tr>
<tr>
<td>Splenectomy, n (%)</td>
<td>38 (37.6)</td>
<td>None</td>
<td>NS</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>7.9±1.3</td>
<td>12.4±2.1</td>
<td>0.000</td>
</tr>
<tr>
<td>TP, g/dl</td>
<td>7.6±0.76</td>
<td>7.4±0.68</td>
<td>NS</td>
</tr>
<tr>
<td>Alb, g/dl</td>
<td>4.6±0.64</td>
<td>4.8±0.8</td>
<td>NS</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>47.9±39.5</td>
<td>14.5±8.2</td>
<td>0.000</td>
</tr>
<tr>
<td>AST, U/l</td>
<td>56.8±47.4</td>
<td>18.5±7.4</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Thalassemia (101)</th>
<th>Controls (65)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (g/l)</td>
<td>2529±2218</td>
<td>38±26</td>
<td>0.000</td>
</tr>
<tr>
<td>MDA (μmol/l)</td>
<td>1.90±0.91</td>
<td>1.10±0.32</td>
<td>0.000</td>
</tr>
<tr>
<td>Ferroxidase (U/l)</td>
<td>1362±358</td>
<td>96.5±19.8</td>
<td>0.000</td>
</tr>
<tr>
<td>IMA (ABSU)</td>
<td>0.723±0.155</td>
<td>0.554±0.154</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* MDA (malondialdehyde); IMA (ischemia modified albumin).
related to oxidative stress including type 2 diabetes [15], hyperlipidemia [16,17], chronic kidney disease [18], obesity [19], and multiple sclerosis [21]. Furthermore, recent in vitro studies have demonstrated that generation of hydroxyl radicals (·OH) by the Fenton reaction was associated with a rapid rise of IMA concentration [13]. Consistent with these observations and taking into account the fact that oxidative stress, production of ROS, impaired antioxidant defense mechanisms, hypoxia, and anemia clearly manifest in thalassemic patients, it is only logical to predict elevated levels of IMA in such patients. In this context, iron-driven oxidative stresses in association with chronic anemia and hypoxia, are the most likely causes that lead to the modification of the N-terminus of serum albumin and thus the formation of increased levels of IMA in thalassemic patients. Albeit, the exact mechanism still need to be elucidated.

High serum ferritin is a predictable consequence of continuous blood transfusion in thalassemic patients [25]. Though it may also increase during acute inflammation, it remains the most common indicator of iron overload in thalassemic patients [25]. In agreement with these observations, high levels of serum ferritin noted in our patients are strongly suggestive of a state of iron overload. As a result of iron overload, redox active fractions such as NTBI and LPI are expected to increase in circulation [4,9]. These fractions can catalyze the production of ROS contributing to significant tissue injury. Under such conditions, it is very possible that such redox active forms of iron could contribute to the formation of IMA in thalassemic patient; a finding that has been noted by this study. In further support of this finding, significant correlation was observed between IMA and serum ferritin as evidenced by data from univariate (p = 0.000) (Table 3) and multivariate regression analysis (p = 0.002) (Table 4 and Fig. 1).

Ceruloplasmin, a copper-containing plasma protein, exhibits antioxidative ferroxidase activity through different mechanisms. Ceruloplasmin ferroxidase activity converts iron from the ferrous to the ferric form [26] thus preventing the formation of ROS through the Fenton reaction and hence minimizing oxidative damage of lipids, proteins, and DNA [26]. Conversion of ferrous iron to its ferric form is known to enhance iron uptake by transferrin thus facilitating its transport, storage, and/or utilization. Furthermore, ferroxidase activity promotes the efflux of iron from tissues into plasma as means of minimizing iron-induced tissue damage [27–29]. It has been suggested that under conditions of iron overload and as a result of transferrin saturation, iron could bind with albumin thus contributing to the formation of more redox active forms of iron like NTBI [30–32]. Based on these findings, increased ferroxidase activity as observed in thalassemic patients [24] (Table 2) could be explained in the context of indirectly enhancing oxidative stress and hence the significant correlation with IMA (Table 4, Fig. 1). It is worth emphasizing that this is the first study reporting on the association between ferroxidase activity and IMA in thalassemic patients.

MDA, a product of lipid peroxidation, significantly elevates in thalassemic patients through different mechanisms including excess amount of iron binding to erythrocytes [8] and free α-globin chain precipitation and the consequent generation of intracellular ROS [6,33,34]. It has also been suggested that increased liver lipid peroxidation as a result of ferritin accumulation could raise the rate of leakage of MDA into the circulation [7]. Hence, the association between ferritin, MDA, and IMA becomes predictable.

Table 3

<table>
<thead>
<tr>
<th>Parametersa</th>
<th>Age</th>
<th>Ferritin</th>
<th>Ferroxidase</th>
<th>MDA</th>
<th>IMA</th>
<th>ALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin</td>
<td>r = .426</td>
<td>p = .000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDA</td>
<td>r = .410</td>
<td>r = .525</td>
<td>r = .759</td>
<td>p = .000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT</td>
<td>r = .255</td>
<td>r = .252</td>
<td>p = .001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMA</td>
<td>r = .566</td>
<td>r = .425</td>
<td>r = .359</td>
<td>p = .000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST</td>
<td>r = .010</td>
<td>r = .000</td>
<td>r = .000</td>
<td>p = .000</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* MDA (malondialdehyde); IMA (ischemia modified albumin); ALT (alanine transaminase); AST (aspartate transaminase).

Table 4

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Beta (b)</th>
<th>SEb</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin</td>
<td>0.566</td>
<td>0.000</td>
<td>0.002</td>
</tr>
<tr>
<td>MDA</td>
<td>-0.090</td>
<td>0.025</td>
<td>NS</td>
</tr>
<tr>
<td>Ferroxidase</td>
<td>0.202</td>
<td>0.000</td>
<td>0.029</td>
</tr>
<tr>
<td>ALT</td>
<td>0.186</td>
<td>0.001</td>
<td>NS</td>
</tr>
<tr>
<td>AST</td>
<td>-0.138</td>
<td>0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

b: regression coefficient; SEb: standard error of b.

ferritin and MDA as previously reported [7] and as observed in this study (Table 5 and Fig. 2) can be readily explained.

In conclusion, findings reported by this study suggest that IMA is a general rather than a tissue-specific oxidative stress marker. The significance of association of IMA with ferritin and ferroxidase indicates the importance of including it as a new marker of oxidative stress in thalassemia.

Acknowledgments

This work was supported by research grants from the University of Sharjah (No. 090506) and the Hashemite University in Jordan (No. AM/16/13/10). The authors wish to thank Mrs. Kefah Abu Ashaikh of Zarqa Blood Bank for her technical expertise.

References
