Investigation of Antioxidant Status in Iraqi Patients with Beta-Thalassemia Major

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Abstract

Background: Oxidative stress is the most common complication in Beta-thalassemia patients due to iron overload that is resulted from long life blood transfusion leading to growth retardation, delay of sexual maturation, and later involvement of the liver, heart, and endocrine system. Aim of study: Assess of some antioxidant parameter status in serum and saliva of patients with beta-thalassemia major in comparison with control subjects. Methodology: Total antioxidant (TAO), glutathione (GSH), uric acid (UA), urea (U) and albumin were investigated in serum and saliva samples of all subjects. Results: Albumin level in serum is significantly (P < 0.00001) decreased in patients group (3.27 ± 0.17 g/dl) from that in control group (3.59 ± 0.13 g/dl). However, TAO level was significantly elevated (P = 0.031) from (3.14 ± 1.38 U/ml) in the saliva of control group up to (4.09 ± 1.76 U/ml) in the saliva of patients group as well as significant difference (P = 0.02) in salivary fraction index (Sf) only for TAO which is elevated from 0.47 in control group up to 0.56 in patients group. Conclusion: The findings of this study indicated that the serum level of albumin can be used as a good marker to reflect the liver status in TM patient in addition to other conventional markers. Also this study suggested that saliva can be act as an alternative sample to assess the antioxidant status in TM patients but need further investigation.

Keywords: Beta thalassemia major, Iron overload, Antioxidant, Saliva.

Introduction

Thalassemia is one of the commonest hemolytic anemia resulted by genetic disorder in beta globin chain production [1]. Thalassemia major presents as a progressive anemia during 6 to 24 months of age as γ-chain synthesis diminishes without a concomitant increase in β-chain synthesis due to genetic abnormalities involving both β genes and characterized by reduced Hb level (<7 g/dl) [2]. In thalassemia major, the excess unpaired alpha-globin chains aggregate to form inclusion bodies that damage RBC membranes, leading to intravascular hemolysis and premature destruction of RBC precursors, causing ineffective erythropoiesis [3, 4]. Blood transfusion is commonest treatment for beta-thalassemia major that is performed repeatedly with different interval period depending on the severity of anemia in order to compensate the ineffective erythropoiesis as well as to maintain Hb concentration [5, 6]. Frequently blood transfusion can caused several vicious effects such as HCV, HBV and hypersensitivity reactions [7], however, iron overload still remains the most life-threatening complication in individuals with beta thalassemia as a consequence of recurrent transfusion and ineffective erythropoiesis which lead to oxidative damage in several internal organs including heart, liver, and endocrine system [2, 8]. Although iron is essential for metabolic processes, it has a catalytic role to produce powerful reactive oxidant species (ROS) and free radicals, which lead to oxidative damage [9, 10].

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To protect themselves from pro-oxidant agents, which are generated both during cellular metabolism and as a result of pathologic processes, cells have both enzymatic and non-enzymatic defense systems. The oxidative state of the cell is related to the balance between the formation of oxide-reducing agents and the antioxidant defense system [9, 11]. Since evaluation and maintenance of antioxidant defense can be useful in protecting β-thalassemia patients from more serious complications of the disease, therefore the present study is designed to investigate some antioxidant markers in the serum and saliva of TM patients in comparison with normal subjects.

**Material and Method**

Subjects involved in this study include eighty subjects; 60 of them were patients with homozygous beta-thalassemia major (TM) (35 males, and 25 females) at age range 4-30 years, all of them were blood transfusion-dependent, also all of them were on iron chelation therapy. Additionally, twenty normal subjects (10 males, and 10 females) with age range corresponding to that of patients were involved and considered as control group. Blood and saliva samples were collected.

The concentration of urea, uric acid, and albumin were determined in serum and saliva for both groups by using a specific kit from (AGAPPE diagnostics-Switzerland, GmbH) based on colorimetric method [12-14], as well as The level of TAO was determined in the serum and saliva of all subjects (normal and patients) by using colorimetric detection kit provided from (Elabscience Biotechnology Co, Ltd, China).

However, the concentration of GSH was determined in the serum of all subjects by using competitive-ELISA kit provided from (Elabsence Biotechnology Co, Ltd, China). All data are expressed as mean ± standard deviation (M±SD) and differences in the level of biomarkers are statistically analyzed by student-test and p value less than 0.05 is considered as significant.

**Results**

Five intrinsic antioxidants are measured in the serum of all subjects in patients and control groups which include albumin, urea (U), uric acid (UA), glutathione (GSH), and total antioxidant (TAO). The results revealed non-significant difference in serum level of most antioxidants except for that of albumin which is significantly (P < 0.00001) decreased in patients group (3.27 ± 0.17 g/dl) from that in control group (3.59 ± 0.13 g/dl) as shown in (Table 1).

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Serum level (M±SD)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>TM Patients</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.59 ± 0.13</td>
<td>3.27 ± 0.17</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>33.09 ± 4.4</td>
<td>33.2 ± 3.3</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>5.29 ± 0.79</td>
<td>5.3 ± 0.26</td>
</tr>
<tr>
<td>GSH (μg/ml)</td>
<td>34.05 ± 9.17</td>
<td>34.98 ± 9.17</td>
</tr>
<tr>
<td>TAO (U/ml)</td>
<td>6.41 ± 2.01</td>
<td>6.86 ± 2.51</td>
</tr>
</tbody>
</table>

In similar manner, level of these antioxidants are measured in the saliva of all subjects in patients and control groups. The results demonstrated that only TAO level was significantly elevated (P = 0.031) from (3.14 ± 1.38 U/ml) in the saliva of control group up to (4.09 ± 1.76 U/ml) in the saliva of patients group (Table 2).

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Salivary Level (M±SD)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>TM Patients</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>0.99 ± 0.06</td>
<td>0.95 ± 0.16</td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>24.2 ± 4.9</td>
<td>22.04 ± 8.07</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>5.14 ± 1.79</td>
<td>4.84 ± 1.91</td>
</tr>
<tr>
<td>GSH (μg/ml)</td>
<td>12.7 ± 3.7</td>
<td>13.8 ± 4.7</td>
</tr>
<tr>
<td>TAO (U/ml)</td>
<td>3.14 ± 1.38</td>
<td>4.09 ± 1.76</td>
</tr>
</tbody>
</table>
According to results obtained from table-1 & 2, salivary fraction index (Si) is calculated by dividing the concentration of each antioxidant in saliva (A) by its concentration in serum (B): [Si = A/B]. Again, the results illustrated in (Table-3) recorded significant difference (P = 0.02) in (Si) only for TAO which is elevated from 0.47 in control group up to 0.56 in patients group.

Table 3: The salivary fraction index (Si) of intrinsic antioxidants in patients and control groups

<table>
<thead>
<tr>
<th>Antioxidant</th>
<th>Salivary fraction (Si) (M±SD)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>TM Patients</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.27 ± 0.01</td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>Urea</td>
<td>0.73 ± 0.10</td>
<td>0.66 ± 0.22</td>
</tr>
<tr>
<td>Uric Acid</td>
<td>0.98 ± 0.34</td>
<td>0.91 ± 0.35</td>
</tr>
<tr>
<td>GSH</td>
<td>0.39 ± 0.15</td>
<td>0.39 ± 0.10</td>
</tr>
<tr>
<td>TAO</td>
<td>0.47 ± 0.14</td>
<td>0.56 ± 0.10</td>
</tr>
</tbody>
</table>

Discussion

The patients with β-thalassemia major usually suffer from iron overload, iron has a catalytic role to produce powerful reactive oxidant species (ROS) and free radicals, which lead to oxidative damage [8]. Antioxidants play an essential role in protection of the cells from oxidative damage and can be evaluated by measurement of either individual antioxidants levels in cells and plasma or total antioxidant capacity. The latter can be estimated by measuring total reducing activity of body fluids such as serum and plasma [11, 15].

The present study was performed to assess five intrinsic antioxidants in the serum and saliva of all subjects in patients and control groups which include albumin (Alb), urea (U), uric acid (UA), glutathione (GSH), and total antioxidant (TAO). In the serum, only albumin concentration was significantly (P < 0.00001) decreased in TM patients (3.27 ± 0.17 g/dl) from that in control (3.59 ± 0.13 g/dl) as shown in (Table-1). However, only TAO level was significantly elevated (P = 0.031) from (3.14 ± 1.38 U/ml) in the saliva of control group up to (4.09 ± 1.76 U/ml) in patients group (Table-2). After calculating the salivary fraction index (Si) of these antioxidant markers, only TAO was significantly elevated from 0.47 in control group up to 0.56 in TM patients (Table-3).

Three ideas can be extracted from these findings; the first one indicated that all antioxidants markers involved in this study were detectable in saliva, the second idea confirmed that salivary fraction index of each marker nearly remains in a constant percentage relatively to its concentration in serum whether in healthy or diseased subjects. However, the third idea indicated that salivary TAO marker act as a predictive value that reflect the antioxidant status in the TM patient and can be used for monitoring prognosis of complications in such patients. There are limited studies about assessment of serum total antioxidant capacity in thalassemic patients. Some of these studies reported no significant differences in TAC between thalassemic and control groups, in spite of increased level of Oxidative Stress Index [16].

However, other studies reported that endogenous antioxidants such as ferritin, uric acid (UA) and bilirubin can result in increased level of TAC in the patients with Beta-thalassemia major [17, 18].

In contrast, Ghon et al showed the depletion of antioxidants in thalassemic patients [8], also a significant decrease of Total Antioxidant Capacity (TAC) was observed in thalassemic patients compared to controls, and a significantly lower concentration of TAC in thalassemic patient with chelation therapy compared to patient without therapy was also reported [19]. Recently, Tolba et al in study on Egyptian TM patients found decreased antioxidants level in their serum represented by (Paraoxonase 1, ary esterase, reduced glutathione and catalase) along with hypertriglyceridermia, hypercholesterolemia, low HDL levels, and also increased malondialdehyde level, so that all these factors contributing to the development of atherosclerosis in TM patients [20].
Several hypotheses were postulated to explain the enhanced total antioxidant status (TAS) in TM patients. One of them suggested that elevation antioxidant is a compensatory response arising from excessive oxidative stress that may be relevant to chelation therapy by deferoxamine \[21, 22\]. Other studies suggested that endogenous antioxidants such as ferritin, UA, Albumin and bilirubin can result in increased level of TAS in the patients with Beta-thalassemia major because iron overload could potentially induce hepatic toxicity that increased bilirubin level due to decrease in activity of cytochrome c oxidase disrupting the mitochondrial respiration, and consequently hepatic damage could lead to decrease in vitamin E serum level in the absence of transaminase augmentation \[17-18, 23-24\].

In respect with the elevation of the salivary fraction index (Sf) of TAC in TM patients that is found in this study (Table-3), no corresponding studies investigated this marker except very few researchers investigated individual antioxidant markers in saliva for various diseases rather than thalassemia major for instances; it has been found that salivary concentrations of urea and UA are similar to plasma and may change as a result of metabolic disorders such as kidney disease, gout or metabolic syndrome \[25, 26\].

Similarly, salivary variations in total antioxidant status, its components (superoxide dismutase, catalase, UA, glutathione peroxidase, glutathione reductase, glutathione) and other oxidative stress markers (malondialdehyde, protein thiols, pro-inflammatory cytokine) have been observed in saliva \[27, 28\]. Furthermore, several studies showed increased in serum and saliva malondialdehyde levels in patients with beta thalassemia major and was significantly higher in thalassemic patients with periodontitis than that in the healthy subject and also than that in thalassemic patients without periodontitis \[29, 30\]. This over production of malondialdehyde at the inflammatory site can be related to the greater degree of oxidative stress in patients with periodontitis \[31\].

On the other hand, uric acid concentration in saliva might be better index of uric acid production in the body than the uric acid concentration in blood or urine \[32\]. Recent study found an increase in serum and saliva uric acid in patients with beta thalassemia major, but its concentration was significantly higher in thalassemic patients without periodontitis than that with periodontitis and healthy groups. This elevation of uric acid may be due to counteract the increased oxidative stress in those patients represented by the increased level of serum and saliva oxidative stress biomarker malondialdehyde \[33\].

In Conclusion, the findings of this study indicated that the serum level of albumin can be used as a good marker to reflect the liver status in TM patient in addition to other conventional markers. Also this study suggested that saliva can be act as an alternative sample to assess the antioxidant status in TM patients and need further investigation.

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References


