Full Length Research Paper

Biochemical factors relevant to kidney functions among Jordanian children with beta-thalassemia major treated with deferoxamine

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Thalassemia is one of the systematical diseases that occur worldwide and is the commonest form of hemoglobinopathy in Jordan. The most important cause of mortality and morbidity in these patients with thalassemia is organ failure related with the shortened red cell life span, rapid iron turnover and tissue deposition of excess iron. These are the major factors responsible for functional and physiological abnormalities found in various forms of thalassemia. This study aied to examine the biochemical factors related to kidney functions such as glucose, urea, creatinine, sodium and potassium levels among Jordanian children with β-thalassemia major treated with deferoxamine. Forty two patients (aged 12 to 28 years) with β-thalassemia major (20 males and 22 females) that undergo periodical blood transfusion and they are on deferoxamine (DFO) as chelating agent were involved in this study. All patients were free from hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). The diagnoses of β-thalassemia major were made based on the clinical, hematological and hemoglobin electrophoresis profiles for the patients. Haemoglobin (Hb) electrophoresis for the father and mother and genetic study of the b globins genes in some disputable cases were also done. Forty controls of matched age and gender (20 males and 20 females) were also included in this study. Results showed that the significant differences (p < 0.05) appeared between the experimental and control groups over all the measured physiological variables (urea, creatinine, uric acid, sodium and potassium) except for blood glucose and chloride. It is concluded that the functional abnormalities of the kidney in patients with β-thalassemic patients can be attributed to chronic anemia, iron overload as well as to DFO toxicity and enhancement of the oxidative stress induced by excess iron deposits. These functional abnormalities would have any long-term effects on the patients.

Key words: β-Thalassemia major, renal function, desferrioxamine, iron overload.

INTRODUCTION

Beta-thalassemia major is one of the most common hereditary hematologic disorders characterized by severely impaired β-globulin synthesis. Beta thalassemia major (BTM) is a common health problem in the Middle East, Africa, the Indian subcontinent, and Southeast Asia. BTM is a hereditary severe anemia resulting from defects in beta-globin synthesis (Modell et al., 2001; Rund and Rachmilewitz, 2005). Beta thalassaemia major is the most prevalent type of thalassaemia as it is common in certain populations. It produces severe anemia in its...
homozygous state (Widad et al., 2003). About 190 million people throughout the world have genetic mutations associated with different hemoglobinopathies, and more than 90 million of them carry defective genes leading to thalassaemia (Ambekar et al., 2001; Das et al., 2004).

According to Yesilipek (2007), there are over 200,000 beta thalassemia patients in the Mediterranean area alone. According to the Jordan Ministry of Health (MOH), there are about 1400 registered thalassemic patients in Jordan till this time and noteworthy that 4 to 6% of Jordan’s population has the characteristic of the disease but are not infected, of whom health care has a large burden on health budget and family economics and social complain. The disease is associated with hemolysis in the peripheral circulation, and deposition of excess iron in the tissues, are some of the causes of clinical manifestations (Yesilipek, 2007).

Iron deposition in the heart, liver, and multiple endocrine glands results in severe damage to these organs, with variable endocrine organ failure. Profound anemia and excess iron deposition leads to dysfunction of cardiovascular, reticuloendothelial, and other organ systems (Muncie and Campbell, 2009). However, the most serious complication of iron overload is life-threatening cardiotoxicity. Cardiac events due to iron overload are still the primary cause of death (Papanikolaou et al., 2005). Iron overload causes most of the mortality and morbidity associated with thalassemia. Iron-chelating therapy is largely responsible for doubling the life expectancy of patients with thalassemia major; it has been proven to prevent liver and heart damage, allow for normal growth and sexual development in children with thalassemia, and increase life span (Rund and Rachmilewitz, 2005). There are many reports on complications of β-thalassemia in different organs (Low 2005; Al-Rimawi et al., 2005; Angelopoulos et al., 2006; Cetin et al., 2003; Asma et al., 2003).

In the absence of chelating therapy, the accumulation of iron results in progressive dysfunction of the heart, liver, and endocrine glands. In the last 30 years, conventional treatment of β-thalassaemia major, based primarily on regular blood transfusions and iron chelation therapy with deferoxamine (DFO) and know a day with desferrioxine (exjade) has markedly improved the prognosis of the disease. Adequate administration of parenteral DFO reduces or prevents iron accumulation and iron-mediated organ damage, resulting in a consistent decrease of morbidity and mortality (Wong and Richardson 2003). There are little information available about renal involvement in this disease. In recent years, there are evidences of aminoaciduria, hypercalciuria, phosphaturia, magnesiuria, hyperuricosuria, low urine osmolarity, and excess urinary secretion of markers of tubular damage such as N-acetyl-D-glucosaminidase in patients with beta-thalassemia major (Aldudak et al., 2000; Sumboonnanonda et al., 1998; Sadeghi et al., 2008).

Renal failure is a terminal event in thalassemia major and is usually secondary to heart failure and/or hepatic failure. Acute renal failure following deferoxamine overdose or hemolysis has been reported (Prasannan et al., 2003). There are limited studies on renal involvement in β-thalassemia, mainly involving patients on deferoxamine, reporting both glomerular and tubular dysfunction. In the present study, our aim was to evaluate the renal manifestations in patients with thalassemia major. In order to evaluate the effect of DFO and iron overload on the renal functions among Jordanian thalassemic children treated with DFO, we examined, for the first time, a number of biochemical variables such as glucose, urea, creatinine, uric acid and electrolytes as Na, K, and Cl.

MATERIALS and METHODS

Study patients

Forty two patients (aged 12 to 28 years) with β-thalassemia major (20 males and 22 females) that underwent periodical blood transfusion and they were on DFO as chelating agent were involved in this study. The diagnoses of BTM were made based on the clinical, hematological and hemoglobin electrophoresis profiles and the results of β-globin chain synthesis at Thalassaemia Unit at Princess Rahma Educational Hospital, Irbid, Jordan. In addition, forty healthy individuals of matched age and gender were also included as controls. Furthermore, approval permission was obtained from the patients and the control persons and their parents. This study was conducted, ethical approval was obtained by the Institutional Review Board of Princess Rahma Educational Hospital. Medical histories such as clinical and transfused records of all 42 BTM patients were obtained from the hospital files. Informed consent was provided for each patient and healthy control and their parents’, who participated in this study. All patients and controls were interviewed and filled out standardized questionnaires during this study. In addition, all patients and controls were tested and found free from hepatitis B virus (HBV), hepatitis C virus (HCV) and human immunodeficiency virus (HIV). None of the studied patients had undergone splenectomy or other supplement treatment. All patients received regular blood transfusion after the age of one year old, usually given regularly every 2 to 4 weeks, to maintain a pre-transfusion hemoglobin level above 10 g dl⁻¹. None of the subjects was treated with vitamin E and/or vitamin C supplemtations before the study. All patients were also started on subcutaneous infusion of DFO as chelating agent (45 mg kg⁻¹ day⁻¹), for 8 to 10 each week) at age of two or three years old prior to presentation to us.

Blood collection

Five (5 ml) of venous blood sample was drawn into heparin from each BTM patient before the transfusion and from each healthy control. Three (3 ml) were centrifuged at 3,000 rpm for 10 min at room temperature. The serum samples were stored at 4°C until needed for analysis of urea, creatinine, uric acid, sodium, potassium, chloride, ferritin and blood glucose level. The remaining 2 ml were used for studying some hematological parameters such as hematocrit, hemoglobin levels and leukocyte counts.

Experimental

Serum urea, creatinine, glucose and uric acid were examined using
Table 1. Hematological and biochemical data of s-thalassemia major patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n=20)</th>
<th>Male (n=20)</th>
<th>Female (n=20)</th>
<th>Female (n=20)</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Patients</td>
<td>Control</td>
<td>Patients</td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>36±3.8</td>
<td>29±2%*</td>
<td>35±2.6</td>
<td>27±4.2*</td>
<td>0.000*</td>
</tr>
<tr>
<td>Ferritin (µg/l)</td>
<td>78±21</td>
<td>2564±762*</td>
<td>62±18</td>
<td>2389±684*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>12.9±0.7</td>
<td>9.2±1.6*</td>
<td>11.1±0.9</td>
<td>8.4±2.2*</td>
<td>0.001*</td>
</tr>
<tr>
<td>Leukocytes x 10⁶/L</td>
<td>10.56±2.31</td>
<td>10.42±3.26</td>
<td>9.44±2.67</td>
<td>10.2±2.46</td>
<td>0.463</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>37.27±5.18</td>
<td>39.14±3.42</td>
<td>36.9±6.60</td>
<td>41.13±5.37</td>
<td>0.001*</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>33.66±8.05</td>
<td>36.62±5.53</td>
<td>34.85±6.20</td>
<td>37.87±6.40</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

*The mean difference is significant in comparison with control untreated group (P < 0.05).

Table 2. Laboratory characteristics and significance testing in the thalassemic and control groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental N=42</th>
<th>Control N=32</th>
<th>P-value t-test</th>
<th>P-value Mann-Whitney U-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>113.36±52.52</td>
<td>98.75±13.54</td>
<td>0.13</td>
<td>0.432</td>
</tr>
<tr>
<td>Urea</td>
<td>33.5±5.50</td>
<td>29.05±7.30</td>
<td>0.005*</td>
<td>0.009*</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.352±0.113</td>
<td>0.296±0.0533</td>
<td>0.012*</td>
<td>0.012*</td>
</tr>
<tr>
<td>Uric acid</td>
<td>4.42±1.083</td>
<td>3.57±0.473</td>
<td>0.009</td>
<td>0.008*</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>143.02±2.50</td>
<td>137.66±5.15</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>4.670±0.331</td>
<td>4.194±0.345</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>Chloride (mmol/L)</td>
<td>103.24±3.24</td>
<td>103.16±4.10</td>
<td>0.924</td>
<td>0.887</td>
</tr>
</tbody>
</table>

*Result is significant at the 5 % level.

Commercial analytical kits from Sigma (St. Louis, Mo, USA). Sodium, potassium and chloride were measured using the ion selective electrode (ISE).

Statistical analyses

Analysis was conducted using Statistical Package for Social Science for Windows version 11.0 (SPSS, Chicago, IL, USA). Means and standard deviations were calculated and student's t-test was used to compare the two groups. P-values less than 0.05 were considered statistically significant.

RESULTS

The clinical utility of biochemical screening using multiple parameters has often been used to assess the functions of many organs in the body. The aim of the present study was to investigate the biochemical factors relevant to kidney functions among Jordanian children with β-thalassemia such as urea, creatinine, Cl, Na and K. The abnormality of these factors is known to have dangerous impact on the health of the thalassemic patients. Table 1 shows some hematological and biochemical results of the examined patients and the control group. It is clear from the results that a significant decrease of hemoglobin concentration was noticed in both males and females in comparison with controls. On the other hand, ferritin concentration was significantly higher in both males and females (2564 ± 762, 2389 ± 684, respectively) in comparison with controls (78 ± 21, 62 ± 18, respectively).

Table 2 shows the means and standard deviations for the studied variables relevant to kidney functions in the two groups. The results revealed that significant differences (p < 0.05) appeared between the thalassemic and the control group over all the measured physiological variables urea, creatinine, uric acid, Na and K, except for glucose, and chloride. Our results revealed a significant increase in serum urea level in experimental group compared to control group, even though it is within normal range (33.5 ± 5.50, 29.05 ± 7.30 mg/dl, respectively). The concentration of serum creatinine is the most widely used and commonly accepted measure of renal function in clinical medicine. Our results showed significant increase in creatinine concentration in the experimental group compared to the control group even though it is within normal range (79.8 and 60.5 mmol/L, respectively). Comparison of the results obtained from male and female patients showed no significant differences between them for all the variables studied. This indicates that there are no gender differences among thalassemic Jordanian patients in the studied group.

DISCUSSION

Patients with beta-thalassaemia major are prone to metabolic complications, including different organ
dysfunction which can occur as single or multiple involvements. Although the actual mechanism is not definitive, the most likely explanation is related to anemia and iron overload, in addition to lipid peroxidation, oxidative stress and free radical release (Walter et al., 2008).

In patients with beta-thalassaemia major, the most important cause of mortality and morbidity is organ failure due to deposits of iron. In our study, we investigated the kidney functions test in patients with beta-thalassaemia major. The determination of biochemical indices of renal function might help in the prevention of serious kidney damage. A rise in iron indices observed in our beta-thalassemia patients may be due to erythrocyte hyperhemolysis and to chronic blood transfusion. Similar results were found in the study of Asma K et al. (2003), the significant increase of serum ferritin in the patients indicated an existing iron overload. The acute iron overload found in beta-thalassemia can lead to an iron intestinal hyperabsorption and to an abnormal molecular iron form (non-transferrin-bound: NTBI) accumulation. NTBI has hepatocellular and cytoxic properties. Furthermore, NTBI contributes to the formation of free radicals and increases hemolytic process (Borgna-Pignatti et al., 2004). The released iron could play a central role in the oxidation of membrane cells and senescent cell antigen formation, one of the major pathways for erythrocyte removal. We revealed no significant difference of blood glucose in thalassemic patients compared to controls (113.36 ± 52.52, 98.75 ± 13.54, respectively). We suggest that the duration of iron chelating therapy can prevent the pancreatic hemosiderin deposition and the damage to β cells leads to diabetes, and intensive combined chelation therapy may have a positive effect on glucose metabolism. More studies (Brittenha et al., 1994; Brittenham, 1992) have indicated that adequate iron-chelation therapy can prevent complications, including diabetes. Serum levels of urea and creatinine as waste products formed during the digestion of proteins and in urine as the vehicle for ridding the body of nitrogen is used as indicators for renal function.

Our results revealed a significant increase in serum urea level in experimental group compared to control group (33.5 ± 5.50, 29.05 ± 7.30 mg/dl, respectively) even Though it is within normal range. The concentration of creatinine in serum is the most widely used and commonly accepted measure of renal function in clinical medicine (Perrone et al., 1992). Our results showed significant increase in creatinine concentration in the experimental group compared to the control group (0.352 ± 0.113 and 0.296 ± 0.0533, respectively). The increasing level of urea and creatinine in thalassemic patients possibly due to higher iron deposition in their kidneys, shortened red cell lifespan and excess iron which causes functional and physiological abnormalities in various organ systems in thalassaemia patients.

β-Thalassaemia patients have a high prevalence of renal tubular abnormalities such as the kidney, suggesting that the damage might be caused by the anemia and increased oxidation induced by excess iron deposits (Oktenli and Bulucu, 2002). Iron overload, usually observed, generates oxygen-free radicals and peroxidative tissue injury as renal tubular (Kassab-Cheikir et al., 2003). Some studies showed that the plasmatic urea and creatinine were significantly decreased in beta-thalassemia compared to controls (Kalman et al., 2005). Oktenli and Bulucu (2002) did not find any marked difference concerning blood urea and creatinine in the patient population and found that a urinary and suggested that the severity of renal abnormalities was correlated with anemia degree.

The least severe abnormalities were found in patients under hypertransfusion and deferoxamine therapy. Renal involvement may occur by 3 mechanisms: deferoxamine side effects, deposition of iron in renal tissue, and vascular thrombosis and renal infarction due to increased platelet aggregation and decreased serum level of protein S and antithrombin III, and deferoxamine-induced kidney injury is more probable, because iron deposition may result in death of cardiac involvement, before kidney failure appears (Eldor et al., 1993). In one study of 19 patients treated with deferoxamine, tubular damage (by measurement of β2-microglobulin) was observed in 13 patients (Cianciculli et al., 1994). Acute kidney failure related to deferoxamine is usually nonoliguric and reversible with discontinuation of the drug (Koren et al., 1989). Based on autopsy reports of patients with thalassemia major, the most common glomerular findings are mesangial cell proliferation, mesangial matrix expansion, and hemosiderin deposition in glomerular and tubular cells. Iron deposition may result in tubulointerstitial fibrosis and atrophy (Buñuel et al., 1993). Glomerular diseases may also develop; immunoglobulin A nephropathy was reported in a patient with thalassemia major (Harada et al., 1994).

Electrolyte levels are tightly controlled by several hormones and by the kidneys, which are primarily responsible for retaining and removing electrolytes when necessary and keeping them in a constant state of balance. An electrolyte imbalance can lead to serious health issues, including eventual death if not corrected. The most common imbalances occur with sodium and potassium. Such physiological variables related to glomerular filtration of the kidney as Na and K as the major cations of the extracellular and intracellular fluid were also studied. Our findings showed that there is significant increase in the serum Na and K in the patient group (143.02 ± 2.50, 4.670 ± 0.331, respectively) compared to the control (137.66 ± 5.15, 4.194 ± 0.345, respectively).

Disturbances in monovalent cation transport are manifested by osmotic swelling or shrinkage and this can be a consequence of rare genetic defects in cation
transport. Enhanced permeability of cations in thalassemia has been described previously (Wilairat et al., 1992). Increased serum level of potassium in β-thalassemia major was attributed to the rapid erythrocyte turnover (Cetin et al., 2003). There is also a relationship between abnormal K leak and hemoglobin precipitation on the membrane (Nathan and Gunn, 1966). Oxidative damage is responsible for the K-loss in β-thalassemia by increasing the activity of K-Cl cotransport (Wikramasinghe et al., 1984). Unchanged serum K concentration in both male and femalethalassemic patients did not seem to be in agreement with earlier studies. The hypernatraemia in patients is associated with increased plasma osmolality, in contrast with previously reported normal concentration. Abnormal membrane function plays a relevant role in the alteration of membrane cation transport as observed in thalassemic red blood cell counts (RBCs). The defective sodium, potassium transport in red cell and serum is associated with disturbed Na-K-ATPase (membrane bound) activity. Changes in the levels of serum sodium, potassium, calcium reflects the defective membranal transport of the cations in the red cell membrane of thalassemia. These results provide a confirmation that abnormal cation homeostasis may contribute to the pathogenesis of thalassemia.

Our results revealed that there is significant increase in uric acid in the patient group compared to the control group (Mann-Whitney U-test). Hyperuricemia is caused either by accelerated generation of uric acid through purine metabolism or by impaired excretion in the kidney, or by high levels of fructose in the diet (Chizyński and Różycka, 2005; Nakagawa et al., 2006). We found significantly higher levels of uric acid in thalassemic group, which was predictable due to the higher cellular turnover secondary to the use of hydroxyurea (Becker et al., 2005).

Conclusion

Renal disorders are not rare in patients with beta-thalassemia major and they may increase in terms of frequency with age, increased duration of transfusion and deferoxamine usage.

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