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Biochemical Factors Relevant to Kidney Functions among Jordanian Children with β-Thalassemia Major Treated with Deferoxamine

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Abstract: 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. The aim of this research was 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 12-28 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 HBV, HCV and HIV. The diagnoses of β-thalassemia major were made based on the clinical, hematological and hemoglobin electrophoresis profiles for the patients. 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 physiological variables measured (urea, creatinine, uric acid, sodium and potassium) except for blood glucose and chloride. Researchers conclude 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 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, patients

INTRODUCTION

β-Thalassemia Major (BTM) is a community health problem in many countries. Including 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). According to the Jordan Ministry of Health (MOH), there are about 1400 registered thalassemic patients in Jordan till this time and noteworthy that 4-6% of Jordan's population has the characteristic of the disease but who are not infected of whom health care has a large burden on health budget and family economics and social complains. This disease is commonly associated with the shortened erythrocyte lifespan and excessive destruction of erythrocytes. Therefore, blood transfusion is needed continuously (every 2-5 weeks) to maintain a pretransfusion hemoglobin level above 10 g dL^{-1} as a life saving for BTM patients (Rund and Rachmilewitz, 2005). However, frequent blood transfusion can lead to iron overload which may accumulate in key organs such as liver, heart and endocrine glands of BMT patients due to the lack of physiological pathway for iron excretion (Olivieri, 1999; Andrews, 1999; Melody *et al*., 2004).

This massive accumulation may cause organ dysfunction and failure and ultimately death (Rund and Rachmilewitz, 2005). An overload of iron in the patients tissues that accumulates in the liver, heart and endocrine glands can be fatal with or without blood transfusions; affecting the normal functioning of these organs (Jensen, 2004). The major complications of blood transfusions are those related to transmission of infectious agents or the development of iron overload (Allen *et al*., 2008). Survival of patients has greatly improved following the introduction of desferrioxamine and regular iron chelating when serum ferritin level is maintained below 2,000 μg L^{-1} (Franchini, 2006).

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Hereditary high serum ferritin level in thalassemia major was related to higher frequencies of blood transfusion and suboptimal use of desferrioxamine mostly due to poor compliance. So, as a result patients must undergo chelating (iron-removing) therapy for up to 12 h a day with subcutaneous doses of the iron binding agent (Prasannan *et al*., 2003). Accumulation of iron may cause tissue damage and ultimately organ dysfunction and failure (Modell *et al*., 2001; Rund and Rachmilewitz, 2005). 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 many reports on complications of β-thalassemia in different organs (Low, 2005; Al-Rimawi *et al*., 2005; Angelopoulos *et al*., 2006; Aldudak *et al*., 2000; Cetin *et al*., 2003).

In the present study, the 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. Researchers 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 12-28 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. Their mean age was 17 years (aged 12-28 years). The diagnoses of BTM were made based on the clinical, hematological and hemoglobin electrophoresis profiles and the results of β-goblin 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. None of these individuals had history of anemia abnormal complete blood counts and abnormal hemoglobin electrophoresis results. Before 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 HBV, HCV and HIV. None of the studied patients are undergo of splenectomy or other supplement treatment. All patients were received regular blood transfusion after the age of 1 year old usually given regularly every 2-4 weeks to maintain a pretransfusion hemoglobin level above 10 g dL^{-1}. None of the subjects was treated with vitamin E and/or vitamin C supplementations before the study. All patients were also started on subcutaneous infusion of DFO as chelating agent (45 mg/kg/day for 8-10 each week) at age of 2 or 3 years old prior to presentation to us.

Blood collection: About 5 mL of venous blood sample was drawn into heparin from each BTM patient before the transfusion and from each healthy control. The 3 mL were centrifuged at 3000 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 and potassium ferritin and blood glucose level. The remaining 2 mL were used for studying some hematological parameters such as hematocrit, hemoglobin levels and leukocyte counts.

Serum urea, creatinine and uric acid were examined using commercial analytical kits from Sigma (St. Louis, Mo, USA). Sodium, potassium and chloride were measured using the Ion Selective Electrod (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. $p<0.05$ 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, 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

Table 1: Hematological and biochemical data of s-thalassemia major patients

Parameters	Male (n = 20) control	Male (n = 20) patients	Female (n = 20) control	Female (n = 20) patients	p-values
Hematocrit (%)	36±3.8%	29±2%*	35±2.6%	27±4.2%*	0.000*
Feritin (μg L^{-1})	78±21	2564±762*	62±18	2389±684*	0.001*
Hemoglobin (g dL^{-1})	12.9±0.7	9.2±1.6*	11.1±0.9	8.4±2.2*	0.001*
Leukocytes (×10^6 L^{-1})	10.56±2.31	10.42±3.26	9.44±2.67	10.2±2.46	0.463
AST (IU L^{-1})	37.27±5.18	34.14±7.42	36.9±6.60	37.23±5.37	0.001*
ALT (IU L^{-1})	33.66±8.05	32.62±12.53	34.85±6.20	35.87±9.40	p<0.05

*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

Parameters	Experimental (N = 42)	Control (N = 32)	p-value (t-test)	p-value (Mann-Whitney U-test)
Glucose (mg dL^{-1})	113.36±52.520	98.75±13.5400	0.130	0.432
Urea	33.5±5.50000	29.05±7.30000	0.005*	0.009*
Creatinine	0.352±0.113	0.296±0.0533	0.012*	0.012*
Uric acid	4.42±1.0830	3.57±0.47300	0.009	0.008*
Sodium (mmol L^{-1})	143.02±2.5000	137.66±5.15000	0.000*	0.000*
Potassium (mmol L^{-1})	4.670±0.331	4.194±0.3450	0.000*	0.000*
Chloride (mmol L^{-1})	103.24±3.2400	103.16±4.10000	0.924	0.887

*Result is significant at the 5% level

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. The results revealed a significant increase in serum urea level in experimental group compared to control group, even it is within normal range (33.5±5.50, 29.05±7.30 mg dL^{-1}, respectively). The concentration of serum creatinine is the most widely used and commonly accepted measure of renal function in clinical medicine. The results showed significant increase in creatinine concentration in the experimental group compared to the control group even it is within normal range (79.8 and 60.5 mmol L^{-1}, 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

In patients with β-thalassaemia major, impaired biosynthesis of the β-globin leads to accumulation of unpaired α-globin chain. Shortened red cell lifespan and iron overload cause functional and physiological abnormalities in various organ systems. Thus, in patients with β-thalassaemia major, the most important cause of mortality and morbidity is organ failure due to deposits of iron. In the study, researchers investigated the kidney functions test in patients with β-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 the β-thalassemia patients may be due to erythrocyte hyperhemolysis and to chronic blood transfusion. Similar results were found in the study of Asma *et al*. (2003), the significant increase of serum ferritin in the patients indicated an existing iron overload. The acute iron overload found in β-thalassemia can lead to an iron intestinal hyperabsorption and to an abnormal molecular iron form (Non-Transferrin-Bound: NTBI) accumulation. NTBI has hepato and cardio-cytotoxic 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. Researchers revealed no significant difference of blood glucose in thalassemic patients compared to controls (113.36±52.52, 98.75±13.54, respectively). Researchers 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 (Brittenham, 1992; Brittenham *et al*., 1994) 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.

The 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^{-1}, respectively) even 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). The 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 (Sumboonnanonda *et al*., 2003). Iron overload, usually observed, generates oxygen-free radicals and peroxidative tissue injury as renal tubular (Kassab-Chekir *et al*., 2003). Some studies showed that the plasmatic urea and creatinine were significantly decreased in β-thalassemia compared to controls. This result is puzzling but can be partially explained by the low muscle mass of included population. Also, 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 desferrioxamine therapy.

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. The 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 (Aldudak *et al*., 2000). 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 female thalassemic patients not seems to be in agreement with earlier studies. The hypernatraemia in patients is associated with increased plasma osmolality, contrasts with previously reported normal concentration. Abnormal membrane function plays a relevant role in the alteration of membrane cation transport as observed in thalassemic 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.

The 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 (Chizynski and Rozycka, 2005; Nakagawa *et al*., 2006).

Researchers 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

Researchers conclude that renal disorders are not rare in patients with β-thalassemia major and that they may increase in terms of frequency with age increased duration of transfusion and deferoxamine usage.

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