

Study of Some Biochemical Parameters in Iraqi Male Children with Thalassemia

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Abstract

Thalassemia is a term that refers to a group of genetic disorders characterized by a defect in the synthesis of hemoglobin. It is sometimes called Mediterranean anemia. Many biochemical changes in the blood accompany this disease. In this research, some biochemical parameters were measured in thalassemic patients and compared with healthy control group. These parameters include serum Iron, ferritin, TIBC, hemoglobin, uric acid, albumin, calcium, transferrin, and transferrin saturation percentage.

The results of the research showed that there is a significant increase ($P < 0.05$) in serum iron and ferritin in thalassemic patients group in comparison with healthy control group. A significant decrease ($P < 0.05$) in serum uric acid, TIBC, transferrin protein concentration, and hemoglobin. There is no significant difference ($P > 0.05$) in serum albumin and calcium in both groups.

The results of this research can be explained by different mechanisms involving the hemolysis of erythrocytes and consequence precipitation of iron in the tissues. In addition, the hypoxia may be one of the reasons about the biochemical changes in thalassemic patients. Monitoring the measured parameters may be useful in the prognosis and follow up of the thalassemic patients.

Introduction

Thalassemia is a term that refers to a group of genetic disorders characterized by insufficient production of hemoglobin, wherein there is a defect in the synthesis of hemoglobin. It is sometimes called Mediterranean anemia. To understand how thalassemia affects the human body, we must first understand a little about how blood is made. If the body doesn't produce enough of alpha and a beta chains of globin, the red blood cells do not form properly and cannot carry sufficient oxygen. The result is anemia that begins in early childhood and lasts throughout life. Genes involved are those that control the production of alpha and beta globins contained in hemoglobin [1]. The two main types of thalassemia, alpha and beta, are named for the two protein chains that make up normal hemoglobin.

Since thalassemia is not a single disorder but a group of related disorders that affect the human body in different ways. Thalassemia can be classified according to symptoms or to the genes affected. Thalassemia major that is studied in this work is inherited from both parents. Beta thalassemia tends to be more common in people from many ancestry including Mediterranean and Arabian Peninsula.

Thalassemia in Iraq is a real problem due mainly to the deficiency in the equipments and drugs during different periods of lack of security and wars. Thalassemia is a problem in many areas in Iraq [2]. Of 1064 couples recruited from the Public Health Laboratory in Basra, southern Iraq, about 5% had beta-thalassemia trait and the carriers of major beta-globin disorders comprised 11.48%. These defects constitute a real health problem and necessitate a management plan and public health education for early diagnosis and therapy. In Iraq-Najaf

Government, till October-2007 there are 288 patients' files who are still treating in the thalassemia Unit in AL-Zahra'a Teaching Hospital.

The patients are more dependent on blood transfusions, the more likely he or she is to be classified as thalassemia major. Biochemical changes may include; serum iron, increased/normal total iron binding capacity (TIBC), decreased/normal ferritin, increased/normal and increased transferrin saturation percentage (TISP). These factors in addition to others are studied in Iraqi patients in this work in addition to uric acid, calcium and albumin which are not studied extensively in thalassemic patients.

Transferrin acts as iron transport protein between sites of absorption, storage, and use. Ferritin is a ubiquitous protein in which the only clearly defined function is the sequestration and storage of iron [3]. Ferritin synthesis may be induced by iron. The serum ferritin is a universally available and well-standardized measurement that has been the single most important laboratory measure of iron status during the past quarter century. The well-known limitation of the serum ferritin is the elevation in values independent of iron status that occur with acute or chronic inflammation, malignancy, liver diseases, and alcoholism. Therefore, transferrin-bound iron and transferrin saturation must be measured in the same serum sample with ferritin protein to distinguish iron status from inflammation. Just as a high serum ferritin protein may mean inflammation rather than iron overload, a low serum iron may mean inflammation rather than iron deficiency. Only if both serum iron and serum ferritin protein go in the same direction (i.e., both go up or down) can reasonably assess iron status from them.

There is no treatment when asymptomatic. Different methods for treating Thalassemia are available. Partial splenectomy was associated with a dramatic reduction of mortality in the Iraqi patients [2].

Thalassemia in Iraq was studied in different areas at different fields of study [4], but the complete picture about the chemical changes in Iraqi thalassemic patients is not understood yet.

The aim of the study is to identify variation in biochemical changes in thalassemic patients in comparison with healthy control group. These parameters include serum Iron, ferritin, TIBC, hemoglobin, albumin, calcium, uric acid and TISP.

Materials and Methods

1-Patients:

Forty-five male children with thalassemic have participated in this research. Age range was (6-10 years) from AL-Zahraa Teaching Hospital for Obstetrics and Children during the period from 1-7-2007 till 1-10-2007. Thirty non-thalassemic children were taken as control group.

2-Biochemical (measurements):

Blood was aspirated from individuals in the morning and collected in plain tubes for serum in order to estimate the following parameters.

Hemoglobin electrophoresis shows; Hgb A decreased, Hgb F increased, and Hgb A2 variable.

Biochemical Analysis

Serum Iron was estimated by using colorimetric method[5], total Iron Binding Capacity were estimated by using colorimetric method by the following method: An excess of iron is added to the serum iron to saturate the transferrin. The unbound iron is precipitated with basic magnesium carbonate. After centrifugation the iron in the supernatant is determined). Hemoglobin was estimated by using colorimetric method. Calcium was estimated by using colorimetric method using O-Cresolphthaleine complex compound, at alkaline pH, Albumin was estimated by using colorimetric method by binding to the BCG dye to produce a blue green color. Uric acid was used for estimation by using colorimetric method. The Ferritin Quantitative Test is based on a solid phase enzyme-Linked

immunosorbent assay (ELISA). The assay system utilizes one rabbit anti-ferritin antibody for solid phase (microtitre wells) immobilization and a mouse monoclonal anti-ferritin antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution. Transferrin saturation percentage (TISP) was calculated by dividing serum iron concentration by TIBC (17) concentrations, using the formula: serum iron ($\mu\text{mol/L}$)/transferrin (g/L) x 3.98. The formula is based on the maximal binding of 2 mol Fe^{3+} /mol of transferrin and a molecular weight of 79,570 Dalton for transferrin [6]. As shown in the formula :

$$\text{Transferrin}(g/L) = \frac{S.\text{Iron}(\mu\text{mol}/L)}{\text{Transferrin.saturation}\% * 3.98}$$

Biostatistical analysis:

The results were expressed as (mean \pm standard deviation). Pooled t-test was used for the comparison of a significant difference between the healthy and control groups in the measured parameters. Correlation coefficient (r) was also used for searching about any correlation between the parameters.

Results

The results of the research expressed as mean \pm standard deviation are shown in Table (1). There is a significant increase ($P < 0.05$) in serum iron, ferritin, and TISP in thalassemic patients group in comparison with healthy control group.. A significant decrease ($P < 0.05$) in serum uric acid, TIBC, transferrin protein concentration, and hemoglobin. There is no significant difference ($P > 0.05$) in serum albumin and calcium in both groups.

The correlation coefficient values (r-value) of serum iron versus different measured parameters for healthy and thalassemic groups are shown in Table (2). There was no strong correlation, either positive or negative, between serum iron and the parameters except for the TISP, which is strongly correlated with serum, iron in both groups.

Discussion

Some hematological and biochemical characteristics of our thalassemic patients are listed in Table (1) and compared with those of healthy controls. Iron indices, with the exception of TIBC and transferrin protein concentration, were markedly increased, and the mean concentration of serum ferritin was more than seven times higher than normal (Table 1). In states of iron overload or excess, the iron composition of ferritin increases and this may be the most important cause for the elevation of serum ferritin.

The results of our research on Iraqi thalassemic patients are comparable with the result of Fairbanks (1999) [7]. In that research the following results were obtained in thalassemic patients; serum iron is normal or increased, TIBC is normal, TISP is 30-100%, Serum ferritin is Increased.

Some researches showed a higher level of ferritin in thalassemic patients than healthy children and the ferritin level was twenty times higher than normal . Serum Ferritin was found to be 5506 \pm 635 microg/l in thalassemic patients in one study [8].

. In one study carried out in one of Iraqi neighboring country , Iran, the serum ferritin showed a higher concentration (3503 \pm 201 ng/ml) in thalassemia major in many thalassemic patients which is absent in our Iraqi sample group [9]. These differences may be due to the difference in mutation defects, causes thalassemia in Iraqi's, differs from those reported in the surrounding countries and the difference between races and subpopulations in transferrin saturation and ferritin data [10]

Serum ferritin concentration results from the leakage of tissue ferritin. While tissue ferritin clearly plays a role in intracellular iron handling. The level of ferritin in plasma represents the balance between its secretion, which is directly related to intracellular iron synthesis, and its clearance, mainly in liver and other organs [11].

Serum ferritin protein levels >400 ng/ml define iron overload in most clinical laboratories, but, in fact, such interpretation requires confirmation by finding a high percentage of saturation with iron of iron binding capacity (transferrin). While other researches have suggest a higher serum ferritin values (>2000 mg/dl) which are much more likely to be an indicator of iron overload as shown in different disorders including those in thalassemic patients with haemochromatosis [11]. Hence, the Iraqi patients in this work are less likely to correlate with haemochromatosis as a syndrome but instead they suffer from mild increase in serum iron (Table 1) and subsequent events of this increaser which are not well documented. These changes in some hematological and biochemical changes may be mainly due to iron overload and precipitation of iron as different ions or complex in hepatocytes. In one research, all the patients were iron overloaded. Markers of free radical injury such as malondialdehyde (MDA) and antioxidant enzymes levels were significantly elevated in thalassemic children while mean glutathione peroxidase levels were decreased in patients compared to controls. All these markers are significantly correlated with serum ferritin levels. Another significant fact is obtained from the effective chelation therapy, when initiated, the serum ferritin falls more rapidly than body iron. This may happen partly because of the improvement in liver function and partly because serum ferritin may reflect predominantly reticular endothelial iron rather than parenchymal iron in the liver and other organs [12]

The other important prove for the result of our work is the TISP that is an approach to saturation (95.6 ± 22.9) as shown in Table (1). Suominnen *et al* (1998) (34) found that plasma ferritin iron increases markedly in those with fully saturated transferrin. Furthermore, there was a significant difference ($p = 0.01$) between mean serum ferritin in thalassemic patients with endocrine complications and thalassemic patients without endocrinopathies in many previous researches [13]. This indicates the involvement of the harmful effect of increase iron storage and precipitation on the endocrine glands that must be studied in the future works in details.

From Table (1) a high serum ferritin is accompanied by a high percentage of saturation of a normal serum transferrin which usually indicates iron overload. These two parameters are positively correlated (Table (2) at which iron overload clearly appears clinically and biochemically in our patients. Transferrin level showed a decrease in thalassemic patients as compared with healthy controls Table (1). This result may be due to the fact that transferrin is a reverse acute phase reactant. Thus the decrease in hemoglobin in thalassemic patients is accompanied by an acute phase response confirmed by the decrease in transferrin concentration and increase in serum ferritin level as an antagonist cause of the elevation of serum ferritin in addition to the increase in serum iron in thalassemic patients. For example, during the acute phase response, inflammatory cytokines such as interleukin 1 beta and tumor necrosis factor alpha increase the synthesis of both subunits of ferritin [14]. Hence, serum ferritin can be elevated in inflammation.

In Table (2) there is no significant difference in blood hemoglobin indicating that thalassemia is not related to iron within hemoglobin molecule but instead it correlates with iron liberated from the hemoglobin molecules after hemolysis and degradation. Statistically significant correlations were found between serum Fe, serum ferritin (5).

Under various pathological conditions associated with iron overload, including thalassemia, there is an evidence of an increase in low molecular weight iron in serum and in the intracellular transit pool of iron. This is the same in our thalassemic patients (Table 1). This promotes peroxidative damage to cell and organelle membranes" in organs that

accumulate excess iron, including liver, pituitary gland, pancreas, and heart (40). Iron plays a key role in the formation of toxic oxygen radicals that can attack all biological molecules. Hence, specialized molecules for the acquisition, transport (transferrin), and storage (ferritin) of iron in a soluble nontoxic form have evolved [15].

Transferrin saturation can be elevated by increased iron stores and a variety of other conditions. In patients with severe iron overload, plasma can contain transferrin completely saturated with iron and also a chelatable low molecular weight iron fraction not associated with transferrin. Hence, over saturation in some other thalassemic patients may be due to the presence of these species of non-transferrin bound iron where the TISP range is (95.6 ± 22.9) as shown in Table (1). Nonspecific, non-transferrin-bound iron is rapidly cleared from the plasma, mainly by the liver [16].

Non-transferrin-bound iron (NTBI) appears in the serum of individuals with iron overload and in a variety of other pathologic conditions. Because NTBI constitutes a labile form of iron, it might underlie some of the biologic damage associated with iron overload. Thalassemic sera contained NTBI in 80% of the cases (range, 0.9-12.8 micromole/L). [17]

Total iron-binding capacity (a measure of plasma transferrin levels) is approximately 56 umol/liter; thus, transferrin is about one-third saturated with iron, with approximately 10% present as a diferric transferrin. [18]. Hence, there seems to be a control mechanism that guarantees that rate of iron release from stores which perfectly matches the one with which the iron is taken up by tissues, but the nature of this regulation is unknown.

Table (1) showed a significant decrease in serum hemoglobin as compared with healthy control group. This result is expected mainly due to the hemolysis of RBC and the release of iron from the degraded abnormal hemoglobin molecules chain. (Hb) Anemia can occur in severe iron overload. A number of studies also indicate that RE cells release significant amounts of erythrophagocytosed iron in the form of hemoglobin, or ferritin. It has been speculated that hemoglobin release results from macrophage cell death after the ingestion of too many erythrocytes, whereas others argue that hemoglobin release represents a normal physiological process. Interestingly, Moura et al. (1998b) [19] noted that most early release consists of hemoglobin, whereas ferritin and low-molecular-weight iron are the main forms released subsequently.

It has also been proposed that iron and oxygen radicals may play a key role in the progression of chronic renal failure, the fact that may be related to the changes in kidney excretion of different substances. Proteinuria, resulting from the glomerular injury, may perpetuate renal injury, and it has generally been assumed that the tubulointerstitial injury is induced by albumin. Patients with Hemoglobinopathies including thalassemia would develop albuminuria and this may affect the blood concentration of albumin. We concluded that renal disorders are not rare in patients with beta-thalassemia major. The albuminuria or decrease in serum albumin are not shown in our work neither any correlation between albumin and iron status. Many works obtained the same results [20] also noticed no correlation between serum ferritin with serum albumin.

Nutritional deficiencies are common in thalassemia secondary to the use of desferrioxamine, the hemolytic anemia, and iron overload that accompanies this disease. Calcium in our work is changed and this result differs from the results obtained from other thalassemic patients group in Mosul-Iraq [21] at which the mean serum level of calcium had no significant difference as compared with healthy control group. In the Mosul study, the thalassemic patients was 105 patients while in the present study only 45 patients are involved due to the variation in sampling between the centers.

Some calcium metabolism markers were measured in another study. Serum levels of calcium, phosphate, parathyroid hormone (PTH), calcitonin and 25-OH vitamin D were measured. Average serum levels of PTH and vitamin D were significantly lower in thalassemic patients than in control group. [22]

It is suggested that increasing the circulating IGF-I concentration through aggressive nutritional therapy and/or GH/IGF-I therapy with supplementation with vitamin D and/or calcium might improve bone growth and mineralization and prevent the development of osteoporosis and consequent fractures in thalassemic patients.

Secondary gout is a well-recognised complication of disorders characterized by increased nucleic acid catabolism and disordered renal function. However, in the present work, there is a significant decrease in serum uric acid in comparison with control group (Table 1). This results agrees with a study carried out in Turkey . While, In Italian studies that carried out by Gallerani *et al* (1989) [23] showed that uric acid levels in Beta Thalassemia were higher than in the control group.

Hyperuricemia and microscopic hematuria are more common in thalassemia intermedia than thalassemia major. Microscopic hematuria in thalassemia intermedia might be related to either hypercalciuria or hyperuricosuria (9).

Conclusion

Our study should be qualified for its relatively small sample size ($n = 45$) and its cross-sectional design. While causality cannot usually be detected in cross-sectional studies, associations can be examined. Moreover, the markers of inflammation were not measured in this study, nor were other measures of malnutrition. Therefore, it is important to understand iron metabolism not only at the molecular and cellular levels but also at the level of the whole organism. In thalassemia patients, the iron absorption is increased and the excretion rates of iron should be achieved to maintain a "safe" level of body iron. Monitoring of iron status requires: estimation of the iron content of different body organs, and assessment of the function of the organs particularly damaged by iron overload namely; heart, liver, and endocrine glands,. Thus, the monitoring of iron status is of importance to overcome the possible consequences that could occur in vital organs. These aspects are of importance and they are the targets of our future works.

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Table (1): The biochemical parameters of thalassemic patients as compared with healthy control group.

Biochemical Parameter	Thalassemic M±SD	Control M±SD	Significance
S.Ferritin (ug/L)	556.5±103	73.8±31.2	Significant
S.Calcium (umol/L)	2.58±0.13	2.46±0.22	NonSignificant
S.Albumin (g/L)	41.5±10.5	43.8±5.7	NonSignificant
S.TIBC (umol/L)	51.9±8.9	61.6±8.3	Significant
S.Iron (umol/L)	49.1±12.3	20±5.5	Significant
Hemoglobin (g/L)	72±17	135±21	Significant
TISP %	95.6±22.9	32.9±9.1	Significant
Transferrin Conc.g/L)	0.131±0.023	0.155±0.021	Significant
S.Uric acid (umol/L)	278.8±53.6	319.9±67.9	Significant

(*): Significant only when p<0.05.

Table (2): Correlation coefficient of serum iron versus different biochemical parameters concentration in both thalassemic patients and healthy control group.

The Compared Parameters	Thalassemic patients	Healthy Control
S.Iron vs. Ferritin	0.66	0.54
S.Iron vs. TISP	0.67	0.89
S.Iron vs. Transferrin	0.52	0.15
S.Iron vs. Albumin	0.19	-0.04
S.Iron vs. Uric acid	0.04	0.10
S.Iron vs. Calcium	0.08	0.27
S.Iron vs. TIBC	-0.52	-0.47
S.Iron vs. Hb	0.17	0.10

دراسة بعض المتغيرات الكيموحياتية في مصول الاطفال العراقيين المصابين بمرض الثالاسيميا

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الخلاصة

يطلق مصطلح الثالاسيميا على مجموعة من الاضطرابات الوراثية التي تؤثر في عملية تصنيع الهيموكلوبين ويسمى هذا المرض أحيانا بفقر دم البحر الأبيض المتوسط. يرافق هذا المرض الكثير من التغيرات الكيموحياتية في الدم. في هذا البحث قيست بعض المتغيرات الكيموحياتية المهمة في المرضى وقورنت نتائجهم بمجموعة السيطرة. شملت القياسات العملية المتغيرات التالية: الحديد، و الفيريتين، و سعة ارتباط الحديد الكلية، و الهيموكلوبين، و حامض اليوريك، و الألبومين، و الكالسيوم، و الترانسفيرين، و نسبة تشبع الترانسفيرين. أظهرت النتائج ارتفاعا معنويا ($P < 0.05$) في تراكيز الحديد و الفيريتين، بينما لوحظ انخفاضاً معنويا في تراكيز حامض اليوريك و سعة ارتباط الحديد الكلية و الهيموكلوبين و الترانسفيرين و نسبة تشبع الترانسفيرين و لم تتغير تراكيز الألبومين و الكالسيوم في مصول مرضى الثالاسيميا مقارنة مع مجموعة السيطرة. يمكن تفسير نتائج هذا البحث من خلال ميكانيكيات مختلفة تتضمن تحليل كريات الدم الحمر وترسب الحديد في الأنسجة. كذلك ان قلة نقل الأوكسجين للأنسجة قد يكون سببا في التغيرات الكيموحياتية التي لوحظت في هذا البحث. يمكن الاستنتاج بان قياس ومتابعة تراكيز هذه المتغيرات قد يكون مفيدا في عملية متابعة تطور المرض.