

The Necessity of routine hematological and blood biochemistry monitoring in Yemeni thalassemia major patients.

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Abstract

Background and objectives: Thalassemia Major is a type of chronic, inherited, hemolytic microcytic anemia that is characterised by defective globin chain synthesis and ineffective erythropoiesis, hemolysis, and increased red blood cell turnover. In the current study, we explored the relationship between hematological standard laboratory and biochemical parameters in Thalassemia Major patients. Material and method: A prospective study was a case-control study carried on fifty Thalassemia Major patients attending pediatric departments in Al-Kuwait Hospital and thirty matched healthy individuals. The range age between five to 14 years. Results: The results showed Thalassemia Major patients had Microcytic, hypochromic red cells, and their total hemoglobin, Hb A2 level, Mean Corpuscular Volume (MCV), were much lower than healthy individual ($p < 0.0001$). On the other hand, the mean levels of plasma ferritin, Lactic dehydrogenase (LDH), aminotransferase (ALT, AST), and thiobarbituric acid reactive substances (TBARS) were significantly increased in thalassemia Major patients ($p < 0.0001$) compared to healthy individual. Conclusion: Based on findings of the present study it can be concluded that Thalassemia Major causes multiple abnormalities in hematological and biochemical parameters.

Keywords: Thalassemia Major; hematological monitoring; Anemia; Iron overload; LDH, CK; Aminotransferase; Oxidative stress.

Introduction:

Thalassemia Major is an inherited hemoglobin disorder, microcytic anemia and the most prevalent type of thalassemia as it is common in certain populations.

Thalassemia major gene occur in Middle Eastern countries with varying frequency.¹ Yemen has numerous patients affected with this disease, and the number of patients is increasing.²

The Yemen Society for Thalassemia and Genetic Blood Disorders was founded in 2000, the association has recorded 719 patients suffering from thalassemia and other hereditary blood diseases have arrived hospital in different Yemeni governorate from 2000 to April 2008. About 115 of these cases (16%) registered were diagnosed with thalassemia,² in addition there was an initial pilot study

which showed that features suggestive of β -thalassemia (β -thal) were represent (4.43%) and features suggestive of -thal trait were found representing (8.6%) of patients attending clinics in Sana'a City,³ as consanguineous marriage and marriage between members of the same tribe are common in Yemen.³ Beta-thalassemias are caused by point mutations or, more rarely, deletions in the beta globin gene on short arm of chromosome 11, leading to reduced (β +) or absent (β 0) synthesis of the beta chains of hemoglobin (Hb). Ineffective erythropoiesis, hemolysis, and increased red blood cell turnover ensue.^{4,5} Blood transfusion is critical for survival in these patients. Over the course of the past three decades, hyper transfusion therapy in these patients has shown significant increase in life expectancy and quality of life.

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Unfortunately this type of therapy also increased the frequency of complications due to iron overload. They had significant anemia, which resulted in growth retardation, delayed puberty, and retarded bone age.⁶

Affected individuals are dependent on repeated blood transfusions and most succumb before 20 years of age, death is usually attributable to cardiac failure resulting from iron deposition in the myocardium⁷. The aim of this study is to

investigate the association of thalassemia on some biochemical and hematological parameters.

Materials and Methods:

Study design:

The study involved 50 Yemeni children patients who were attending Al-Kuwait Hospital in the capital city Sana'a, were clinically diagnosed by physician as Thalassemia Major based on hematological criteria (complete blood counts), hemoglobin (Hb) electrophoresis quantitation of Hb A2 and Hb F of the patients from early years of life. All the patients were examined regularly once or twice a month by clinicians. They were regularly receiving blood transfusions every month. Transfusion characteristics and duration of transfusion were similar in all patients, aged under 14 years (30 males and 20 females) compared to 30 healthy, age matched volunteers were randomly selected to serve as control subjects. This work done after Informed consent was obtained from the parents of all patients and control individuals, and local ethical committee approval.

Three to five ml of blood from healthy individuals and thalassemia patients was collected just before the transfusion by venopuncture in EDTA or heparinized tubes during their visits the Pediatric Department of The Al-Kuwait University Hospital and The Yemen Society for Thalassemia and Genetic Blood Disorders of the capital city Sana'a. Hematological parameters and red cell index were estimated. Collected blood was left standing at room temperature until it clotted, then the sample centrifuged at 3000 rpm for 10 minutes to separate the

plasma from the cells and buffy coat. The plasma was removed and stored frozen at -20 °C. Then the plasma was used to estimate basic serum biochemical parameters.

Analytical Methods:

Estimation of plasma Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total bilirubin, and direct bilirubin, were by (BioSystem-Spain), Creatinine and lactic dehydrogenase (LDH) using Enzymatic Kits (Human gesellschaft for Biochemical and diagnostic mbH, Germany), while alkaline phosphatase (ALP) and creatine kinase (CK) were determined by (Biosystem – France). Ferritin was determined by Abbott AXSYM system automated Microparticle Enzyme Immunoassay (MEIA) technology (USA). Plasma was analyzed for lipid peroxide by spectrophotometric quantitative assay of thiobarbiturate as thiobarbituric acid reactive substances (TBARS).⁸

Statistical analysis:

Data were presented as mean \pm SD. Normal distribution of the variances was tested before analyzed, significant differences were considered at $p < 0.05$ using SPSS version 15.

Results:

As shown In Tables (1 and 2) the hematological and biochemical parameters of study subjects were a comparison between Thalassemia Major patients and healthy matched individuals.

Hematological profile:

Table (1) shows, Thalassemic Major patients had significantly higher plasma ferritin levels ($p < 0.001$), and platelet count ($p > 0.05$). Meanwhile RBC indices show microcytic anemia characterized by reduced Hb level (7.5 g/dl), Hematocrit % ($p < 0.001$), mean corpuscular volume (MCV) 65 fl ($p < 0.001$), mean corpuscular Hb (MCH) 22 pg, mean corpuscular hemoglobin concentration MCHC ($p < 0.05$), and decreased Hb A2 level.

Table 1: Mean \pm SD of Hematological parameters of the study groups

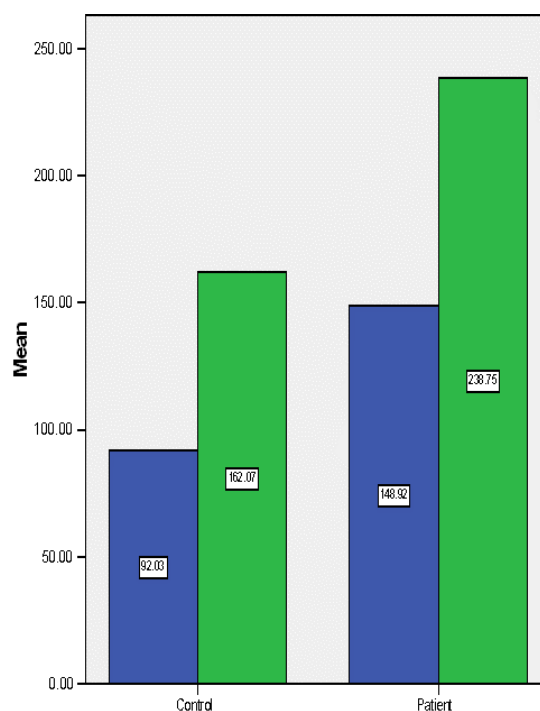
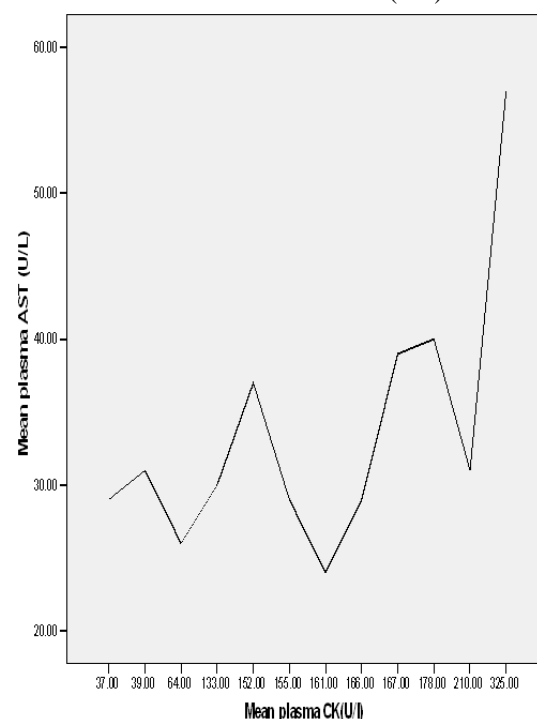
Parameters	Patient (n=50)	Control (n=30)	P. Value
Ferritine (ng/ml)	3554.8 \pm 3137*	75.9 \pm 45	<0.001
Hb (g/dl)	7.50 \pm 1.8 *	12.5 \pm 1.3	<0.001
Hematocrit %	25.4 \pm 6.5 *	37.5 \pm 2.0	<0.001
RBC count (x10 ⁶ / μ l)	3.32 \pm 0.71*	4.8 \pm 0.579	<0.001
WBC count (x10 ³ / μ l)	11.7 \pm 16.5	7.5 \pm 1.9	>0.05
PLT count (x10 ³ / μ l)	286.2 \pm 140.4	281.8 \pm 63.5	>0.05
MCV	65.4 \pm 2.5*	78.6 \pm 5.6	<0.001
MCH	22.4 \pm 4.6	26.58 \pm 3.5	0.1
MCHC	311.0 \pm 15.1*	331.7 \pm 16.7	0.02

Biochemical profile:

Among the biochemical parameters, the plasma mean levels of AST, ALT, LDH were significantly higher in Thalassemic Major patient compared to healthy individuals ($p < 0.001$), as well as plasma mean levels of

ALP, CK ($p < 0.05$), total bilirubin, direct bilirubin, and creatinine ($p < 0.01$), Table (2) and Figure (1). There was a high significant increase ($p < 0.001$) in the level of plasma TBARS

(as a marker of lipid peroxide) in patients with Thalassemia Major as compared to control individuals. In addition, there was a significant positive correlation between plasma levels of CK, LDH and AST ($r = 0.72$; $p = 0.009$), ($r = 0.52$; $p = 0.02$) respectively, (Figure 2), whereas, the plasma TBARS level was significantly negative correlated with MCHC ($r = -0.75$; $p = 0.01$) and WBC count ($r = -0.78$; $p = 0.01$) respectively.

The mean levels of LDH ($p < 0.0001$) and CK ($p = 0.01$) in thalassemia patient and healthy**Figure (1): Plasma lactic Dehydrogenase (LDH) and Creatine Kinase (CK)****Figure (2): Correlation between Plasma levels of AST and CK in Thalassemic Major patients ($r = 0.72$; $p = 0.01$).****Table 2: The Mean \pm SD Biochemical parameters of the studied groups**

Parameters	Patient (n=50)	Control (n=30)	P.Value
ALP (U/L)	345.4 \pm 137.1*	246.6 \pm 51.8	0.03
ALT (U/L)	47.7 \pm 16.5*	27.7 \pm 8.0	<0.001
AST (U/L)	36.5 \pm 7.4*	18.7 \pm 3.1	<0.001
T.bilirubin (μ mol/l)	1.3 \pm .71*	0.7 \pm 0.2	0.008
Direct.bilirubin (μ mol/l)	0.8 \pm 0.4*	0.4 \pm 0.1	0.005
CK (U/L)	148.9 \pm 78.8*	92.0 \pm 35.0	0.01
LDH (U/L)	324.2 \pm 135.9*	162.1 \pm 25.0	< 0.001
Creatinine (mg/dl)	0.7 \pm 0.2*	0.5 \pm .06	0.002
TBARS (μ g/ml)	28.1390 \pm 8.6*	4.5 \pm 2.8	<0.001

Discussion:

Thalassemia Major, like many sickle-cell diseases, is both dangerous and chronic. Therefore, patients face many problems throughout their life in fighting this chronic disease. Thalassemia patients need a constant treatment involving a blood transfusion every three weeks, as well as clinical visits every week and physical examinations every other week. In the few past years, most patients of Thalassemia major died young. Most of them died at the age of 5 years.

In the present study Thalassaemia Major patients had Microcytic, hypochromic red cells, their total and A2 hemoglobin levels were much lower than healthy individual. The β -Thalassaemia Major patients is characterized by severe anemia starting during the first year of life and requiring life-long transfusion therapy for survival. The aim of transfusion is to maintain a hemoglobin level that inhibits ineffective erythropoiesis, marrow expansion and allow normal growth.^{6,9} In all thalassemias, clinical features that result from anemia, transfusion, and absorptive iron overload are similar but vary in severity.⁹

Serum ferritin level in Thalassaemic major patients was significantly higher more than ten times of healthy individual. Further studies had confirmed the intracellular storage of iron is primarily accomplished by the intracellular proteins, ferritin, and hemosiderin. About two-thirds of the iron stores in the human body exist in the form of ferritin. Serum ferritin (SF) is most commonly measured as an indicator of iron stores, beside the direct relationship between serum ferritin concentration and iron store in the body.^{10,11} β -thalassemic Major patients who had multiple transfusion had increased serum iron and ferritin levels, are well know. Although iron is essential for living organisms to survive, its reactive properties require strict regulation in order to prevent toxic effects. The common adverse effect of iron overload include organ failure, however, tissue

iron levels progressively increase with age and may cause life-threatening complications.¹¹ Iron overload causes most of the mortality and morbidity associated with thalassemia, and ferritin levels below 2500 mg/ml are associated with improved survival.¹⁰

These were clear In current study where thalassemic Major patients were had higher LDH and CK than those healthy individuals. Patients with high ferritin levels and poor compliance to treatment with chelating agents, are at high risk of cardiac hemochromatosis and its complications, chelation was not given because the Disveral injection device expensive.^{12,2} Whereas, another study mention despite availability of iron chelation, iron-mediated cardiac toxicity remains the leading cause of death in Thalassemia Major patients. Cardiac toxicity effects may be severe, with cellular degeneration and fibrosis of the myocardium, disturbances of cardiac rhythm and remains the leading cause of death in β -thalassemia major patients.^{13,7}

The higher mean plasma ALT, AST, ALP, in addition to total and direct bilirubin in Thalassemia Major patients than healthy group in this study confirm that this can be a good marker of liver status in patients, this in agreement with previous studies which mention the prevalence and severity of liver diseases of transfusion dependent thalassemia major patients were significantly associated with higher serum ferritin, liver enzymes, and liver iron content (LIC).¹⁴ Transfusion-related infections (primarily Hepatitis B and C, or HIV) are now considered the major cause of death in developing countries where proper blood testing facility is not available. Adverse effect of iron overload include organ failure which increasing with age may cause advanced liver disease, jaundice, cirrhosis, and hepatotoxicity.¹⁵ High plasma CK, LDH demonstrate significant linear positive association with AST in this study (Figure 2), may account for the thalassemic major patients offers one explanation for life-threatening complication, and include liver and heart abnormalities and failure.^{16,15}

Moreover, Thalassemia Major patients in current study showed also significant increasing in the mean level of plasma creatinine than healthy individuals. Recent study shown that to Shortened red cell lifespan and excess iron cause functional and physiological abnormalities in various organ systems in thalassaemia patients. β -Thalassemia Major patients have a high prevalence of renal tubular abnormalities. The severity correlated with the degree of anaemia, being least severe in patients on hypertransfusion and iron chelation therapy, suggesting that the damage might be caused by the anemia and increased oxidation induced by excess iron deposits.¹⁷

This study revealed a high significant elevation in plasma TBARS of Thalassemic Major patient. TBARS is a product of lipid peroxidation is generated in excess amounts in supporting the fact that large amount of membrane bound iron is present in thalassemic erythrocytes. Peroxidative damage of lipids is indicated by the increase in serum TBARS levels.¹⁸ In Thalassemia patient the increased susceptibility of their red blood cells to oxidative stress plays an important role in hemolysis, result from the presence of excess unpaired globin chains, then high intracellular concentration of normal hemoglobin (Hb) encountered in these cells, as manifested by their low mean corpuscular hemoglobin concentration (MCHC),¹⁹ this is in agreement with our observation in current study, where MCHC reduced in high significant ($p < 0.001$) and supported by the negative correlation between the plasma TBARS and MCHC ($r = -0.75$; $p = 0.01$) in patient group. The excess globin chain leads to produce unstable hemoglobin leading to generate free oxygen radical species and then causes the formation of methemoglobin and hemichromes. The heme molecule undergoes degradation within the RBC leading to iron loading. Iron causes oxidative damage may contribute to shortened life span of erythrocytes via Fenton reaction.²⁰

Conclusion:

Based on findings of the present study it can be concluded that Thalassemia Major causes multiple abnormalities in

hematological and biochemical parameters. Repeated blood transfusions letting Thalassemic Major patients suffer from high serum ferritin and TBARS (oxidative stress) levels. We suggest the appropriate chelating therapy to remove excess iron, proper and regular blood testing facility is necessary to improve morbidity and reduce mortality of patients.

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