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IMMUNOLOGICAL AND BIOCHEMICAL STUDIES ON SOME RELATED BIOMARKERS IN THALASSEMIA PATIENTS IN THI-QAR PROVINCE, IRAQ

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Abstract

The current study was designed to determine some immunological and biochemical biomarkers in patients with thalassemia major (TM) and thalassemia intermedia (TI), 20 patients with thalassemia and 20 healthy volunteers as control group. The results have shown a significant increase in WBCs also a significant decrease in neutrophil activity and IFN-γ in TM and TI groups as compared to controls. The levels of Hb decrease in TM and TI groups as compared to controls, while a significant increase has been observed in platelets count. The results have also revealed a significant elevation in Iron and ferritin in TM and TI groups when compared with controls while Transferrin was decreased in the patients when compare with controls. The liver enzymes (AST and ALT) have shown significantly elevated activities in TM an TI groups.

Key words : Thalassemia, WBCs, IFN-γ, Liver enzyme.

Introduction

Thalassemia is the name of a group of genetic, inherited disorders of the blood. More specifically, it is a disorder of the hemoglobin molecule inside the red blood cells (Bhuiyan *et al.,* 2012). World Health Organization (WHO) estimates that at least 6.5% of the world populations are carries of different inherited disorders of hemoglobin (Karim *et al.,* 2016). Thalassemias are principally classified according to the individual globin gene or genes affected (*i.e.*, α-, β-, γ-, δ-, δβ-, and γδβ—thalassemias) with α- and β-thalassemias being the most commonly encountered types worldwide [14]and result in severe anemia in the homozygous and compound heterozygous states (Al-Samarrai *et al.,* 2008). Its characterized by reduced synthesis of one or more of the globin chains leading to imbalanced globin synthesis which is the major factor in determining the severity of the disease in the thalassemia syndromes (Belsare *et al.,* 2015). Various complications caused by this disease including growth retardation, endocrine dysfunction, hypothyroidism, progressive liver failure and abnormal kidney function (Rooks *et al.,* 2012). All these effect are due to ineffective erythropoiesis and hemochromatosis (Reading *et al.,* 2014).

The present study was designed to evaluate immunological, hematological and biochemical aspects of thalassemia patients at the Thi-Qar Province and their correlation with iron overload.

Materials and Methods

The present study was carried out in Department of pathological analysis, College of Science, Thi-Qar University.

Selection of Cases

Cases were selected 20 amongst the patients diagnosed with beta thalassemia and intermedia thalassemia by Hb electrophoresis) aged between 1 to 15 years admitted in wards of Thalassemia and blood diseases center at Thi-Qar province 20 healthy control were selected.

Collection of Sample

Five millimeters of Fasting blood were withdrawn by disposable syringes with stainless needles, divided into

two portions. First portion was transferred to plain tube containing EDTA (ethylenediaminetetraacetic acid disodium) for determination of hemoglobin by Hematology analyzer (Genex, Count 60), and white blood cells count, phagocytic activity by the Nitroblue Tetrazolium stain. The second portion was transferred to plain polyethylene tube containing gel as a clot activator for serum separation.

The second portion was centrifuged (Hermle Z-200-A / Germany) at 4000 rpm for 10 minutes, and the serum was separated immediately to four labeled eppendroft tubes, the serum was used to determination of Gamma interferon by using Enzyme Linked Immunosorbent Assay (ELIZA) and Iron (Randox/ England), Ferritin (BioMeriux /France), Transferrin (LTA/Italy), AST (Randox/England) and ALT (Randox/England) by spectrophotometer (APEL PD-303 / Japan).

Statistical analysis

Statistical analysis was carried out using SPSS statistical package (version 20). Analysis of variance (ANOVA) of the data was used to detect overall difference in group means.

Differences among group means were assessed using least significance difference (LSD).

Results and Discussion

Determination of some immunological parameters in whole blood of TM, TI patients and control groups

Results in table 1 shows the mean value of WBCs (*mm*3) show an increase ($p<0.05$) in the patients comparing with control group, this due to, the patients have a greater degree of susceptibility to infections and increased risk of septic complications associated with a mortality rate than healthy which could explain the high increasing in WBC count to thalassemia patients (Sari *et al.,* 2014). As well as the significant decrease ($p<0.01$) in the mean value of neutrophil for patients when comparing with control group may attributed to cellular and humeral dysfunction due to iron overload and a chemotactic impairment of neutrophils because transfusion overload (Abo-Shanab *et al.,* 2015). Interferon gamma (IFN-γ) plays an important role in the pathogenesis of thalassemia, the results in this table observe significant decrease in concentration of IFN-γ to patients because a major cause of morbidity and mortality in thalassemia patients was infections, lead to be the result of immunological changes (El-Beshlawy and Youssry, 2009).

Determination of hemoglobin and platelets levels in whole blood of TM, TI patients and control groups

Hemoglobin synthesis requires the coordinated production of heme and globin. Heme is the prosthetic group that mediates reversible binding to oxygen by hemoglobin. Globin is the protein that surrounds and protects the heme molecule (Stryer, 1997).

The mean levels of Hb in three studied groups are shown in (Table 2). The results revealed that hemoglobin levels are significantly decreased in TM and TI patients compared to control. Thalassemia is hereditary disorder characterized by defective production of hemoglobin. An imbalance in the alpha and beta globin chains necessary for the production of hemoglobin is caused by the inheritance of a defective gene (Marwaha *et al.,* 2004).

In beta thalassemia major the production of beta globin chains is severely impaired, because both beta globins are mutated. The severe imbalance of globin chain synthesis (alpha > > beta), the excess unpaired alpha globin chains aggregate to form precipitates that damage red cell membranes, this leads to excessive destruction of red blood cells (Scott *et al.,* 1993). Platelets have main role in stopping the bleeding, it is assemble at the site of interrupted endothelium. The activation of platelets is associated with fibrin secretion (Karmakar *et al.,* 2015).

The level of the platelets count of the three groups studied is shown in (Table 2). The result showed significant increase in platelets of the two patients groups (TM and TI) compared with control group. These results are in agreement with (Comporti *et al.,* 2002), who have suggested that the enhanced thrombin generation leads to activation of platelets. In thalassemia patients there is unbalance between α and β globin chains, any increase in one of any chain can cause a degradation in the normal globin chain and lead to destruction of cell membrane, also the damage may be happened because of free iron ion that finally leads to lipid peroxidation of cell membrane. This damage may partly explain the enhanced aggregation of some proteins; these proteins have a capacity to enhance thrombin generation via the assembly of the prothrombinase complex so that platelets are activated.

The level of platelets in major thalassemia patients is less than intermedia thalassemia patient. This result can be attributed to the fact that the bone marrow in intermedia patients is more able to produce platelets as they are less affected than thalassemia major patients.

Biochemical tests

Iron study in serum of TM, TI and control groups

Iron is essential part of hemoglobin and myoglobin.

Table 1: WBCs, Neutrophil Activity and IFN-ã levels in whole blood of TM, TI patients and control groups.

Variables	Statistics	Major	Intermedia	Control
WBCs	Mean± SD (*mm*3)	9745±13.92	8332±16.72	7384±13.77
	ANOVA	<0.001		
Neutrophil Activity	Mean± SD	8.442±0.29	6.917±0.89	17.35±1.53
	ANOVA	<0.001		
IFN-γ	Mean± SD (pg/ml)	5.022±0.841	3.001±0.427	11.331±1.043
	ANOVA	<0.001		

Table 2: Hemoglobin and platelets levels in whole blood of TM, TI patients and control groups.

Variables	Statistics	Major	Intermedia	Control
Hb	Mean± SD (g/dl)	8.04±0.49	8.15±0.53	12.88±0.16
	ANOVA	<0.001		
Platelets	Mean± SD (plt/dl)	364.47±28.7	374.6±18.39	243.07±18.84
	ANOVA	0.002		

Table 3: Iron Study in serum of TM, TI and control groups.

Variables	Statistics	Major	Intermedia	Control
Iron	Mean± SD (µg/dl)	218.94±32.33	203.54±12.49	90.88±13.6
	ANOVA	<0.001		
Ferritin	Mean± SD (ng/dL)	3943.99±93.1	1800.6±85.73	117.19±17.64
	ANOVA	<0.001		
Transferrin	Mean± SD (mg/dl)	208.288±13.92	191.55±16.9	330.65±22.5
	ANOVA	<0.001		

Table 4: AST and ALT activity in Serum of TM, TI and control groups.

Variables	Statistics	Major	Intermedia	Control
AST	Mean± SD (U/l)	38.907±8.1455	32.035±8.7620	13.203±3.4323
	ANOVA	<0.001		
ALT	Mean± SD (U/l)	50.379±15.5082	29.115±9.1569	18.437±2.3600
	ANOVA	<0.001		

Dietary iron available in two forms either heme or non heme. Iron is released from hemoglobin and associated with transferrin then it is transfer to the bone marrow for new hemoglobin synthesis or to ferritin to be stored (Elaine *et al.,* 2016). The results of iron study in two groups of thalassemia patients (TM and TI) and control group are revealed in (Table 3). The results showed highly significant increase in iron and ferritin levels for the two patients groups compared with control group, while transferrin levels are significantly decreased for the two patients groups compared with control.

Periodic blood transfusion as a treatment of thalassemia often leads to accumulation of excess iron may be an acquired condition. It is well known that blood transfusion provides the body with approximately 250 mg of iron, while the body cannot excrete more than1 mg/day of iron typically added to the body's stores (Fleming and Ponka, 2012). The cirrhosis of liver is associated with increase in serum ferritin levels. However, as in primary iron overload, the majority of morbidity and mortality ultimately results from progressive heart and liver failure (Herbert *et al.,* 1995). Serum ferritin protein is an acute phase reactant, rising with any inflammation process from infection through chronic disease. To determine whether a high serum ferritin protein is due to iron overload or inflammation, it is also necessary to determine serum iron and transferrin (Al-Kataan *et al.,* 2009).

Transferrin has a much longer half-life in plasma than iron and shows short term of fluctuation (Zilva and Pannall, 1984). Consequently, it can be said that the high levels of ferritin accompanied with high level of serum iron and the low level of transferrin in two patients groups compared with control may be an evidence for iron overload in these patients.

Liver enzymes in serum of TM, TI and control groups

AST is widely distributed in the heart, liver, kidney and erythrocytes, and damage to any of these tissues may cause elevated levels (Fleming and Ponka, 2012). (Table 4) show the results of AST of two groups of thalassemia patients (TM and TI) and control group. These results show high significant increase in the two patients groups compared with control. The effect of iron overload on heart can cause congestive cardiomyopathy and other problems *i.e.* (pericarditis, restrictive cardiomyopathy, and angina without coronary artery disease) (Harmatz *et al.,* 2000). Liver fibrosis and cirrhosis are well known complications of thalassemia so they lead to elevate this enzyme [22].ALT is an enzyme found primarily in the liver but also in the heart and other tissues, it is more useful in diagnosing liver function than AST.The results of ALT in two groups of thalassemia patients (TM and TI) and control group are shown in

(Table 3-3 and Fig. 3-3). The results reveal highly significant increase in ALT activity of the patients groups compared with control. Many studies have established an association between elevated ALT activity and infection with hepatitis C (Adams *et al.,* 2013). Hepatitis C infection is a common cause of liver disease in thalassemia major patients (Hajarizadeh *et al.,* 2013). Abnormal elevated of ALT activities may arise as a result of iron overload which is a common blood transfusional symptom in thalassemia as reported by (Abdel-Hamid *et al.,* 2016).

The positive correlation that observed between ferritin and ALT in our study may support this explanation.

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