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Research Paper

Evaluation of Hepcidin and Interleukin-6 in Patients with Beta-Thalassemia Major

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ABSTRACT:

BACKGROUND:

In β- thalassemia major, irregularly transfused patients, hepcidin usually high due to increased iron load and the alleviation of ineffective erythropoiesis; rather than regularly transfused patients. Elevation of interleukin-6 in β- thalassemia had been reported in few theses.

OBJECTIVE:

To evaluate the level of serum hepcidin, interleukin-6 and ferritin in patients with β-thalassemia

PATIENTS AND METHOD:

This was a case control study conducted at thalassemia center of Ibn Al-Atheer Teaching Hospital in Nineveh Province during a period of six months between February and August 2022. It included 180 participants divided into two groups: Case group included 90 patients diagnosed as β-thalassemia major, aged between 5-40 years and control group included 90 healthy persons matched in age and gender to the case group.

RESULTS:

In this study, means of IL-6, hepcidin, and ferritin were significantly higher with P-value (0.001, 0.001, 0.001) respectively, while mean of Hb was significantly lower with P-value (0.001) in case group than that in controls. Strong negative correlation detected between ferritin and hepcidin, weak negative correlation found between ferritin and IL-6, and weak positive correlation between hepcidin and IL-6.

CONCLUSION:

Iron overload and alleviation of ineffective erythropoiesis made Hepcidin, interleukin-6 and ferritin higher in β-thalassemia major patients. Hepcidin and interleukin-6 levels have significant inverse correlation with serum level of ferritin. There was direct linear relationship between Interleukin-6 and hepcidin.

KEYWORDS: β- thalassemia major, hepcidin, interleukin-6, ferritin, transfusion.

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INTRODUCTION:

Thalassemia is a genetic disorder of hemoglobin which consider one of the commonest single gene disorder, with a carrier rate of 7% among the world population. They occur at particularly high frequencies in populations of the tropical and subtropical belt, and consist mainly of the α- and β-thalassemia, and the hemoglobin variants S, C, and E[(1)]. Thalassemia is an inherited as autosomal recessive; these abnormalities may result either in the reduction or absence of one or more of the globin chains of hemoglobin[(2)]. The pathophysiology of β-thalassemia is due to partial (Beta+) or complete loss of β globin chain (Beta 0) which result in excess unbound α globin chain and un equal globin chain ratio[(3)]. Patients with β-thalassemia major often diagnosed during the first few years of life as severe anemia (Hb level from 2-8g/dl), if which untreated lead to failure to thrive, jaundice, bone and skeletal deformity , blood film shows hypochromia, microcytosis, anisopoikilocytosis, nucleated RBC[(4)]. Iron overload consider the most common and serious problem in transfusion dependent thalassemia. In β-thalassemia major, hepcidin production mostly low, although of iron excess so iron absorption is

high due to inhibition of hepcidin gene expression [5]. Hepcidin is a 25 amino acid peptide hormone that suppress iron entrance into the plasma from the 3 sources of iron: dietary absorption in the duodenum, the release of recycled iron from macrophages and the release of stored iron from hepatocytes [6]. Interleukin-6 (IL-6) is a pro and anti-inflammatory cytokine, it has also metabolic, regenerative and neural role [9]. The aim of this study is to evaluate the level of serum hepcidin, IL-6 and ferritin in patients with β-thalassemia major, to study the correlation between them with other clinical, hematological and biochemical parameters, and to recognize the patients who are more prone to risk of iron overload in relation to hepcidin level.

PATIENTS AND METHOD:

Study design, setting, and time: This was a case - control study conducted at thalassemia center of Ibn Al-Atheer Teaching Hospital in Nineveh Province during a period of six months between February and August 2022.

Study Population and sample size: The study included 180 participants aged between 5-40 years and divided into two groups:

Case group: Included 90 patients diagnosed as β-thalassemia major by clinical, hematological and some of them molecular.

Control group: Included 90 healthy individuals matched in age and gender to the case group.

This study was approved by an official permission by the ethics committee at Iraqi board for medical Specialties commission and by the directorate of health in Mosul. Informed consent was obtained from all recruited patients. Patients underwent bone marrow transplant, patients diagnosed with hepatitis B or C, patients with other type of hemoglobinopathy, double heterozygous, and those who refused to be part of this study were excluded from this study.

Data were collected as demographic including (age, gender, educational level, occupation, parent consanguinity), age at diagnosis, age at starting transfusion, frequency of transfusion in the last year, date of the last transfusion, hemoglobin level before transfusion, regularity of blood transfusion, compliance for treatment, underwent splenectomy or not, presence of splenomegaly and/or hepatomegaly.

Workup: Six milliliters of venous blood were collected by venipuncture method from every patient, (3 mls) of blood was placed in EDTA tube for checking of complete blood count (CBC), and (3 mls) for determination of serum hepcidin, IL-6, and other biochemical analysis, putted in plain tube was allowed to clot at room temperature for about half hour then putting in centrifuge at 2000 rpm for 15 minutes to separate serum and used for serum hepcidin, IL-6 and biochemical analysis.

Serum hepcidin assay was done by CUSABIO ELISA Kit.

Estimation of serum ferritin was done by an in vitro immunoassay kit for quantitative determination of ferritin in human using Minividus machine from Bio Merieux (France).

Estimation of serum IL-6 was done in an in vitro chemiluminescence immunoassay for quantitative determination of IL-6 in human serum using (CILIA) machine.

Statistical analysis: All statistical analysis were done using the Statistical package for Social Sciences software, version26(SPSS), and a p value of <0.05 was considered as statistical significant.

RESULTS:

In this study, means of IL-6, hepcidin, and ferritin were significantly higher while mean of Hb was significantly lower ($P < 0.05$) in case group than that in controls. No statistical significant differences in age, SGOT, and SGPT between study groups ($P \geq 0.05$) as shown in table (1).

Table 1: Comparison between some parameters in this study.

Variable	Study group		P – Value
	Case Mean ± SD	Control Mean ± SD	
Age (Year)	19.8 ± 5.4	20.2 ± 4.1	0.577
Hb (g/dl)	8.1 ± 1.0	12.15 ± 1.4	0.001
IL-6 (pg/ml)	21.0 ± 21.3	3.4 ± 1.9	0.001
Hepcidin (ng/ml)	262.4 ± 82.5	17.6 ± 9.7	0.001
Ferritin (ng/ml)	4239.8 ± 3147.9	20.8 ± 12.0	0.001
SGOT (U/L)	41.6 ± 34.2	39.2 ± 13.6	0.536
SGPT (U/L)	33.0 ± 42.7	31.5 ± 11.4	0.748

In patients diagnosed as β-thalassemia major, mean of Hb was significantly the lowest in patients with more frequent blood transfusions to reach 7.9 g/dl in those with transfusion every two weeks. No significant differences in other clinical parameters according frequency of transfusion as shown in table (2).

Table 2: Comparison in certain parameters according frequency of transfusion.

Variable	Frequency of transfusion in the last year			P - Value
	Every two weeks Mean ± SD	Every three weeks Mean ± SD	Every month Mean ± SD	
Hb (g/dl)	7.9 ± 0.8	8.5 ± 1.2	9.0 ± 1.4	0.032
IL-6 (pg/ml)	15.2 ± 13.2	14.9 ± 14.3	17.25 ± 15.4	0.765
Hepcidin (ng/ml)	236.2 ± 73.5	255.6 ± 85.1	299.8 ± 82.4	0.121
Ferritin (ng/ml)	4460.2 ± 3055.8	3100.8 ± 2331.1	3310.4 ± 2416.7	0.836
AST (U/L)	29.6 ± 24.8	33.2 ± 31.7	34.1 ± 30.9	0.933
ALT (U/L)	28.0 ± 31.4	31.6 ± 34.4	26.1 ± 36.2	0.896

As shown in table (3), strong negative correlation detected between ferritin and hepcidine (r= - 0.782, P= 0.001), weak negative correlation found between ferritin and IL-6 (r= - 0.371, P= 0.041), and weak direct relation between hepcidin and IL-6 (r= 0.327, P= 0.027).

Table 3: Correlation between biochemical parameters.

Variable	Hepcidin (ng/ml)	
	R	P - Value
IL-6 (pg/ml)	0.327	0.027
Ferritin (ng/ml)	- 0.782	0.001
Ferritin (ng/ml)		
IL-6 (pg/ml)	- 0.371	0.041

DISCUSSION:

This study showed that IL-6, hepcidin, and ferritin were significantly higher while mean of Hb was significantly lower in case group than that in controls. In β- thalassemia major, irregularly transfused patients, hepcidin usually high due to high iron level and the amelioration of ineffective erythropoiesis, rather than regularly transfused patients [7] .Elevation of IL-6 had been reported in few thesis, which is most probably stimulated by multiple cells damage secondary to iron overload, others theory could be due to stimulation by alloantibodies after blood transfusion, elevation of IL-6 will stimulate hepcidin synthesis leading to abnormalities in iron metabolism [10]. Hepcidin affect iron status in the body by affecting the distribution of it on many tissues and organs. Any change in hepcidin production can lead to change in the levels of iron , continuous elevation of hepcidin can cause iron deficiency anemia, while it is deficit can lead to excess iron deposit in the liver and internal organs [11]. The high level of IL6 might be due to inadequate chelation therapy which was not available in thalassemic center for more than one year at time of this study and absence the follow up for complications of the disease by their families. The normal level of liver enzymes might be due to exclusion of hepatitis B and C virus patients in this study and absence of drug induced hepatotoxicity [12].

CONCLUSION:

This study was done for the first time in thalassemia center in Nineveh province.

Hepcidin , IL6 and ferritin are high in β-thalassemia major patients compare to healthy control due to high iron overload and alleviation of ineffective erythropoiesis.

Hepcidin , IL6 level are significantly inversely correlated with ferritin.

There was direct linear relationship between IL6 and hepcidin in β-thlassemia patients.

IL6 was significantly high in cases with increase liver enzyme .IL6 was significantly increase with splenomegaly and splenectomy.

Large proportion of patients are not compliance to treatment.

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