Study Some Biochemical Parameters in Beta Thalassemia Major Patients in Males and Females Adults in Baghdad

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Summary

Our study aims to investigate in levels of Ferrtin and IL-23 in beta-thalassemia major patients (males and females), and compared with the control group (healthy individuals). The total number of samples were (88) blood samples, beta-thalassemia major patients (44) and healthy individuals (44). All the samples submitted to ELIZA test to determination Ferrtin and IL-23 in blood samples (males and females) (patients and healthy group). According to our results, showed there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in Ferrtin concentration as compared with the control group at ($P<0.01$). In addition, Our study showed no there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in IL-23 level as compared with the control group at ($P<0.01$). Finally, there is a high increase in Ferrtin concentration of blood samples in males and females, while there are little changes (non-significant) in IL-23 concentration of blood samples in males and females.

Keyword: *Ferrtin, IL-23, beta-thalassemia major.*

Introduction

Thalassemias are genetic disorders, included decreased production of the hemoglobin (alpha or beta chain or both). Hemoglobin function is to carry the oxygen and formed from two proteins. Decreasing of these proteins leads to the inability of RBC to transfer the oxygen; leads to anaemia. Thalassemia is occurred due to deletion or mutation of some gene [(1) (2).]

Thalassemias have two types (Alpha and Beta). Alpha thalassemia is occurring due to the deletion of the alpha-globin gene or disorder of the synthesis of alpha-globin [(3) (4).] In addition, Beta-thalassemia results from point mutations in the beta-globin gene. It is divided into three categories based on the zygosity of the beta-gene mutation (minor, major and intermediate), these types result in different severity [(5) (6).]

The clinical signs of thalassemia appear before a child's second birthday. Very acute anaemia, furthermore, jaundice, frequent infections, a poor appetite, fussiness, failure to thrive, paleness, and the skin becomes yellow, organs enlargement, acute anaemia required blood transfusions [(7).]

Ferritin is protein work inside the cells. Bacteria, animals, archaea and human can produce Ferritin. Ferritin acts as a buffer control level of iron [3]. Ferritin is present in the cell and secreted at small amounts into the serum. Ferritin is indirect indicator of the total amount of stored iron therefore; the ferritin test is used as a laboratory test for iron concentration [(8) (9).]

Interleukin-23 is a pro-inflammatory mediator formed from two subunits (p19 and p40). It have immunological role in the body. IL-23 is stimuli Th17 cells that have great role in inflammation reaction [(10).]

Our study aims to investigate the relationship and changes in the levels of ferritin and interleukin 23 in the blood of patients with major beta-thalassemia

Material and Methods

Study design:

This prospective study for beta-thalassemia major patients had been conducted from December 2018 until June 2019 in AL Karama teaching hospital in Iraq Baghdad. All the patients had been chosen according to the following inclusion and exclusion criteria included All the patients are suffering from β-thalassemia major, Age more than 18 years old, Non-splenectomized, No obvious clinical infection, hepatitis and unmarried.

Sample size:

The study included eighty-eight (88) patients divided into two groups:

1- The first group (patient): Were forty-four (44) of the β-thalassemia major patients.

2- The second group (control group): was forty-four (44) apparently healthy.

Data collection:

The questionnaire is prepared for all the patients are included data related to name, gender, age, diagnosis of disease and other information.

Venous blood withdrawal:

The venous blood (5) ml was taken from control and patients group included in the study. The collected blood was divided almost (3ml) put in K3-EDTA tubes while other (2ml) keep in plain tubes, then centrifuged for five min at (300) rpm for separation the serum then stored at (-20) C.

Determination of Ferrtin hormone:

Assay Protocol:

1- Adding Pipet Ferritin Standard, control, and samples 25 µl by Pipet into the wells

3- Adding of the biotin Reagent (100) µl on all the wells and mixed.

4- Incubation of the plate for thirteen minute at (25°C)

5- Lifting of the liquid from all wells then washing wells by wash buffer.

6- Adding of the Enzyme Reagent (100) µl on all the wells and mix

7- Incubate for half hour at (25°C).

8- Discarded liquid from all wells and washing wells by using wash buffer.

9- Adding TMB substrate (100) µl on all the wells and incubation fifteen minutes at (25) C.

10- Adding of the stop solution (50) µl on all the wells and mix

11- The absorbance is read at (450) nanometer for fifteen minutes after mixing the stop solution.

Determination of IL-23 hormone:

Assay Procedure:

1- Put control, sample and standard in the wells

2- Adding Sample/Standard dilution buffer (0.1) ml into the zero well

3- Adding diluted sample (0.1) ml into test sample wells

4- Covering and incubation at 37°C for 1.5 hours

5- Left the cover and remove the plate content.

6- Adding Biotin-AB working solution (0.1) ml on the wells, adding of the solution on all wells

7- Covering and incubation at 37°C for one hour

8- Left the cover, and washing it by using wash buffer.

9- Adding of SABC working solution (0.1) ml into all the wells and incubation at 37°C for thirteen minutes

10- Lifting and washing by using wash buffer.

11- Adding TMB substrate (90) µl on all the wells, covering it and incubation at 37°C for thirteen minutes

12- Adding Stop solution (50) µl on all the wells and mixing, the color will be yellow directly.

13- OD Absorbance is read at (450) nanometer by using micro plate reader after mixing with the stop solution.

Statistical Analysis

The Statistical Analysis System software (2012) was applied for detection of the effect of two factors in our study. LSD test was used for compare between the means. Q test was used for compare between percentage (0.01 and 0.05).

Results

According to our results, showed there are significant difference in and males with beta-thalassemia major (3063.26±396.46) and females with beta-thalassemia major (3663.73±523.42) in Ferrtin concentration as compared with the control group (157.24±48.12) and (20.45±3.65) respectively at (P<0.01) as the table (1).

Table (1): Effect of gender and group in Ferrtin

Group	Mean ± SE	
	Male	Female
Patients	3063.26 ± 396.46	3663.73 ± 523.42
Control	157.24 ± 48.12	20.45 ± 3.65
T-test (P-value)	1029.10 ** (0.0001)	1329.50 ** (0.0001)

* S: Significant, ** H.S., Highly Significant, NS: Non- Significant.

Based on our study, showed no there are significant difference in and males with beta-thalassemia major (19.58±2.79) and females with beta-thalassemia major (13.99±3.26) in IL-23 level as compared with control group (17.34 ± 1.22) and (21.10 ± 1.04) respectively at (P<0.01) as the table (2).

Table (2): Effect of gender and group in IL-23

Group	Mean ± SE	
	Male	Female
Patients	19.58 ± 2.79	13.99 ± 3.26
Control	17.34 ± 1.22	21.10 ± 1.04
T-test (P-value)	7.496 NS (0.550)	8.457 NS (0.096)

*S: Significant, **H.S., Highly Significant, NS: Non- Significant.

Discussion

Many of the changes are occurring in patients with major beta-thalassemia, which include physiological and biological changes. These changes are included levels of hormones and some intermediate compounds in the activation of some reactions in the body such as ferritin and interleukin-23 [11].

Ferritin is a complex protein composed from two subunit heavy chain and light chain. Ferritin is regulate level of iron in the serum [12].

According to our results, showed there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in Ferrtin concentration as compared with the control group and respectively at (P<0.01) as the table (1).

Level of the ferritin protein is a reflex level of the iron. In another mean, the ferritin concentration gives a general view of how much iron in the body. If the ferritin level is low, that indicates the iron is low. If the ferritin concentration is high, the iron is high. The high concentration of ferritin is associated with beta major thalassemia due to repeated blood transfusion lead to increasing of iron in the body results in increases of ferritin and that agreement with results of [13] [14].

Interleukin-23 (IL-23) plays an important role in the development and maintenance of T-cell helper, wherever it has a great immune- protective role in the body [15].

Based on our study, showed no there are significant difference in and males with beta-thalassemia major and females with beta-thalassemia major in IL-23 level as compared with the control group and respectively at ($P<0.01$) as the table (2), There are little increase in males and a little decrease in females.

Some studies such as [16] and [17] showed increasing of IL-23 level, However, another study such as [18] showed little increases non- significant of IL-23 level.

Our study showed no statistical differences in interleukin-23 in males and females as compared with the control group. Perhaps these slight differences between the groups are due to individual factors, un-clinical infection conditions, and other unknown factors.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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