International Journal of Micro Biology, Genetics and Monocular Biology Research

Vol.3, No.2, pp.1-8, December 2017

Published by European Centre for Research Training and Development UK (www.eajournals.org)

EVALUATION OF CELLULAR IMMUNITY FOR B-THALASSEMIA MAJOR PATIENTS IN WASIT THALASSEMIA CENTER

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ABSTRACT: *The objective of this research work is to evaluation of cellular immunity for β-thalassemia major patients in Wasit Thalassemia center. Methods: hematological parameters including (Hb, WBCs), Phagocytic activity by Nitroblue Tetrazolium stain (NBT) and Enzyme linked Immuno Sorbent Assay (ELIZA) applied for estimation of the serum cytokines included IL-8, TNF-α and IFN-γ from 60 male β-thalassemia major patients and twenty healthy persons as control group. Results: the hematological parameters including Hb concentration shows significant decrease but WBCs count appeared significant increased compared with control group and significant decreased in the neutrophil activity. Interleukin-8, TNF-α and IFN-γ concentration showed significant decreased compared with control group. Conclusions: significant increase in the WBCs count and significant decrease in the Hb concentration, neutrophil activity and Interleukin-8, TNF-α and IFN-γ concentration compared with control group.*

KEYWORDS: Thalassemia, Infection, Immunity

INTRODUCTION

Thalassemia refers to a diverse family of genetic disorders characterized by abnormal hemoglobin production, a protein in red blood cells that carries oxygen (1). Thalassemia major is an inherited hematological disorder which_is_caused anemia in affected children. It is an autosomal recessive disease located_on chromosome 11. It affects synthesis of the B globin chain of hemoglobin, which is either decreased or absent, resulting in an early turnover of Red Blood Cell (RBC) (2).

Thalassemia patients cannot have an adequate hemoglobin in their bodies and they consequently suffer from anemia (3).

Iron overload develop in transfusion-dependent patients because they have no physiologic process to remove excess iron from multiple transfusions. Therefore, they need treatment with an iron chelator starting between five and eight years of age (4).

phagocytic activity by using Nitroblue tetrazolium stain (NBT):

Neutrophil are white blood cells that form a major part of our innate or non-specific immune system. They are circulating phagocytes created continuously in the bone marrow and have a half life of 6-9 hour. They also include more than 55% of our total circulating white blood cells and more 85% of circulating phagocytes. Neutrophils also marginated in the tissues in large numbers, and this storage allows them to mobilize rapidly in response to the incidence of an infection or inflammation where they phagocyte invading bacteria (5). Neutrophils are the cellular lineament of acute inflammation and rapidly accumulate in large number at the sites of infection. During their short life span (hours to days) neutrophils perform many

ISSN 2059-9609(Print), ISSN 2059-9617(online)

functions of host defense, including phagocytosis and killing of bacteria with reactive oxygen intermediates and the other mechanisms (6).

Interleukin-8 :

IL-8 was primarily recognized as a neutrophil-activating protein on the basis of two in-vitro effects, chemotaxis and the release of granule enzymes (7). These effects indicated that IL-8 was a novel type of chemoattractant. These stimuli enhance three main responses in neutrophils: (i) shape change and directional migration, (ii) exocytosis of storage proteins, and (iii) the respiratory burst (8).

Tumor necrosis factor - Alpha :

Tumor necrosis factor (TNF) plays a pivotal function in various immune and inflammatory processes, including cellular activation, proliferation and survival, as well as cell death by necrosis and apoptosis. TNF cellular source depends on the nature of the stimulus. TNF is produced originally by cells of hematopoietic origin, including myeloid lineage such as macrophages and monocytes when stimulated by innate sensors, such as the Toll-like receptor (TLR) system. TNF also produced from T and B lymphocytes in response to antigenic stimulation (9).

Gamma Interferon :

Interferon gamma (IFN-γ) is a cytokine discovered in 1965, with diverse roles in the innate and adaptive immune responses. IFN-γ function has been highly conserved throughout evolution and across multiple species. It has a myriad of effects in both host defense and immune regulation, including antiviral activity, antimicrobial activity and antitumor activity, and T cells NK cells are the primary generator of IFN-γ (10).

The Aims of this study are:

1. Study of Phagocytic activity in male beta-thalassemia major patients.

2. Study interleukin-8, Gamma interferon and Tumor necrosis factor alpha levels in the serum of the patients.

MATERIAL AND METHOD:

Sixty male β-thalassemia major patients over than 3 years old attending at thalassemia center at AL- Kut Hospital in Wasit province for regular blood transfusion from October 2016 to february 2017 were assigned in the study.

Blood samples were collected from the patients pre-blood transfusion and the clinical history was taken from each patient including: name, age, blood group, address, family history and treatment. The patients infected with hepatitis B, C and patients with Splenectomy were excluded.

Twenty healthy persons were examined as a control for all parameters of the patients group.

ISSN 2059-9609(Print), ISSN 2059-9617(online)

The blood samples is divided into two part

A- The blood sample was put in the anticoagulant tube for estimation of white blood cells count, HB and phagocytic activity by the Nitroblue Tetrazolium stain.

B- Another part of blood sample put in a plain tube after centrifuged to separate the serum to estimation interleukin-8, Tumor necrosis factor alpha and Gamma interferon by using Enzyme Linked Immunosorbent Assay (ELIZA).

RESULTS

A total of 60 male thalassemia major patients and 20 control group were studied. The mean age was 10.10 ± 4.265 in the patients and 11.15 ± 3.133 in the control group. Results in table (1) shows significant decrease ($p<0.05$) in the mean value of Hb concentration (g/dl) in male thalassemia major patients while the mean value of WBCs (mm^3) appeas significant increase ($p<0.05$) in the patients when comparison with control group and significant decrease ($p<0.01$) in the mean value of neutrophil positive for N.B.T in male patients compared with control group. Table (2) shows the laboratory data of the patients and controls, the mean IL-8 concentration (pg/ml) in the serum of patients shows significant decrease in male thalassemia major patients (3.217±1.070 pg/ml) when comparison with control group (8.360 ± 0.954 pg/ml), the mean concentration of TNF-α appears significant decrease in male thalassemia major patients (1.703± 0.706 pg/ml) when comparison with control group (5.876±1.618pg/ml) and the results observe significant decrease in the mean concentration of IFN-γ for male thalassemia major patients (4.034± 0.957 pg/ml) when comparison with control group (9.834±1.137pg/ml).

Table (1): the mean concentration of the Hematological parameter in male thalassemia major patients and control group.

Parameter	**Control n=20**	**Patient n=60**	**P value**
Hb (g/dl)	13.48	8.6882 S	*P* <0.001
WBCs (mm^3)	6695	8175 NS	*P*= 0.009
Neutrophil Activity	14.45	6.266 S	*P* <0.001

NS: refers to the parameters with non-significant compared with control group.

ISSN 2059-9609(Print), ISSN 2059-9617(online)

Table (2): the mean concentration of IL-8, TNF-α and IFN-γ(pg/ml) in male thalassemia major patients and control group.

Items	Patients /Mean ± SD (pg/ml)	Control /Mean ± SD (pg/ml)	P value
IL-8	3.217 ± 1.070 S	8.360 ± 0.954 S	*P* <0.001
TNF-α	1.703 ± 0.706 S	5.876 ± 1.618 S	*P* <0.001
IFN-γ	4.034 ± 0.957 S	9.834 ± 1.137 S	*P* <0.001

S : refer to the parameter with significant compared to the control.

DISCUSSION

Discussion of hematological parameter

In our study, the hematological parameter appears significant decrease in the mean value of Hb for male thalassemia major patients compared with the control group table (1), these results was agreed with pervious study in Wasit province by Alwan who observed significant decrease in the mean value of Hb concentration in thalassemia patients than in control group (11). Also the studies mentioned by Ali in Thi- Qar, Ala ́a in Baghdad and Dhawan in India observed the same results (12, 13, 14). The Hb concentration decreased in the thalassemia patients because the abnormal shaped RBCs are rapidly destroyed by the reticuloendothelial system, particularly the spleen leading to chronic anemia and iron overload (15).

The mean values of WBCs appear significant increase in the male thalassemia major patients than the mean value of WBCs in control group table (1), this results was agreed with Alwan in wasit province, Shanna in Erbil and Dwyer they observed the number of white blood cells was increased in the thalassemia patients ($p<0.05$) when compared with control group (11, 16, 17).

Discussion for neutrophil activity:

In this study, the evaluation of neutrophil activity in male thalassemia major patients (which was assessed NBT test), shows a significant decrease in 60 thalassemia patients when comparison to the 20 control group table (1). This study was agreement with Ghaffari et al study which showed significant decrease in phagocytic activity for patients than in control group (18). The study of Cantinieaux et al appeared the polymorphonuclear neutrophils (PMN's) phagocytic activity was decreased, and they considered this abnormality because the cellular and serumal dysfunction due to iron overload (19). Also Cantinieaux et al study showed PMN's phagocytic activity of neutrophils was impaired, and they considered that as a result of cellular and serumal abnormalities due to iron overload (20). Palacios et al and Kutukculer et al showed a chemotactic impairment of neutrophils in thalassemic patients, and concluded that the defect found might be caused by transfusion overload (21, 22).

ISSN 2059-9609(Print), ISSN 2059-9617(online)

Discussion for cytokines:

Discussion for IL-8:

In this study the results appear significant decrease in the mean concentration of IL-8 in the serum of male thalassemia major patients when compared with control group table (2). this results was disagreement with the study of Oztürk et al who found that plasma IL-8 levels in the patients who had blood transfusions over 100 times were significantly higher than those of under 100 times when compared with normal control group (23), because there are direct positive correlation between IL-8 concentration and total number of blood transfusion and they take blood samples after blood transfusion but in our study the blood samples was taken before blood transfusion. Also Shfik et al demonstrated increased serum levels of IL-8 in multiple blood transfused of β-thalassemia major patients (24). The disagreement of our results with the results of previous researcher may be due to the blood samples in our study was taken from the patients before blood transfusion, while all the previous study was taken the blood samples after blood transfusion because they want to study the level of IL-8 in the serum of thalassemia patients after multiple blood transfusion. Also the immunogenetic difference race cause these difference in the results between Iraqian population and another people.

Discussion for TNF-α :

The present study found the serum levels of TNF-α are statistically significantly decreased in male thalassemia major patients compared with control group table (2). This result was disagreement with study of Abo Shanab et al who observed the concentration of TNF-α in the serum of thalassemia patients was significantly increased (25). The study of Shfik et al demonstrated increased serum levels of TNF-α in multiple transfused patients with β-thalassemia major (24). Also Uguccioni et al appeared increased serum levels of TNF-α were reported in homozygous polytransfused beta-thalassemia major (26). The difference in the results between this study and other because all previous researchers take the blood sample after the blood transfusion but in this study the samples taken before blood transfusion.

Discussion for IFN-γ:

In this study, the serum levels of IFN-γ in the male thalassemia major patients was significant decrease compared to the control group.

A major cause of morbidity and mortality in thalassemia patients was infections, assumed to be the result of immunological changes (El-Beshlawy and Youssry, 2009) (27). Interferon-gamma (IFN-γ) plays an important role in the pathogenesis of thalassemia (Moshtaghi et al) (28). This is agreement with the study of Roshdy et al who showed decreased levels of IFN-γ in thalassemia patients than control group (29), also Gharagozloo et al study demonstrated a significant decreased in IFN-γ production by activated lymphocytes from patients with β-thalassemia compared to the normal group (30) and Rasoul et al study showed significant decreasing in IFN-γ in beta thalassemia major patient (31).

ISSN 2059-9609(Print), ISSN 2059-9617(online)

CONCLUSION

Significant increase in WBCs count and significant decrease in the Hb concentration, neutrophil activity and Interleukin-8, TNF-α and IFN-γ for male β-thalassemia major compared with control group.

RECOMMENDATION

1. A Genetic study before marriage for male and females are highly recommended to avoid the expression of hidden gene for thalassemia.

2. A Study of the mutation or changes in the gene which makes individuals more susceptible to disease.

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Rasoul Baharlou, Mohammad Hassan Davami, Abbas Ahmadi Vasmehjani, Morteza Ebrahimi (2016): Increased IL-17 and TGF-β serum levels in peripheral blood of

ISSN 2059-9609(Print), ISSN 2059-9617(online)

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ISSN 2059-9609(Print), ISSN 2059-9617(online)