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Laboratory Evaluation of Clinical Utility of Serum Interleukin- 8 in Thalassemic Patients

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Abstract

Introduction: Various types of immunologic defects have been found in β -thalassemic patients, of which the impairment of neutrophil phagocytic and killing functions are the most important. Interleukin-8 is an important chemotactic and activating peptide for neutrophils and can be detected in the circulation. This cytokine can be produced by a variety of cell types, including large granular lymphocytes, macrophages, endothelial cells, fibroblasts, and synovial cells. Changes in IL-8 plasma levels may be relevant to the pathophysiology of β -thalassemia.

Aim of the study: It aimed to measure the level of IL-8 and assess granulocyte recruitment, as markers of immunomodulation, in poly-transfused thalassemic patients attending Fayoum University Hospitals

Subjects and Methods: The study was performed on 50 patients with β -thalassemia who were selected from the El Fayoum University Hospital. The patients are divided into 2 subgroups, the first one includes 21 thalassemic patients who received MORE than 10 packed red blood cells (PRBCs) transfusion, while the second includes 21 thalassemic patients who received LESS than 10 PRBCs transfusion. 32 age and sex-matched healthy subjects were included as a control group. Full history taking, to exclude any other chronic disease was done. Measurement of IL-8 using a Human IL-8 ELISA Kit and Rebeck's skin window technique: to assess granulocyte recruitment was performed.

Results: It was found that the concentration of IL-8 in β -thalassemic patients was higher than the normal control (P -value <0.001). Also, it was found that IL-8 plasma concentration in patients who had blood transfusion >10 times was significantly higher than those of <10 times (P -value <0.001). As regards neutrophil recruitment (RSWT), there were statistically significant differences between patients who had blood transfusions >10 times and controls ($P<0.05$). Conversely, the results showed no significant difference as regards neutrophil recruitment between patients who had blood transfusion <10 times and controls ($P=0.03$).

Conclusion: These parameters (serum IL-8 and Rebeck skin window test) could be used as useful markers of immune modulation in thalassemia patients.

Keywords: β -thalassemia; IL-8; Rebeck skin window test.

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1. Introduction

β -thalassemias are heterogeneous autosomal recessive hereditary diseases characterized by reduced (β^+) or absent (β^0) - globin chain synthesis resulting in a relative excess of unbound alpha globin chains. The Imbalance in the α -non- α -

globin chain is the basis of β -thalassemia [1].

α -Globin accumulates in the erythroid precursors forming inclusion bodies that, bound to the membrane skeleton, cause oxidative membrane damage and extensive premature destruction by

apoptosis of the RBC precursors in the bone marrow (ineffective erythropoiesis). Hemolysis plays a secondary role [2].

It has been found that infections are the second most common cause of death after heart failure in patients with β -thalassemia major. Infections in thalassemia are related to the disease itself, altered immune system secondary to blood transfusions, iron overload and splenectomy [3].

IL-8 is a pro-inflammatory chemokine that is produced by, macrophages, endothelial cells, and epithelial cells in response to infection or tissue injury, where one of the functions of IL-8 is to induce chemotaxis of granulocytes, primarily neutrophils. At the site of infection, IL-8 can promote its resolution by inducing phagocytosis, oxidative burst, and the release of DNA webs known as neutrophil extracellular traps which trap and kill the invading microbes. IL-8 also activate the angiogenic response as it induces vascular endothelial cell proliferation, survival, and migration,

causing the formation of new blood vessels [4].

The concentration of IL-8 is elevated in the majority of β -thalassemic patients. This production could be caused by transfusion-related continuous antigenic stimulation and iron overload with consequent macrophage activation. Macrophages (and fibroblasts) can be responsible for IL-8 production either directly or indirectly via TNF- α synthesis [5].

Defects in PMN chemotaxis and bactericidal activity have also been shown in β -thalassemia. Recently it was found that intravascular IL-8 inhibits PMN migration by inhibiting the leukocyte-endothelial adhesion process. Thus, high circulating IL-8 concentrations in β -thalassemia may contribute to the chemotaxis defect shown by PMN. On the other hand, high IL-8 can activate PMN, which is in keeping with the finding of an increased superoxide anion production by neutrophils from β -thalassemic patients [6].

2. Subjects and methods

2.1. Subjects

This study was conducted on 50 patients with β -thalassemia who were selected from the El Fayoum University Hospital, in addition to 32 healthy subjects' age and sex-matched.

The study subjects were divided into two groups:

First group: Cases; Thalassemic patients receiving blood transfusions subdivided into:

- a. Subgroup Ia (n=21): Thalassemic patients who received more than 10 blood transfusions. Thalassemic patients usually receive blood every 2-3 weeks according to the severity of

the case. There were 10 females and 11 males whose ages ranged from 6 to 36 years.

- b. Subgroup Ib (n=29): Thalassemic patients who received less than 10 blood transfusions. There were 20 females and 9 males whose ages ranged from 1 to 30 years.

Second group (n=32): This included 32 sex- and age-matched healthy subjects whose ages ranged from 1 to 26 years mean (9.4±6.589).

Inclusion criteria

All thalassemic patients were admitted to Fayoum University Hospitals.

Exclusion criteria

Thalassemic patients, who are diagnosed with any additional chronic disease other than thalassemia.

2.2. Methodology

All subjects were subjected to the following tests:

3. Results

Serum level of IL-8, is significantly higher in group Ia of thalassemic patients (22.9 pg/ml), compared to controls (1.5 pg/ml). Also, there is a highly statistically significant difference with a *P-value* <0.001 between study group Ib and control group regarding serum level of IL-8, showing a significantly higher mean in group Ib thalassemic patients (4.8 pg/ml), compared

- Full history taking, to exclude any chronic disease other than thalassemia.
- Measurement of IL-8: samples were collected on sterile EDTA vacutainer tubes, allowed to clot at room temperature then centrifuged and serum was separated and stored at -20 degrees Celsius until assayed. For the quantitative determination of IL-8 in serum, we used an IL-8 ELISA Kit; implementing the sandwich-ELISA technique.
- Rebeck skin window technique: to assess granulocyte recruitment.
- The top layer of skin is scraped off and a clean glass coverslip is applied to the area and surmounted with hypoallergic surgical tape. Leukocytes accumulate at the site and adhere to the coverslip. At 4 hours, the coverslip was removed, air-dried, and stained like a blood smear. A second coverslip was applied similarly and kept in place until the 24th hour of traumatic inflammation had supervened. Both neutrophils and monocytes are counted in 10 fields. Controls also were studied as described with respect to their leukocytic response to the trauma of the technic alone.

to controls (1.5 pg/ml). Furthermore, our study showed that there is a highly statistically significant difference with a *P-value* <0.001 between study group Ia and study group Ib as regards serum level of IL-8, showing significantly higher mean in group Ia thalassemic patients (22.9 pg/ml), compared to group Ib (4.8 pg/ml) (**Table 1, Figure 1**).

Table 1: Comparisons of IL-8 between controls and thalassemia cases.

Variables	First group, Cases		Second group Controls (n=32)
	Subgroup Ia (n=21)	Subgroup Ib (n=29)	
IL-8 (pg/ml) Mean (range)	22.9 (11.2-45.1)	4.8 (1.4-11.2)	1.5 (1.4-1.7)
<i>P1</i>	<0.001	<0.001	
<i>P2</i>	<0.001		

P1: *P-value* (versus control); *P2*: Ia versus Ib.

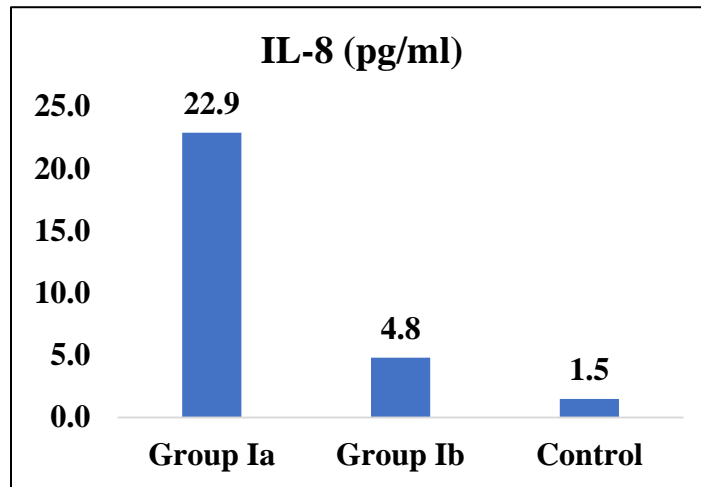


Figure 1: Comparison of IL-8 in controls and thalassemia cases.

Our data, also showed that there is a highly statistically significant difference with a *P-value* <0.001 between study group Ia and study group Ib as regards the total number of transfusion units, showing a significantly higher mean in group Ia

thalassemia patients (198.3 units), compared to group Ib (5.58 unit). Therefore, as study group Ia receives more transfusions than group I, this emphasizes the fact that more transfusions increase the burden of immunomodulation (**Table 2**).

Table 2: Comparisons between the groups of thalassemia cases.

Variables	subgroup Ia (n=21)	subgroup Ib (n=29)	<i>P-value</i>
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Unit transfused	198.3 (88-450)	5.58 (3-9)	<0.001
Age at first transfusion(years)	4.23 (1-12)	5.55 (0.6-17)	0.24
Duration of transfusion (years)	9.66 (4-24)	7.45 (0.6-19)	0.16

Regarding the Rebeck skin window test, there is a statistically significant difference as regards neutrophil recruitment between group Ia and controls ($P < 0.05$), neutrophil recruitment was 89% at 4 hours and 82% at 24 hours in group Ia compared to 96% at 4 hours and 97% at 24 hours in controls. Also, the results showed No significant difference as regards neutrophil recruitment between group Ib and controls

($P=0.03$), neutrophil recruitment was 94% at 4 hours and 97% at 24 hours in group Ib compared to 96% at 4 hours and 97% at 24 hours in controls. Moreover, there is a statistically significant difference as regards neutrophil recruitment between group Ia and group Ib ($P < 0.05$), neutrophil recruitment was 89% at 4 hours and 82% at 24 hours in group Ia compared to 94% at 4 hours and 97% at 24 hours in group Ib (**Tables 3**).

Table 3: Comparison of RSWT results among study groups.

Variables	First group, Cases				Second group		P-value (Significance)		
	subgroup Ia (n=21)		subgroup Ib (n=29)		Control (n=32)		P1	P2	P3
	4 hrs	24 hrs	4 hrs	24 hrs	4 hrs	24 hrs			
Neutrophils	89% (85-92)	82% (76-91)	94% (92-95)	97% (93-98)	96% (96-97)	97% (93-100)	<0.001	0.03	<0.05
Monocytes	11% (8-15)	18% (9-24)	6% (5-8)	3% (2-7)	4% (3-4)	3% (0-7)	Statistical analysis was performed on Neutrophils		

P1: Ia versus control; P2: Ia versus control, P3: Ia versus Ib.

4. Discussion

Thalassemia Major is characterized by mutations of the β -globin gene with defective β -chain production. Hemolysis and ineffective erythropoiesis cause anemia in TM that requires blood transfusion to survive. Leucocyte-depleted PRBCs are used to avoid the transfusion reaction that occurs in repeatedly transfused patients.

Furthermore, the patient's ABO, Rhesus, Kell, Kidd and Duffy systems should be typed before the beginning of transfusion to avoid the development of red cell antibodies [7].

TM have an increased risk for serious infections, suggesting a basic defect

in the host defense [8]. Neutrophil chemotaxis, specific antibody response, and cell-mediated immunity have been reported to be defective in TM [9]. Blood Transfusion has been involved in causing significant changes in the recipient's immune response.

IL-8 is a pro-inflammatory cytokine that promotes chemotaxis and degranulation of neutrophils [10]. IL-8 can promote the resolution of infection by inducing phagocytosis, oxidative burst, and the release of DNA webs known as neutrophil extracellular traps that trap and kill invading microbes. The second function of IL-8 is to activate the angiogenic response. IL-8 signaling in vascular endothelial cells induces cell proliferation, survival, and migration, which ultimately culminate in the formation of new blood vessels. In this manner, IL-8 serves to both resolve the inflammatory stimulus and promote healing [4].

This study measured the level of IL-8 and assessed granulocyte recruitment, as a marker of immunomodulation, in poly-transfused thalassemic patients. This study includes 50 patients with thalassemia major and 32 age and sex-matched controls. The patients are divided into 2 subgroups, the first one (subgroup Ia) includes 21 thalassemic patients who received MORE than 10 packed red blood cells (PRBCs) transfusion, while the second subgroup Ib includes 21 thalassemic patients who received LESS than 10 PRBCs transfusion.

As regards to serum level of IL-8, our data showed that there is a highly statistically significant difference between

study group Ia, group Ib and the control group showing significantly higher mean in group Ia thalassemia patients (22.9 pg/ml), group Ib (4.8 pg/ml) compared to controls (1.5 pg/ml). One study by Hablas et al., (2018) revealed that there was a significant increase in the serum IL8 level in the β -TM children than in controls [6]. Consistent with our findings, Asadov, (2020) also found that in patients with thalassemia concentration of IL-6 and IL-8 increased in comparison with the norm [11]. Akcali et al., (2015) showed that IL-8 were higher in patients with thalassemia major (TM) than in systemically and periodontally healthy comparison groups [7]. These results agreed with Ozturket al., (2001) who found that IL-8 concentration is higher in β -thalassemic patients [12]. Increased IL-8 production might be ascribed to the transfusion-related continuous antigenic stimulation and iron overload with consequent macrophage activation.

The results of RSWT among different study groups show a statistically significant difference as regards neutrophil recruitment between group Ia and controls, neutrophil recruitment was 89% at 4 hours and 82% at 24 hours in group Ia compared to 96% at 4 hours and 97% at 24 hours in controls. Also, the results showed No significant difference as regards neutrophil recruitment between group Ib and controls. Moreover, the results showed a statistically significant difference as regards neutrophil recruitment between group Ia and group Ib, neutrophil recruitment was 89% at 4 hours and 82% at 24 hours in group Ia compared to 94% at 4 hours and 97% at 24 hours in group Ib.

Farmakis et al. (2003) searched the pathogenic aspects of immune deficiency associated with β -thalassemia and found that β -thalassemia has numerous quantitative and functional defects, involving T and B lymphocytes, immunoglobulin production, neutrophils and macrophages, chemotaxis, and phagocytosis, as well as the complement system [13]. Akcali et al. (2015) showed that patients with thalassemia major (TM) may have various immunologic defects in neutrophil and macrophage phagocytic and killing functions and production of some cytokines [7]. Hablas et al. (2018) also found that there were significantly lower haemoglobin, MCV, MCH, neutrophil percentages and platelet count and significantly higher WBC count, and reticulocyte, eosinophil, monocyte and lymphocytes percentages in patients than in controls [6]. Bazi et al. (2018) suggested that persistent antigenic stimulation and oxidative stress from excessive iron are the two main pathophysiologic factors of TM impacting the immune system [14]. Regarding innate immunity, functional

activity of neutrophils, and natural killer cells (NKC) is decreased in TM.

Our data showed that although serum levels of IL-8 are significantly elevated in multiply transfused thalassemic patients, however, levels of neutrophil recruitment were significantly reduced. This could be explained by the following mechanism, during acute inflammation neutrophil elastase cleaves CXCR1, a receptor for IL-8, on the surface of neutrophils, and circulating neutrophils selectively downregulate CXCR2, the only other neutrophil receptor for IL-8. Downregulation of CXCR2 and cleavage of CXCR1 would result in severe hyporesponsiveness to IL-8, an important neutrophil chemoattractant [15]. This could be further confirmed by the fact that IL-8 must bind to its receptor on neutrophils to accomplish its chemotactic function. In other models, strong constitutive expression of a chemokine resulted in desensitization and hypo-responsiveness of leukocytes to the chemokine, even if it is expressed in a tissue-specific pattern (Kucharzik et al., 2005).

Conclusion

Our data highlights the importance of IL-8 and granulocyte recruitment, as markers of immunomodulation, in poly-transfused thalassemic patients. Our data highlights the importance of IL-8 and granulocyte recruitment, as markers of immunomodulation, in poly-transfused thalassemic patients. Multi-transfused β -thalassemic patients had higher serum IL-8

concentrations compared with those of healthy controls. Moreover, they also had an immune deficiency in the form of quantitative and functional defects of neutrophils and macrophage chemotaxis. Therefore, these parameters could be used as useful markers of immune modulation in thalassemia patients

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