

Evaluation of Interleukin 8, Interleukin 2 Receptor and Serum Ferritin in 60 Patients with BetaThalassemia Major: Relationship to Splenectomy

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ABSTRACT:

BACKGROUND:

β -Thalassemia is an inherited hemoglobin disorder characterized by the absence or reduced synthesis of the β -globin chain. Several immunological defects can be found in patients with β -thalassaemia. Increased IL-8 level was documented in patients with β -thalassaemia and it is probably due to overstimulation of macrophages.

OBJECTIVE:

Evaluation of serum levels of IL-8, soluble IL-2 Receptor, and serum ferritin in the splenectomized and the non-splenectomized β -thalassemia major patients.

METHODS:

A cross sectional study was conducted on 60 patients with homozygous β -thalassemia major who were attending Al Karama Teaching hospital from May 2011 to August 2011. Place of work was Al-Kadhmia and Al Karama teaching hospital. Patients were divided into two groups; thirty splenectomized, thirty non-splenectomized, and thirty healthy age and sex matched as control group.

RESULT:

The mean level of IL-8 was increased in both groups of thalassaemia with significantly higher in the splenectomized 333.06 ± 255.50 pg/ml compare to the non-splenectomized 118.61 ± 46.59 pg/ml and the control 38.39 ± 9.52 pg/ml, P -value <0.05 . The mean level of sIL-2R was not increased above the reference range in the control subjects, the non-splenectomized and the splenectomized, which was 732.96 ± 28.96 pg/ml, 723.42 ± 74.15 pg/ml and 777.38 ± 86.93 pg/ml respectively. The mean serum ferritin level in the splenectomized was 2050.1 ng/ml which was significantly higher compared to the non-splenectomized 1212.2 ng/ml, P -value <0.05 . There was significant correlation between IL-8 and serum ferritin, P -value <0.05 while there was no significant correlation between s IL-2R and serum ferritin, in both groups of Thalassaemia P value > 0.05 .

CONCLUSION:

- β -thalassemia major patients had increased level of IL- 8 which was more prominent in splenectomized patients, while no increase in IL-2R levels in both groups of Thalassemia.
- IL-8 levels correlated with ferritin levels, while no significant correlation between IL-2R levels and ferritin levels in both groups of Thalassaemia.
- Splenectomized thalassaemic patients display higher ferritin levels compared to the non-splenectomized.

KEY WORDS: interleukin 8, ferritin , thalassemia , splenectomy .

INTRODUCTION:

Thalassemia primary defect is usually quantitative, consisting of the reduced or absent synthesis of normal globin chain ⁽¹⁾ . The commonly adopted criteria for splenectomy is a blood consumption greater than 50% above the mean requirement of the splenectomized

population , i.e. more than 200-250 ml /kg /year of pure red cells, to maintain a pre-transfusion hemoglobin around 9g / dL ⁽²⁾.

Risks associated with splenectomy include; Infection; the most frequently responsible bacteria are Streptococcus pneumoniae, Hemophilus influenza, E. coli, and Staphylococcus aureus ⁽³⁾. Thalassemia patients are prone to many bacterial and viral infections. Repeated infections also stimulate the immune system and may result in increased

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immunoglobulin levels ⁽⁴⁾. Changes in serum level of cytokines, like increased level of IL-8⁽⁵⁾. Many cytokines are referred to as interleukins, a name indicating that they are secreted by some leukocytes and act upon other leukocytes. The chemokines, a group of low molecular weight cytokines that affect chemotaxis. These molecules play an important role in the inflammatory response ⁽⁶⁾. Interleukin-8 is a chemokine produced by lymphocyte, macrophage, granulocyte, endothelial cells and others, it functions as a chemoattractant of neutrophils, and also a potent angiogenic factor ⁽⁷⁾. IL-2 is a protein produced mainly by helper T-cells that stimulates both helper and cytotoxic T-cells to grow ⁽⁸⁾. The binding of secreted IL-2 to these IL-2R-positive T cells induces clonal T-cell proliferation ⁽⁹⁾. Serum ferritin is a useful parameter for assessing changes in body iron, the thalassemia International Federation guidelines recommend maintaining serum ferritin levels around 1000 µg/L.⁽¹⁰⁾

AIM OF THE STUDY:

1. Evaluation of serum levels of Interleukin 8, and soluble Interleukin 2 Receptor in the splenectomized and the non-splenectomized Beta-thalassemia major patients, and compare with the control.
2. Evaluation of serum ferritin level in the splenectomized and the non-splenectomized Beta-thalassemia major patients.

PATIENT, MATERIALS AND METHODS:

A cross-sectional study was conducted on 60 patients with homozygous beta-thalassemia major who were attending Al Karama Teaching hospital for receiving blood transfusion and treatment, from May 2011 to August 2011. Places of work were in Al-Kadhemia and Al-Karama teaching hospital.

Patients were divided into two groups:

The splenectomized; Comprised 30 patients 16 males and 14 females. Their ages ranged from 5 to 27 years. The non-splenectomized; Comprised 30 patients 13 males and 17 females. Their ages ranged from 3 to 24 years.

Thirty healthy age and sex matched subjects were included in the study as control group, comprised 14 female and 16 male and their age of ranged from 3 to 27 years.

- Interleukin 8 principle

IL-8 ELISA kit applies a technique called a quantitative sandwich immunoassay ⁽¹¹⁾.

- Soluble Interleukin 2 Receptor principle

Human sIL-2R kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA) ⁽¹²⁾.

-Serum ferritin principle

The assay principle combines a one-step enzyme immunoassay sandwich method with a final fluorescent detection (ELFA) ⁽¹³⁾.

Statistical Analysis

Statistical analysis was performed with SPSS (Statistical Package for social sciences) version 16 and Microsoft Office Excel 2007. Numeric data were analyzed as mean, standard deviation and standard error of the mean, using student T-test or ANOVA accordingly, while nominal data were expressed as frequencies and were analyzed using chi-square. Spearman rank correlation was used to determine relation between two numeric variables. P-value<0.05 was considered significance.

RESULTS :

Interleukin 8

The mean serum level of IL-8 in control subject was 38.39±9.52 pg/ml whereas in the non-splenectomized, and the splenectomized was 118.61±46.59 pg/ml and 333.06±255.50 pg/ml respectively, the results were statistically significant P<0.05. Figure 1.

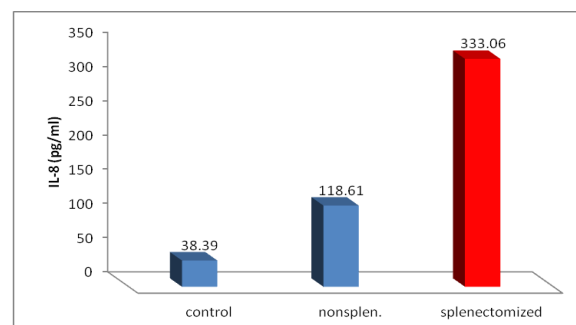


Figure 1: Comparison of mean serum level of interleukin 8 among control, non-splenectomized and splenectomized Thalassaemic patients

Interleukin 2 Receptor

The mean serum level of IL-2R was not increased above the reference range in all the three groups; control subjects, the non-

splenectomized and the splenectomized which were 732.96 ± 28.96 pg/ml, 723.42 ± 74.15 pg/ml and 777.38 ± 86.93 pg/ml respectively. Figure 2

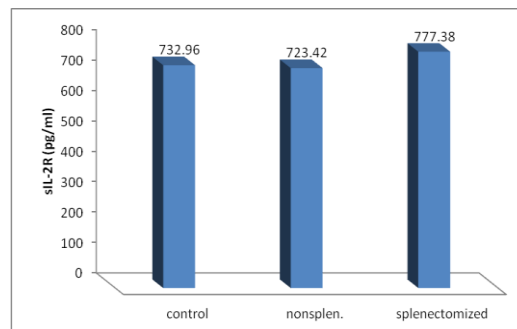


Figure 2: Comparison of mean serum level of soluble interleukin 2 Receptor among control, non-splenectomized and splenectomized thalassaemic patients

Serum ferritin

The mean of serum ferritin level in the splenectomized was 2050.1 ng/ml which was significantly higher

compared to the non-splenectomized 1212.2 ng/ml $P < 0.05$. Figure 3.

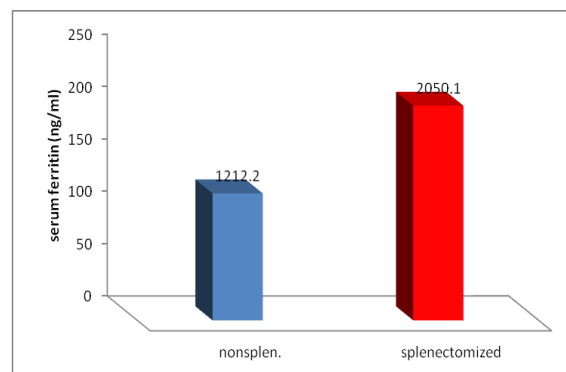


Figure 3: Comparison of mean serum ferritin level between non-splenectomized and splenectomized thalassaemic patients.

Correlation between IL-8 and serum ferritin

There was significant positive correlation between IL-8 and serum ferritin, $P < 0.05$, in the non-

splenectomized and splenectomized groups as shown in figure 4 and figure 5 respectively, using spearman rank linear correlation.

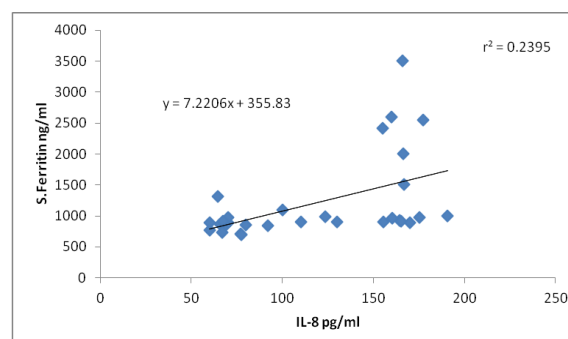


Figure 4 :Correlation between serum ferritin level and IL8 in Non-splenectomized Thalassaemic patients($r=0.489$, $r^2=0.2395$, $P=0.006$).

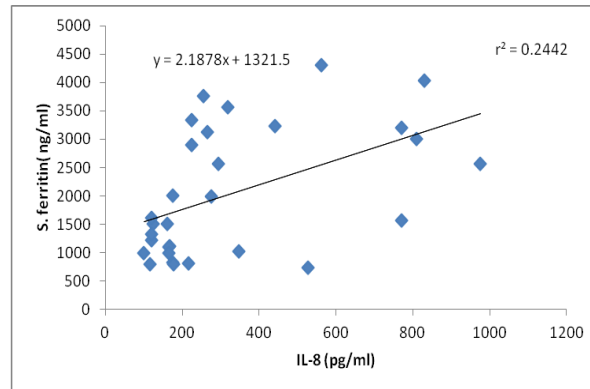


Figure 5: Correlation between serum ferritin level and IL8 in splenectomized Thalassemic patients ($r=0.494$, $r^2=0.2442$, $P=0.006$).

Correlation between s IL-2R and serum ferritin

There was no significant correlation between s IL-2R and serum ferritin, $P>0.05$, in the non-splenectomized and splenectomized groups as shown in figure 6, and figure 7 respectively, using spearman rank linear correlation.

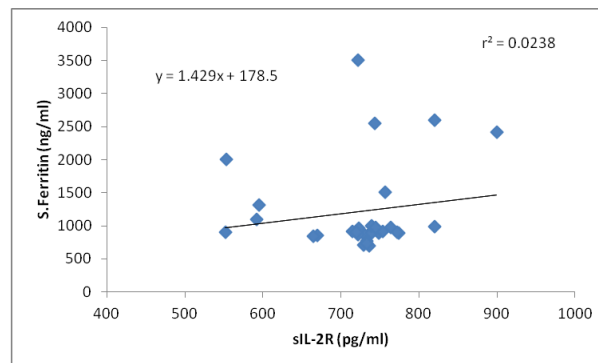


Figure 6: Correlation between serum ferritin level and s IL-2R in non-splenectomized Thalassemic patients ($r=0.154$, $r^2=0.0238$, $P=0.416$).

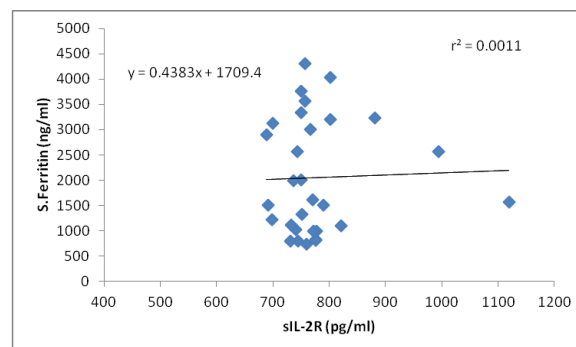


Figure 7: Correlation between serum ferritin level and s IL-2R in splenectomized Thalassemic patients ($r = 0.0364$, $r^2=0.0011$, $P=0.860$)

DISCUSSION:

The current study showed that the mean serum level of IL-8 was markedly increased above the reference range, reference range of IL-8 is <62 pg/ml, in both groups of thalassaemia. Moreover

IL-8 in both groups of thalassaemic patients was significantly higher than that of the control this result was in agreement with other workers^(5,14); this could be ascribed to the transfusion-related continuous antigenic stimulation and iron overload with consequent macrophage activation results in more IL-8 production in thalassaemic patients⁽⁵⁾. In the current study IL-8 was significantly higher in the splenectomized compared to the non-splenectomized, which may be attributed to the significant and permanent increase in both lymphocytes and monocytes, which are the source of IL-8, after splenectomy⁽¹⁵⁾.

Interleukin 2 receptor

The current study showed that the mean serum level of sIL-2R was not increased above the reference range, RR of s IL-2R is <8000 pg/ml, in both groups of thalassaemia, this was comparable with other studies^(5,16). It seems that IL-2 which also called T-cell growth factor and has important role in lymphoma and granuloma formation, but its role in thalassaemia is not clear⁽⁵⁾.

Serum ferritin

The current study showed that the mean of serum ferritin level was increased in both groups of thalassaemia, furthermore the mean of serum ferritin level in the splenectomized was significantly higher compared to the non-splenectomized, similar to the finding of other workers^(17,18,19), who found that splenectomized thalassaemic patients display higher serum ferritin levels, since after splenectomy there will be a potential shift of the splenic iron to extra splenic tissue, and further increase in iron overload should be borne in mind in considering removal of this organ. On the other hand Alan R et al, who found lower serum ferritin levels in splenectomized thalassaemic patients⁽²⁰⁾. This discrepancy between the results may be due to the use of ELISA for estimation of serum ferritin which is less sensitive than the MiniVIDAS instrument used in this study.

Correlation between IL-8 and serum ferritin

There was significant positive correlation between IL-8 and serum ferritin level in both groups of thalassaemia. Similar result were reported by Ozturk et al, who stated that IL-8 correlate with the ferritin level and that IL-8 might have contributed to the abnormalities in iron metabolism and it is probably due to overstimulation of macrophages⁽¹⁴⁾.

Correlation between s IL-2R and serum ferritin

In the current study there was no significant correlation between s IL-2R and level of serum

ferritin in both groups of thalassaemia.

CONCLUSION:

This study has shown that:

- Beta-thalassemia major patients had increased level of IL-8 which was more prominent in splenectomized patients, while no increase in IL-2R levels in both groups of Thalassaemia.
- IL-8 levels correlated with ferritin levels, while no significant correlation between IL-2R levels and ferritin levels in both groups of Thalassaemia.
- Splenectomized thalassaemic patients display higher ferritin levels compared to the non-splenectomized.

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