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Role of Serum Interleukin-10 and Interleukin-27 Levels in the Prognosis of Immune Thrombocytopenia in Iraqi Children

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Abstract

Background: Several studies provide evidence for a role of serum cytokines imbalance including IL-10 and IL-27 in immune thrombocytopenia pathogenesis and prognosis. The aim of this study was designed to investigate the role of serum levels of IL-10 and IL-27 in prognosis the efficiency of treatment in thrombocytopenic Iraqi children

Methods: This case controls study was carried out at Department of Biochemistry, College of Medicine, University of Baghdad, during the period from October 2023 to March 2024. It included 88 children, 63 children previously diagnosed with immune thrombocytopenia, and 25 apparently healthy children who served as control group. The included immune thrombocytopenic children were sub-grouped according to their treatment into three groups: Romiplostim group (group 1), Prednisolone group (group 2), Prednisolone and intravenous immunoglobulin (IVIG) or Prednisolone and mycophenolate group (group 3). Investigations included serum level measurements of IL-10 and IL-27 by using enzyme linked immunosorbent assay ELISA. Platelet count of each included children was measured by Huma Count 30 TS Human, Germany.

Results: The mean (±SEM) values of serum IL-10 and IL-27 levels of immune thrombocytopenic children were insignificantly lower than that of controls. In addition, there was non- significant differences in serum levels of IL-10 and IL-27 among and between the three groups of patient children. The mean value of platelet count of patient children was significantly increased by all types of treatment in whole immune thrombocytopenic children (117.48±18.15*10^9/L).

Conclusion: Measurement of serum IL-10 and IL-27 are helpful biomarker in prognosis of thrombocytopenia irrespective of type of treatment.

Keywords: Immune thrombocytopenic, IL-10, IL-27.

Introduction

Primary immune thrombocytopenia (ITP) is a megakaryocytic/platelet-specific autoimmune disorder characterized by isolated thrombocytopenia (platelet count $\leq 100 \times 10^9$/L) with or without bleeding manifestations, in the absence of other thrombocytopenia-associated conditions (1). The majority of patients present with bleeding signs such as petechiae, purpura, mucous membrane hemorrhages in the mouth and nose, urogenital bleeding, or increased menstrual bleeding (2). Primary immune thrombocytopenia (ITP) therapy aims to boost platelet count, limit bleeding, induce remission, and enhance the patient's quality of life. Corticosteroids are used as first-line therapy to reduce platelet destruction by preventing autoantibody formation and excessive cytotoxic T cell activity. Rescue therapies may also be employed, such as

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nonspecific intravenous immunoglobulins (IVIG) in severe bleeding cases and platelet transfusions or thrombopoietin receptor agonists (TPO-RAs) in life-threatening bleeding (3).

Corticosteroids (prednisone) have various intriguing effects in ITP. Prednisone lowers platelet clearance when given to volunteers who have received antiplatelet antibodies from ITP patients, which is assumed to be owing to decreased phagocytosis by the reticuloendothelial system (4).

One of the most recent therapeutic discoveries that has altered the landscape of chronic refractory ITP treatment is the advent of TPO-RAs. The most commonly utilized of these medications is romiplostim, which promotes megakaryopoiesis while simultaneously increasing platelet production (5). In 2018, the FDA authorized its use in children over the age of one who have had ITP for at least six months and have not responded well to corticosteroids, immunoglobulins, or splenectomy (6).

Interleukin 27 (IL-27), a pro-inflammatory and anti-inflammatory cytokine, is also involved in immunological regulation. Recent studies have shown that IL-27 may decrease inflammatory responses in T cell development and autoimmune disorders. Previous research found that in individuals with immunological thrombocytopenia, cytotoxic T-lymphocyte-mediated platelet disintegration was elevated whereas IL-27 levels were lowered (7). Multiple studies have shown that IL-27 may play a significant regulatory function by reducing acquired immunity, resulting in the formation of T helper cells and an increase in inducible regulatory T cells that release IL-10. IL-27 also suppresses Th17 cells, reducing inflammation (8). Interleukin 10 (IL-10) is a cytokine that reduces inflammation and inhibits immune responses (9) . Is an anti-inflammatory cytokine that modulates immunological function. and is generated by immune cells such as macrophages, T lymphocytes, and natural killer (NK) cells. It is a multifunctional immunoregulatory cytokine (10). Cytokines play various functions in innate immunity and inflammation (11). Macrophages, Th2 cells, and mast cells all release IL-10. Cytotoxic T cells also produce IL-10 to suppress the function of NK cells induced by viral infection (12–14). Activated macrophages, Th1, Th2, Th17, and T-reg cells all release IL-10, which plays an important role in the resolution of peripheral inflammation (15).

Interleukin 10 (IL-10) is a powerful anti-inflammatory cytokine produced by macrophages and T helper-2 type cells (Th2) that may inhibit cell-mediated immune effector pathways involved in the host's defense against intracellular infections (16). IL-10 reduces the production of pro-inflammatory cytokines, including granulocyte-macrophage colony-stimulating factor, interferon γ (IFN-γ), TNF-α, IL-3, and IL-2 (17,18).

Materials and Methods

The case control study was performed at the Department of Biochemistry, College of Medicine/University of Baghdad, and at Welfare Teaching Hospital, Medical City, Baghdad, Iraq, during the period from October 2023 to March 2024. The study encompassed a cohort of 63 children who had been previously diagnosed with immune thrombocytopeni control group and 25 apparently healthy children as controls. The age range of the participants spanned from 1 to 16 years, and they were on treatment. The first line of their treatment was prednisolone alone or in combination with intravenous immunoglobulin (IVIG). The second line of treatment is mycophenolate. The third line is Romiplostim.

This study was approved to be carried out by the scientific and ethical committees of the Department of Biochemistry, College of Medicine, University of Baghdad. Ethical approval was also obtained from the Welfare Teaching Hospital, Medical City, Ministry of Health. Verbal consent was obtained from the relatives of each of the included children to carry out the defined laboratory investigations of this study.

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Inclusion criteria involved children who have had immune thrombocytopenia, aged 1-16 year, of both sexes who are under treatment of prednisolone and intravenous immunoglobulin along with either mycophenolate or romiplostim.

All the children in this study had previously received an ITP diagnosis from registered hospitals, and we divided them into three groups based on their treatments: group 1 (Romiplostim group): It included 18 children who were treated with romiplostim; group 2 (the prednisolone group) included 19 children treated with prednisolone; and group 3 (prednisolone and IVIG, or prednisolone and mycophenolate) included 26 children treated with prednisolone and IVIG, or mycophenolate. The doses of treatment were defined by consultant pediatrician according to disease severity. The control group (Group 4) consisted of 25 apparently healthy children who did not suffer from acute immune thrombocytopenia or any acute or chronic illness.

The exclusion criteria included those patients who received chemotherapy or blood transfusions in the last month and had tumors, infections, malignant blood diseases, hypersplenism, hepatic, and renal diseases. Five milliliters (ml.) from the peripheral vein were aspirated from each patient and control child, left to clot for 15 minutes, and then centrifuged for 10 minutes at 698.75 G. The separated serum was stored at -45 °C until the day of measurements. The serum investigation included measurements of IL-10 and IL-27 using the semiautomatic ELISA Reader Huma Reader by Human Diagnostics German Company and the Washer (COMBIWASH) by Human German Company. The principle of the ELISA technique is based on the biotin double-antibody sandwich technology to assay human interleukin 10 (IL-10) and IL-27. Interleukins are added to the wells, which are pre-coated with the monoclonal antibody, and then incubated. After that, anti-interleukin antibodies labeled with biotin are added to unite with streptavidin-HRP, forming an immune complex. Unbound enzymes are removed after incubation and washing. Substrates A and B are then added. The solution turns blue and changes to yellow with the effect of acid. The shades of the solution and the concentration of human IL-10 or IL-27 are positively correlated. Platelet counts were measured using Huma Count 30 TS by Human, Germany.

For statistical analysis using SPSS version 25.0 software, frequency, percentage, mean, and standard error of mean (SEM) were used to describe the data used for all statistical analyses. ANOVA was used to evaluate the difference in the mean level of numeric data between more than two variables. Pearson correlation regression r was used to evaluate the correlation between numerical data. The significance levels were chosen at $p < 0.05$.

Results

The distribution of gender in ITP and control children's groups was matched. In ITP children (n=63) ,34 were females (54 %) and 29 were males (46 %), in control group, 13 out of 25 (52 %) were females and 12 (48 %) were males. Also, there was non-significant differences in gender distribution in each of ITP and control groups (Table 1).

The mean of age of ITP and control groups was matched and did not differ significantly among and between studied groups. The mean values of platelet count were significantly decreased in three groups of children with ITP compared to controls ($p < 0.05$). However, there was no significant differences among and between groups of patient children in mean value of platelet count, but with important fact that the mean values of the platelet count of the three patient groups were significantly increased after treatment compared to those at primary diagnosis.

The mean value of serum IL-10 concentrations of group 2 and group 3 were lower in comparison with that of control children but did not reach the significant level. Similarly, the mean (±SEM) value of IL-27 concentrations of the three groups of ITP children were lower than that of controls but did not reach the significant level. The mean values of IL-10 and IL -27 did not differ

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significantly among and between groups of patients (Table 2).

There was significant positive correlation between the values of age and duration of disease in Romiplostim group (r= 0.63, p=0.0047) and prednisolone and other treatment group (r=0.44, p=0.03). Duration of disease of romiplostim group was range from 1 to 156 month. There was significant correlation between platelet counts and duration of romiplostim treatment (r=0.69, p=0.0017).

Table 1. Mean (± SD) values of age and platelet counts of the three groups of patients controls.

Parameter	Group 1 (n=18)	Group 2 (n=19)	Group 3 (n=26)	Group 4 (n=25)
Age (years)^{NS}	8.56±1.02	5.47± 0.82	7.46± 0.77	8.32± 0.93
Platelets (count*10^9lL)	97.67±32.62	159.32±39.99	106.50± 25.37	376.16±7.51 *

ANOVA and t-test revealed *significant decrease of platelet counts in patient children's groups than in controls (for all, p=0.001), NS: Non- significant in age among and between studied groups.

Table 2. Mean (± SD) values of IL-10 and IL-27 concentrations of the three groups of patients and controls.

Parameter	Group 1 (n=18)	Group 2 (n=19)	Group 3 (n=26)	Group 4 (n=25)
IL-10 (ng/ml) ^{NS}	71.09± 9.25	55.28± 5.19	61.16± 7.22	67.18± 6.36
IL-27 (ng/ml) ^{NS}	121.33± 21.07	111.25± 21.14	130.72±20.94	150.80± 16.79

NS: Non- significant (p>0.05).

Discussion

The result of our study revealed that the incidence of ITP was more common in females than males. It has been found that the female gender was higher in chronic ITP compared to persistent and newly diagnosed patients, yet the difference didn't lead to a statistically significantly level (19). In a large meta-analysis, Heitink-Pollé et al. identified female gender as a predictor of chronicity (20).

In the present, the mean values of serum IL-10 of ITP groups were lower than that of controls, but did not reach the significant level which may be due to a relatively small sample size. Similarly, about IL-27 with the exception of being higher in Romiplostim group compared to controls. This finding agrees with another study which discovered that active ITP patients had reduced serum IL-10 levels than healthy controls and ITP patients in remission. Moreover, in patients with ITP Serum IL-10 concentrations associated positively with platelet counts, but there was no significant difference (17). In the present study, all the included ITP children were on treatment and their platelet counts were near or above 100 *10^ ^{9} /l which may reflect the remission of disease and may be the cause of non-significant differences found between ITP children and controls.

Although, the platelet counts of ITP children in the present study were significantly lower than those of controls. They showed a significantly higher elevation compared to the base line counts, at first diagnosis in each treatment group. Patients with newly diagnosed ITP had a significantly decreased platelet count compared to those with persistent and chronic ITP (19) .

In addition, the non-significant differences between the ITP children's groups and controls in serum IL-10 and IL-27 may indicate the effectiveness of the first line of treatment (prednisolone plus IVIG) and the second one,

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mycophenolate, in our patients. In agreement with this finding, Čulić et al. observed no significant difference between ITP patients and controls in terms of serum IL-10 levels and the second one, the mycophenolate in our patients. In agreement with this finding, Čulić et al. observed no significant difference between ITP patients and controls with regard to of serum IL-10 levels (21). However, other studies showed that patients had significantly higher levels of serum IL-10 than controls (19). This discrepancy from our findings may be attributed to the fact that most other studies included ITP patients who were newly diagnosed and had not yet started their treatment.

It has been found that IL-10 in chronic ITP patients was decrease to almost the normal plasma concentration seen in healthy controls, which is in agreement with the results of the present study (22–26) .

Interleukin 10 is an immunoregulatory cytokine. Its major biological role is to restrict and terminate inflammatory responses (27).

It has been concluded that IL-27 was significantly higher in ITP patients than healthy controls and in patients with de novo ITP compared to those in remission (28). These authors suggested using IL-27 as a predictor of disease occurrence and to a lesser extent for responsiveness to treatment.

The low platelet counts in the early stage of ITP cannot exert a sufficient anti-inflammatory response to dampen the proinflammatory cytokines, the TNF-α production. Therefore, the significantly increased levels of IL-10 in this stage and before and after IVIg administration could play a counteracting role, diminishing the effects of a high TNF-α (29). In children with acute ITP compared to chronic ITP patients, an increased plasma concentration of IL-10 has been shown to predict the clinical course of an ITP already at the onset of the disease (30).

One of the limitations of this study is the inability to include newly diagnosed children with ITP due to the limited number of cases encountered during the study period.

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It is concluded that measurement of serum IL-10 and IL-27 are helpful biomarker in prognosis of thrombocytopenia irrespective of type of treatment**.**

Limitation

Inability to include of newly diagnosed children with ITP because of limited cases that encountered during the time of study.

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Ethical Information and conflict of interest

The entire work had permitted by the Ethical Committees of local authorities. All participants provided an inscribed informed consent, and the research had conducted in line with the ethical morals identified in the 1975 treaty of Helsinki. The authors declare no potential conflicts of interest related to the present research.

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