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Evaluation of CD4/CD8 ratio in children with immune thrombocytopenic purpura (ITP) after treatment with intravenous immunoglobulin (IVIG)

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ABSTRACT

Intra Venous Immunoglobulin (IVIG) is a plasma-derived product used to treat many autoimmune diseases, including thrombocytopenia, immunodeficiency, and infectious diseases. In this study, the effect of IVIG injection was evaluated on the number of white blood cells, neutrophils, lymphocytes, and platelets. The effect of IVIG was also considered on the percentage of CD4 and CD8 positive cells T cell lymphocytes and their absolute number in pediatric patients with immune thrombocytopenic purpura. The study was a cross-sectional study performed on 32 patients with ITP. In these patients, a blood sample was taken before and one hour after the start of the IVG injection. For all samples, a complete blood cell, platelet count, and differential blood leukocyte count were performed by Sysmex kx-21. Then labeled anti-CD4 and anti-CD8 markers were used to evaluate the type of lymphocytes. SPSS software version 15 and a t-test with a significant level of $p < 0.05$ were used for statistical analysis of the obtained results. Pearson correlation coefficient was also used to evaluate the relationship between patients' age and the total volume of injected IVIG results. Examination of blood cell counts showed a significant decrease in the mean of white blood cells, neutrophils, and lymphocytes after intravenous immunoglobulin injection. However, these changes were not statistically significant for platelets. A comparison of the mean percentage of CD4 and CD8 cells shows a significant increase in the CD4 / CD8 cell ratio after injection. The absolute number of CD4 and CD8 lymphocytes one hour after IVIG injection was significantly decreased, but their proportion increased after injection. Generally, IVIG reduces the absolute number of neutrophils, but this reduction is not associated with infection problems. This decrease is also seen in the number of lymphocytes. However, the change in the number and percentage of CD4 and CD8 cells depends on the sampling time following IVIG injection.

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Introduction

Immune thrombocytopenic purpura is a disease mediated by the immune system due to the production of autoantibodies against platelet glycoproteins (mainly glycoprotein IIb/IIIa and, in rare cases, glycoproteins Ib/IX, Ia, and IIa platelets) (1). These antibodies cause platelet phagocytosis in the reticuloendothelial system, resulting in thrombocytopenia and hemorrhagic manifestations. Patients show symptoms such as petechiae, ecchymosis, and mucosal bleeding (2). In children, ITP usually occurs following a viral infection and resolves spontaneously in 80 to 90% of cases. The manifestations of thrombocytopenia in children are more severe than in adults (3). Steroids, intravenous immunoglobulin (IVIG), and anti-D immunoglobulin are commonly used for treatment in some common cases.

IVIG is a plasma-derived product used extensively in the treatment of autoimmune diseases. In addition, this product is used in immunodeficiency and infectious diseases. In autoimmune diseases, different functions are thought for IVIG, which can be attributed to the inhibitor of FC macrophages or its anti-idiotype properties (1). Recent studies have shown that IVIG can modulate immune system responses by altering the cytokine profile, affecting dendritic cells or T and B cells (4, 5).

So far, many efforts have been made to increase the safety of this product to reduce the risk of transmitting diseases such as hepatitis and AIDS. The use of IVIG, like many other medications, may be associated with side effects, including fever, chills, back pain, nausea,

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and flushing, which are usually transient and are related to the speed of IVIG injection (3). Although the cause is unknown, molecular aggregates, IgG, IgG dimers, or complement system activation appear to be involved (2).

Changes in hematological parameters and the percentage of CD4 and CD8 cells have been investigated in several studies (6). Most of these studies have focused on other autoimmune diseases such as Kawasaki syndrome, Sjogren's (SHOW-grins) syndrome, dermatomyositis, and immunodeficiency. Also, similar studies have been performed on ITP patients with fewer samples (4). Therefore, in this study, we decided to evaluate the effect of IVIG injection on the number of white blood cells, neutrophils, lymphocytes, and platelets in ITP patients. We also assessed the effect of IVIG on the percentage of CD4 and CD8 cells and their absolute number in these patients.

Materials and methods

Patients

This cross-sectional study was performed on 32 children with acute and chronic ITP, all of whom had been referred to a pediatric hospital for intravenous immunoglobulin. The mean age of patients was 3.96 years (2-12 years). Criteria for inclusion of these patients in the ITP diagnosis study were based on isolated thrombocytopenia in peripheral blood, along with the normalcy of other blood cells and the absence of systemic symptoms and bone marrow examination. Patients who tested positive for Coombs or anti-nuclear antibodies were excluded.

Sampling

A blood sample (2 ml peripheral blood) was taken from all patients in the CBC vial containing EDTA anticoagulant a few minutes before IVIG injection (400 to 1000 mg/kg). One hour after IVIG injection, sampling was repeated in the same way. All samples were examined immediately. During the study, three different IVIG products were used according to the manufacturer, which included Sand globulin (ZLBBchring, Switzerland), Ig VENA (KedrionS, p, Italy) and Ingrates (Biotest, Germany).

Study method on samples

First, a complete blood count was performed on all samples using a Sysmex kx-21 device. Differential white blood cell counts were also differentiated manually using Wright's stain. For flow cytometry, monoclonal antibodies labeled with FITC (fluorescein isothiocyanate) and RPE (ficuaritrin) were used, dual and anti-CD4 and CD8 antigens, respectively.

Monoclonal antibodies against these markers were obtained from Dako Cytomation Company. First, in Landa 50 polystyrene tubes, complete blood was poured containing EDTA anticoagulant, CD8 was inserted, and 5 LDs were released. Place at 4°C to 6°C for 20 minutes. At the end of this period, 100 lbs of reagent solution (reagent 1) were added to the previous amount, mixed, and kept at room temperature and in the dark for 10 minutes. At the end of this period, 1 ml of reagent solution (reagent 2) was added to the previous volume and again at room temperature and in the dark for 10 minutes. The tube was centrifuged (300 xg), gently separating the supernatant so that about 50 ul of the solution remained inside the tube. Then, 2 ml of PBS solution was added to the tube and mixed well. The sample was then centrifuged again at the same rate. The supernatant was discarded and re-suspended in 2 ml of PBS buffer.

The maximum time between sampling and performing the relevant experiments was about 24 hours of the study. An isotype control sample was also placed for each test, and the data were analyzed accordingly.

Data analysis

The stored data were analyzed by flow cytometry device with CellQuest software. Dead cells and monocytes were excluded from the study by appropriate gating and FCS / SSC adjustment. In the analysis of samples, at least 10,000 cells were evaluated.

The average (range) is the number of white blood cells, neutrophils, lymphocytes, and platelets given the average (range). The results related to the lymphocyte subtype (CD4 and CD8 positive cells) were reported as mean ± SD percentage.

Student t-test was used to evaluate the significance of changes before and after. In addition, the Pearson correlation coefficient was used to examine the

relationship between age and total volume of IVIG injected with the changes that occurred after IVIG injection.

Results

The results of changes in white blood cells, neutrophils, lymphocytes, and platelets before and one hour after treatment are shown in Table 1 and Figure 1. As the table shows, the mean number of white cells, neutrophils, and lymphocytes after treatment shows a significant decrease compared to before (p <0.001). However, changes in platelet count before and after treatment are not statistically significant (p> 0.05).

Table 1. Changes in the mean of white blood cells, neutrophils, lymphocytes, and platelets before and one hour after treatment with IVIG

Blood Cell	Before Injection	1 Hour After Injection	P-value
	Mean (range)	Mean (range)	
White blood cells	7003 (2900-12400)	5228 (2400-9900)	<0.001
Neutrophils	2814 (384-9920)	1915 (432-7296)	<0.001
Lymphocytes	3779 (1620-8466)	2927 (1364-6264)	<0.001
Platelets	40750 (4000-167000)	4371 (4000-188000)	0.377

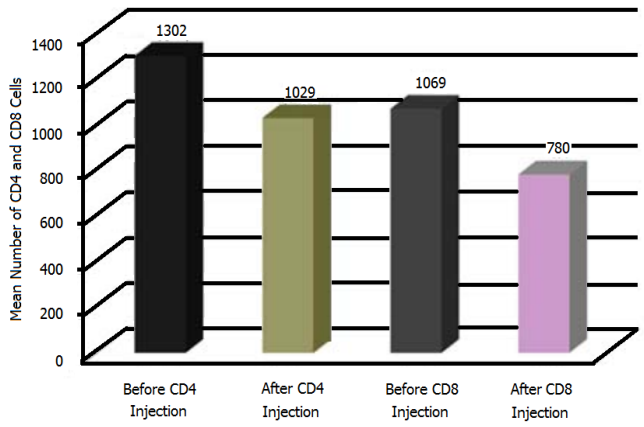

Figure 1. Changes in the mean of CD4 and CD8 lymphocytes before and one hour after treatment with IVIG

In 10 out of 32 patients (31%), the absolute neutrophil count decreased to less than 1500 cells per microliter. Contrary to expectations, platelet count was not always associated with an increase, and in 43% of patients (14 out of 32 patients), platelet count was associated with a decrease after intravenous immunoglobulin injection. Only three patients (21%) received IVIG for the first time, and in all other cases, patients had a history of IVIG. Out of 18 patients whose platelet count was increased after injection, 11 patients (61%) received IVIG for the first time, and only seven patients had a history of injection. Table 2 shows the mean percentage of CD4 and CD8 positive lymphocytes and the number of changes before and 1 hour after treatment. As Table 2 shows, the ratio of CD4/CD8 cells showed a significant increase one hour after treatment (p <0.001), and this increase was mainly due to a significant decrease in the percentage of positive CD8 cells (p = 0.001).

Table 2. Mean changes in the percentage of CD4 and CD8 cells and their ratio before and 1 hour after IVIG injection

Lymphocyte subtype	Before Injection	1 Hour After Injection	P-value
	Mean ± SD	Mean ± SD	
CD4	33.37 ± 6.48	34.44 ± 6.04	0.105
CD8	28.5 ± 8.43	26.6 ± 8.41	0.001
CD4/CD8	1.25 ± 0.37	1.38 ± 0.4	<0.001

The difference between the mean percentage of positive CD4 cells before and 1 hour after IVIG injection was not statistically significant (p> 0.05). Among all the samples tested in 4 patients, the ratio of CD4 / CD8 cells was associated with a decrease due to an increase in the percentage of $CD8^+$ cells in these patients. Still, the increase in platelet count was not significant in these four patients. But in general, due to the decrease in the absolute number of lymphocytes after intravenous immunoglobulin injection, the total number of CD4 and CD8 cells was also associated with a reduction. The number of $CD4^+$ cells is significantly reduced compared to before treatment.

In addition, this decrease in the number of CD8 + cells is also seen (p <0.001), but the ratio of these cells is associated with a significant increase compared to before IVIG injection (p <0.001). The correlation between total IVIG volume injected and white blood cell count was inversely estimated (p <0.05 and r = -0.419). Also, the relationship between age and percentage of CD8 cells was estimated directly before (p <0.05, R = +0.388) and after (R = +0.507) (p <0.05) IVIG injection.

Discussion

One of the most common treatments for ITP disease is IVIG, which has been reported in some

studies with side effects such as neutropenia. In addition to ITP, intravenous immunoglobulin is used in many autoimmune and immunodeficiency diseases (7). In the present study, which was performed on 32 children with acute and chronic ITP, the absolute number of neutrophils and lymphocytes after IVIG injection showed a significant decrease compared to before treatment. Thus, about CD4/CD8 cells, one hour after intravenous injection of immunoglobulin, there was a significant increase, and in most cases, this increase was due to CD8+. However, the percentage of CD4+ cells did not show significant changes.

Although a slight increase in the mean percentage of these cells was observed after injection due to the decrease in absolute lymphocyte count, the total number of CD4 and CD8 cells also decreased after intravenous immunoglobulin injection, possibly due to the presence of antibodies against lymphocyte antigens or Induction of apoptosis in them may be the reason for this.

A study by Baucom *et al.* (8) performed on 14 TP patients reported a decrease in the absolute number of neutrophils after injection. Another study by 49 ITP patients with Paulsen *et al*. (9) found similar results, but the use of anti-D did not cause such a cell reduction. Even *in vitro* studies have shown the use of intravenous immunoglobulin to degranulate and induce apoptosis in neutrophils. Still, no problems with infection have been reported in any of the patients (10-13).

In the present study, no infectious problems were reported in any patients following IVIG injection or a few days after. Anti-neutrophil antibodies or induction of apoptosis in neutrophils may be the reason for this decrease. Regarding the decrease in the number of lymphocytes, several in vitro studies have shown that the use of intravenous immunoglobulin can induce the process of apoptosis in lymphocytes. Antibodies against lymphocyte antigens in IVIG can also cause this decrease.

In a study by Ge *et al*. (14), patients with ITP in whom changes in the CD4 and CD8 lymphocyte subtypes were studied on the first day after IVIG injection, the CD4 / CD8 cell ratio showed a significant reduction compared to before treatment. The decrease was due to an increase in the percentage of CD8 + cells. In addition, the number of platelets in these patients was associated with an increase.

In a study by Langrish *et al*. (15), which was performed on immunocompromised patients, the reduction in CD4/CD8 cell counts twenty hours after IVIG administration was also statistically significant. However, in Li *et al*. study (16) of patients with hypogammaglobulinemia immediately after injection, no changes in the percentage and ratio of CD4/CD8 cells were reported. Therefore, it can be concluded that in the short run, the proportion of these changes or their intensity may be different from what is observed in the long run (17). Studies of cytokine profiles have shown that intracellular TNF-α expression increases after IVIG and anti-D injection (18-20).

In addition, this cytokine can induce cellular apoptosis (21). Therefore, one of the mechanisms that may reduce the percentage of CD8+ cells in the induction of apoptosis in them is due to IVIG injection and mediated by cytokines. Ig CMV has also been shown to induce apoptosis in CD8 + and NK cells (22).

According to the results of this study and in comparison with the data of previous studies, it can be concluded that the use of intravenous immunoglobulin in most cases leads to a decrease in the number of neutrophils. Of course, there are few studies on the number of lymphocytes.

Changes in lymphocyte subgroups vary somewhat depending on the type of disease or the time of sampling following IVIG injection. In this study, the effect of immunoglobulin was studied one hour after the end of the injection. Due to limited access to patients and their hospitalization (due to financial and urban problems), they were able to follow them and study the return of cell count to The limit was not normal, and only in 11 patients, the percentage of CD4 cells and 24 CD8 cells were followed up hours after injection. It may be possible to obtain better information about the function of immunoglobulin in lymphocyte cells by following the response to treatment over a more extended period.

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Authors' contribution

This study was done by the authors named in this article, and the authors accept all liabilities resulting from claims which relate to this article and its contents.

Conflicts of interest

There are no conflicts of interest.

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Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request.

Statements and Declarations

The author declares that no conflict of interest is associated with this study.

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