Effects of high-dose methylprednisolone therapy on lymphocyte subtypes in patients with acute immune thrombocytopenic purpura

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ABSTRACT

The aim of this study was to determine the effect of high-dose methylprednisolone (HDMP) on lymphocyte subtypes, CD4/CD8 ratio and clinical efficacy of the treatment in children with acute immune thrombocytopenic purpura (ITP). The study consisted of 21 children (aged between 1.5-14 years) with ITP treated with HDMP for 7 days. Absolute lymphocyte count, CD4+ and CD8+ T lymphocyte levels were examined on peripheral blood and CD4/CD8 ratio was calculated before and after HMTP treatment (on 0 and 8th days) in all subjects. There was no statistically significant difference for age and sex between the study and the control group. A significant reduction was observed in the percentage of CD4+ lymphocyte (39.0 ± 7.5% vs 29.3 ± 8.1%, p= 0.001), CD8+ lymphocyte (27.1 ± 7.2% vs 23.7 ± 8.3%, p= 0.03), CD4+/CD8+ (1.5 ± 0.5 vs 1.3 ± 0.4, p= 0.02) and the absolute number of CD4+ lymphocyte count (1694.99 ± 1019 vs 1199.12 ± 612, p= 0.038). These findings indicated that HDMP treatment may cause a decrease in the percentage of CD4+ and CD8+ T-lymphocyte and ratio of CD4+/CD8+ lymphocyte in patients with acute ITP. We suggest that the effectiveness of steroids may depended upon the suppression of CD4 T-lymphocyte and sequential monitoring of circulating lymphocyte subtypes may be used to predict the clinical effects of steroid treatment.

Key Words: Immune thrombocytopenic purpura, Lymphocyte subtypes, Steroid.

ÖZET

Akut immüntrombositopenik purpuralı hastalarda yüksek doz metilprednizolon tedavisinin lenfosit alt tipleri üzerine etkisi

Bu çalışmanın amacı; akut immüntrombositopenili (İTP) çocuklarda kullanılan yüksek doz metilprednizolon (YDMP) tedavisinin lenfosit alt tiplerine, CD4/CD8 oranına etkisini ve klinik etkinliğini saptamaktır. Bu çalışma yedi gün süreyle YDMP ile tedavi edilen, yaşları 1.5-14 yıl arasında değişen 21 çocuğu kapsamaktadır. Tanı anında ve YDMP tedavisinden sonra absolü lenfosit sayısı, CD4+ ve CD8+ T-lenfosit sayısı çalışıldı ve CD4/CD8

oranı hesaplandı. Çalışma grubu ve kontrol grubu arasında yaş ve cinsiyet açısından anlamlı fark yoktu. YDMP tedavisi sonunda CD4+ lenfosit yüzdesinde (%39.0 ± 7.5 vs %29.3 ± 8.1, p= 0.001), CD8+ lenfosit yüzdesinde (%27.1 ± 7.2 vs %23.7 ± 8.3, p= 0.03), CD4/CD8 oranında (1.5 ± 0.5 vs 1.3 ± 0.4, p= 0.02) ve absolü CD4 lenfosit sayısında (1694.99 ± 1019 vs 1199.12 ± 612, p= 0.038) anlamlı azalma görüldü. Bu bulgular göstermiştir ki akut İTP'li hastalarda YDMP tedavisi CD4+ ve CD8+ T-lenfosit sayısını ve CD4/CD8 lenfosit oranını azaltmaktadır. Steroid etkisinin CD4 lenfositlerinin baskılanmasına bağlı olabileceğini ve steroid tedavisinin etkinliğinin belirlenmesinde lenfosit alt tiplerinin monitörizasyonunun kullanılabileceğini düşünüyoruz.

Anahtar Kelimeler: İmmüntrombositopenik purpura, Lenfosit alt tipleri, Steroid.

INTRODUCTION

Immune thrombocytopenic purpura (ITP) is a disorder characterized by antiplatelet antibody production resulting in destruction of platelets by the reticuloendothelial system. It has been shown that antiplatelet antibodies detected in sera of patients with ITP belong to IgG and that their major target antigen is platelet membrane glycoprotein IIb/IIIa (GpIIb/IIIa)[1]. Recently, Yamanouchi and colleagues have reported the presence of GpIIb/IIIa-specific and HLA-DR-restricted CD4+ T cells that have the potential to help antiplatelet antibody production by B cells[2].

Steroids can modulate both peripheral blood lymphocyte subsets and immune responses[3]. In the literature a few studies have been reported about the effect of high-dose steroid therapy on lymphocyte subtypes in various diseases[4-6]. No study was reported about its effect on lymphocyte subtypes in acute ITP. In this study we evaluated the effect of high-dose methylprednisolone (HDMP) treatment on peripheral blood T lymphocyte subsets, CD4+/CD8+ ratio, and clinical efficacy of the treatment in children with acute ITP.

MATERIALS and METHODS

The study consisted of 21 children aged between 1.5-14 years (13 males and 8 females) with acute ITP and 21 healthy age-matched control subjects. At the time of diagnosis, all patients met the criteria of ITP: platelet counts less than 100 x 10^9/L; normal or increased number of megakaryocytes in otherwise normal bone marrow; and no other cause of peripheral thrombocytopenia, such as disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, connective tissue disease, or hypersplenism. A written informed consent from the parents was obtained before the study.

All patients with acute ITP received methylprednisolon (30 mg/kg peroral single dose a day for 3 days and 20 mg/kg for 4 days). During the treatment blood pressure and pulse rate were measured at least twice a day. Before HDMP treatment and on the 3^{rd}, 5^{th}, 8^{th} day of the therapy hemoglobin and hematocrit levels, white blood cell counts, red blood cell counts and platelet counts were studied. In all subjects absolute lymphocyte count, CD4+ and CD8+ levels were studied in peripheral blood and CD4/CD8 ratio was calculated before and after HDMP treatment (0 and 8^{th} days). The results were compared with those of the control group.

Complete blood counts were performed by using an automatic analyzer (STKS Coulter) and manual differential counts were performed on Wright-stained PB smears. The numbers of CD4+ (inducer/helper T-cells), CD8+ (cytotoxic/suppressor T-cells) were determined in anticoagulated peripheral venous blood samples. The ratio of CD4 positive cells and CD8 positive cells in peripheral blood was determined by using anti-CD4 (IgG1-

FITC) monoclonal antibody and anti-CD8 (IgG1-PE) monoclonal antibody (Immunotech Kid No: PN IM0747) in flow cytometry tool (Coulter Epics XL flow cytometer, France).

Comparisons of quantitative data were performed by using parametric tests (one way analysis of variance and unpaired Student's t-test). Patients characteristics were evaluated by using Chi-square analysis or the Fisher exact test. P values < 0.05 were considered as significant.

RESULTS

Twenty-one children (13 males and 8 females) with acute ITP were included in this study. The mean age of the patients was 6.14 ± 3.45 years (1.5-14 years). The mean interval between the onset of the symptoms and the diagnosis were 12.8 ± 5.3 days (3-21 days). Twenty-one healthy children (11 males end 10 females) were included as a control group. The mean age of the control subjects was 6.81 ± 3.92 years (ranged between 2-15 years).

Before the treatment, anemia was found in 47% (10/21) ITP patients. After the treatment, the number of patients with anemia was diminished to 40% (4/10). Hemoglobin levels, red blood cells and platelet counts were significantly increased after HDMP treatment (p< 0.05). A significant increase was also observed in white blood cell count after the treatment (Table 1). On the blood smear, neutrophil percentage was increased (50.14 ± 13.7% vs 72.9 ± 10.7%, p= 0.001) and lymphocyte percentage was reduced significantly (48.66 ± 13.74 vs 27.09 ± 10.7, p= 0.001).

Before the treatment, the percentage of CD4+ and CD8+ lymphocytes and the ratio of CD4+/CD8+ were not different between the study and control groups. After HDMP treatment a significant reduction was observed in the percentages of CD4+ (p= 0.001) and CD8+ (p= 0.03). A significant reduction was also observed in the ratio of CD4+/CD8+ (p= 0.02). Before HDMP treatment absolute numbers of CD4+ T-lymphocytes was 1694.99 ± 1019/mm^3 and CD8+ T-lymphocytes was 1135.07 ± 623/mm^3. After HDMP treatment absolute number of CD4+ T lymphocytes decreased to 1199.12 ± 612/mm^3 (p= 0.038) and CD8+ T-lymphocytes decreased to 961.80 ± 610/mm^3 (p= 0.232), total lymphocyte number was reduced after HDMP treatment but this reduction was not significant (p= 0.192) (Table 2).

DISCUSSION

ITP is an autoimmune disease characterized by increased platelet clearance caused by antiplatelet autoantibodies, which bind to circulating platelets, resulting in destruction by the reticuloendothelial system[7]. Altho-

Table 1. Hematological parameters in the study group's patients

DAYS	Hb (g/dL) (mean ± SD) (range)	Red blood cell (count/mm^3) (mean ± SD) (range)	Leukocyte (count/mm^3) (mean ± SD) (range)	Thrombocyte (count/mm^3) (mean ± SD) (range)
0	11.30 ± 1.57 (9.10-14.90)	4479048 ± 579766 (3410000-5350000)	8661.90 ± 2841 (4100-14700)	9571 ± 8053 (1000-29000)
3	10.77 ± 1.20 (8.50-13.60)	4245500 ± 468092* (3360000-4990000)	9485 ± 2512 (4600-16200)	27400±29546* (2000-136000)
5	11.27 ± 1.41 (7.60-13.80)	4475000 ± 486464 (3390000-5350000)	12180 ± 3841* (5000-22800)	112950 ± 136379* (1000-602000)
8	12.03 ± 1.46* (9.10-14.60)	4738952 ± 514840* (3778000-5450000)	16080 ± 6897* (5700-33000)	173571 ± 136845* (5000-478000)

* Compared to before the treatment p< 0.05.

Table 2. Lymphocyte subtypes changes with treatment

Parameters	Before treatment	After treatment	Control	P b-c	P a-c	P b-a
Total lymphocyte (/mm^3)	4428.2 ± 2448	3905.7 ± 1692	4121.5 ± 231	0.12	0.322	0.192
CD4 (%)	39.0 ± 7.5	29.3 ± 8.1	44.07 ± 8.81	0.057	0.000	0.001
CD8 (%)	27.1 ± 7.2	23.7 ± 8.3	28.26 ± 6.99	0.616	0.064	0.03
CD4/CD8	1.5 ± 0.5	1.3 ± 0.4	1.68 ± 0.71	0.540	0.062	0.020

P b-c: P value between the patient group and control group before HDMP treatment.
P a-c: P value between the patient group and control group after HDMP treatment.
P b-a: P value between the patient group before and after HDMP treatment.

ugh earlier studies reported the presence of platelet reactive T-cells in patients with ITP[8,9]. Kuwana et al found that is one of the major target antigens recognized by platelet-reactive CD4+ T-cells[10]. GpIIb/IIIa reactive CD4+ T-cells in patients with ITP have a helper activity that promotes the production of anti-GpIIb/IIIa antibodies capable of binding to normal platelets, indicating that these autoreactive T-cells are involved in the production of pathogenic antiplatelet autoantibodies in patients with ITP. In our study, the initial percentages of CD4+ and CD8+ T-cells were not different between the study and control groups. Koyanagi et al reported that children with acute ITP had no significant difference in serum immunglobulin, CD3, CD4, CD8, CD38+ T-lymphocytes and CD4/CD8 ratio compared to control group[11]. However, when lymphocyte subtypes and serum complement levels were compared with control group, significant decreases were found in other two studies[12,13].

Corticosteroids could modulate both peripheral blood lymphocyte subsets and the immune response[3]. Conventional doses of corticosteroids were known to increase the number of leukocyte count and reduced the number of lymphocyte, eosinophile and monocyte counts[14]. The effects of conventional doses of steroids on lymphocytes were explained by redistribution of lymphocytes out of circulation. Some of the intravascular lymphocytes, mostly T-lymphocytes, were readily shown to migrate to extravasculer site. Tornatore et al reported that definite suppression pattern for total CD4, CD8 lymphocytes were noted after methylprednisolone exposure in young and elderly males[15]. Zweiman et al reported the same suppression on circulating CD4 lymphocyte count in normal humans[16].

HDMP treatment has been used as a therapeutic choice in childhood acute ITP in Turkey for a long time. The effects of HDMP on lymphocyte subtypes were investigated in the hematological and non-hematological diseases except ITP, and contraversial results were reported. Hogevold et al reported that HDMP was given before and 4-12 hours after the total hip replacement surgery[4]. Eight hours after the last steroid dose, reduction of CD4+ and CD8+ T lymphocytes level was recorded. Ten Berge et al showed that significant reduction of CD3, CD4, CD8, total number of absolute T lymphocytes and CD4/CD8 ratio were found 6 hours after the administration of 10 mg, 30 mg and, 60 mg of prednisolone[5]. Verbruggen et al found similar results 6 hours after the administration of 10 mg prednisolone on rheumatoid arthritis patients[14]. Tuncer et al reported that patients with chronic ITP, received 30 mg/kg HDMP treatment for 3 days and 20 mg/kg for 4 days[6]. After the treatment total lymphocyte, absolute numbers of CD4+ and CD8+ lymphocytes were increased, but no significant difference was seen in CD4+/CD8+ ra-

tio. Fukuda et al found that the number of CD4+ cells after HDMP treatment correlated with the clinical efficacy of the treatment[17].

In our study, although the absolute number of CD4+ and CD8+ lymphocyte counts were decreased after HDMP treatment, the reduction was statistically significant only for CD4+ levels. CD4+/CD8+ ratio was significantly reduced. There was no correlation between platelet count and the absolute number of CD4+ and CD8+ T-lymphocytes.

Enhanced T helper cell (CD4+ lymphocyte) interactions in patients with ITP were the primary stimulus for the development of antiplatelet antibody production[18]. We suggest that the effectiviness of HDMP may depend upon the suppression of CD4 T-lymphocyte and sequential monitoring of circulating lymphocyte subtypes may be used to predict the clinical effects of steroid treatment.

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