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Coagulation parameters in children with acute immune thrombocytopenic purpura

Akut immun trombositopenik purpura tanılı çocuklarda koagulasyon parametreleri

Balkan C Kavaklı K Karapınar D

Ege Üniversitesi Tıp Fakültesi Çocuk Sağlığı Anabilim Dalı-Bornova,İZMİR

Summary

Immune thrombocytopenic purpura (ITP) is one of the most common causes of hemorrhagic diathesis during childhood. In this study, we investigated whether there is a change in the coagulation parameters and the effects of different treatment regimens on these parameters in children with acute ITP. Twenty one acute ITP patients were enrolled in the study. Patients were treated randomly with either intravenous immune globulin (IVIG) or intravenous pulse-methylprednisolone (PMP). Bleeding time (BT), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen, von Willebrand factor: ristocetin cofactor activity (vWF:RCoF) were tested at diagnosis. Protein C (PC), free-protein S (f-PS), antithrombin (AT), D-dimer (DD), prothrombin fragment 1+2 (PF 1+2) and lupus anticoagulant (LA) were measured in the patients at diagnosis and after the treatment. Adequate platelet levels were obtained more rapidly in patients treated with IVIG than with PMP. At diagnosis, while AT and PF 1+2 levels were found significantly higher (p=0.0001) in the patients, DD level was significantly lower (p=0.0001). Nine patients (41 %) had LA positivity at diagnosis. After treatment, levels of PC, f-PS and AT increased significantly in PMP group when compared to IVIG group (p=0.004, 0.001, 0.017 respectively). These data show that there are some reactive changes in coagulation and fibrinolytic systems in addition to thrombocytopenia in acute ITP. Different therapy modalities may affect these parameters differently.

Key Words: Immune thrombocytopenic purpura, coagulation, children

Özet

İmmun trombositopenik purpura (İTP) çocukluk çağındaki en sık hemorajik diyatez sebeplerinden biridir. Bu çalışmamızda, akut İTP'li çocuklarda koagulasyon parametrelerinde bir değişiklik olup olmadığını ve uygulanan farklı tedavi modellerinin bu parametreler üzerine olan etkisini araştırdık. Akut İTP tanısı alan 21 hasta bu çalışmaya dahil edildi. Hastalar randomize bir şekilde intravenöz immun globulin (İVİG) veya yüksek doz -pulse- metil prednizolon (PMP) ile tedavi edildiler. Tanı anında tüm hastalarda kanama zamanı (KZ), protrombin zamanı (PZ), aktive parsiyel tromboplastin zamanı (APTZ), fibrinojen ve von Willebrand Faktör: Ristosetin Kofaktör Aktivitesi (vWF:RCoF) çalışıldı. Ayrıca tanıda ve tedavi sonrasında tüm hastalarda protein C (PC), serbest protein S (f-PS), antitrombin (AT), D-dimer (DD), protrombin fragman 1+2 (PF 1+2) ve lupus antikoagulanı (LA) test edildi. İVİG ile tedavi edilen hasta grubunda güvenli trombosit sayılarına PMP grubuna göre daha hızlı ulaşıldığı görüldü. Tanı anında hastaların AT ve PF 1+2 düzeyleri sağlıklı gruba göre anlamlı olarak daha yüksek iken (p=0.0001) DD düzeyleri anlamlı olarak düşük (p=0.0001) bulundu. Tanı anında 9 hastada (% 41) LA pozitif bulundu.

Yazışma Adresi: Can Balkan
Ege Üniversitesi Tıp Fakültesi Çocuk Sağlığı Anabilim Dalı,Bornova-İZMİR
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Tedavi sonrasındaki PC, f-PS ve AT düzeyleri İVİG grubu ile karşılaştırıldığında PMP grubunda anlamlı olarak daha yüksek bulundu (sırasıyla p=0.004, 0.001, 0.017) Bu veriler akut İTP'li çocuklarda trombositopeni yanında koagulayon ve fibrinolitik sistemde de bazı reaktif değişikliklerin olduğunu göstermektedir. Değişik tedavi modellerinin bu parametreler üzerindeki etkileri farklı olabilmektedir.

Anahtar Kelimeler: *İmmun trombositopenik purpura, koagulasyon, çocuk*

Introduction

Immune thrombocytopenic purpura (ITP) usually occurs in healthy, 2 to 10 years old children of either sex (1) and frequently follows a viral infection. Characteristic features include bruising, petechial rash, isolated and often severe thrombocytopenia, and megakaryocytic hyperplasia. In approximately 90 % of children, ITP is an acute, self limiting disease that resolves within 6 months whether or not therapy is instituted (2). However, management of children with acute ITP is controversial, and for years physicians have debated the relative merits of therapy versus no therapy. Intracranial hemorrhage occurs in approximately 1 % of children with ITP who have platelet counts below 20 x 10^9/L. Prior to 1981, the only effective treatment options available to increase platelet counts in patients with ITP were corticosteroids and splenectomy. In recent years, intravenous immunoglobulin (IVIG) and intravenous Rh immunoglobulin have demonstrated efficacy comparable to that of corticosteroids for increasing platelet counts in ITP (3). The main objective of any form of treatment in acute ITP is to raise the platelet count rapidly to a safe level, thereby reducing the risk of intracranial hemorrhage. Oral (4) or IV (5) corticosteroids and/or IVIG (6) are the most common treatments to accomplish this goal.

During acute ITP episodes, many patients have platelet counts lower than 20 x 10^9/L. However, severe bleeding is rarely reported despite very low platelet counts (7). Studies performed in pediatric patients with acute ITP indicate some changes in coagulation parameters which can be interpreted as compensatory mechanisms to maintain hemostasis (8, 9). This preliminary study was designed to investigate the coagulation, fibrinolysis and hypercoagulability parameters before and after the treatment in two patient groups which received either IVIG or intravenous pulse-methylprednisolone (PMP) treatment regimens.

Materials and Methods

Twenty one acute ITP patients with ages 2 - 16 years were enrolled in this study between October 1999 and June2002. During this period these patients were treated randomly with either IVIG or PMP. Written consent was obtained from all of the patients. At diagnosis, the platelet counts in all patients were < 30 x 10^9/L. In 18 patients, the platelet counts were ≤ 20 x 10^9/L. While 11 patients were treated with IVIG, 10 were treated with PMP. The characteristics of patients in two treatment groups are shown in table 1.

Table 1. Clinical and laboratory characteristics of patients.

	IVIG	**PMP**
Number of patients	11	10
Mean age in year (range)	5.6	7.9
	(2.0-12.5)	(3.0-16.0)
Male / Female	4 / 7	4 / 6
Platelet count (10^9/L) (range)	16.4	11.4
	(4.0-27.1)	(1.3-21.8)

IVIG, intravenous immunglobulin; PMP, intravenous pulse-methylprednisolone.

The number of patients, their ages, sexes and the platelet counts before treatment were similar in both groups.

The diagnosis of ITP was based on history, physical examination, thrombocytopenia and an increased number of megacaryocytes in the bone marrow. Bone marrow examination was performed in all patients before the initiation of therapy. Hepatosplenomegaly and lymphadenopathy were not observed and there was no recent history of drug ingestion including aspirin. These patients did not show any symptom of viral infection at diagnosis.

IVIG was administered at a dose of 1 g/kg/d over 6 hours for 2 consecutive days. PMP was administered at a dose of 30 mg/kg/d (max. 1 g) for 3 days, then 20 mg/kg/d for 4 days over 30 minutes (5,8). Vital signs were monitored during the period of therapy.

Hemogram, bleeding time (BT), prothrombin time (PT), activated partial thromboplastin time (APTT), fibrinogen and von Willebrand factor: ristocetin cofactor activity (vWF:RCoF) were performed in all patients at diagnosis (day 0). Hemogram was repeated on days 1, 2, 3, 7, 14, 21 and 30. Protein C (PC), free-protein S (f-PS), antithrombin (AT), D-dimer (DD), prothrombin fragment 1+2 (PF 1+2) and lupus anticoagulant (LA) were also tested on days 0 and 3 in all patients.

BT was tested by Ivy method using Simplate ®. Hemogram was performed by Cell-Dyne 3700 Automatic Counter (Abbott / Haveltown, PA / USA) in venous blood containing EDTA. Standard citrated blood samples were obtained for coagulometric measurements on days 0 and 3. Fibrinogen, PT, APTT, AT, PC, f-PS, LA and DD were measured with IL-Test™ kits (Instrumentation Laboratory / Milano, Italy). All of these measurements were performed by ACL-Futura-Plus Coagulometer (Instrumentation Labarotory / Milano, Italy). vWF:RCoF was tested with Chrono-Log Agregometer (Haveltown, PA / USA). Measurements of PF 1+2 was performed by ELISA and read on a Biotek ELISA Plate Reader. PF 1+2 levels were measured by using Enzygnost ® kits (Dade-Behring / Germany).

Data from 65 healthy children were used as the control group. The results were compared to those of healthy children. Values from the IVIG and PMP treatment groups were compared to control and to each other by means of non-parametric Mann Whitney U Test. The results from the same group obtained on different days were compared by using non-parametric Wilcoxon Ranked Sign Test. Statistical analysis was performed using SPSS WIN 6.0 Software. The results are expressed as mean ± standard deviation. P value of < 0.05 was considered as statistically significant.

Results

The BT was prolonged in 20 patients (90 %). These values returned to normal on day 3 in all patients. Fibrinogen PT, APTT, and vWF:RCoF which were tested before the therapy were normal in all patients.

Platelet counts were < 30 x 10^9/L before the therapy in all patients. There was no statistical difference between IVIG and PMP groups before the treatment. Platelet counts of 8 patients (73 %) in IVIG group and 9 patients (90 %) in PMP group were ≤ 20 x 10^9/L. The mean platelet counts of the two groups observed on therapy days are shown in figure 1.

The platelet counts in IVIG group were significantly higher (p=0.01) than the PMP group on day 2. However, there was no significant difference on days 1, 3, 7 and 30. Coagulation and fibrinolysis parameters of the control group and treatment groups before the treatment are shown in table 2.

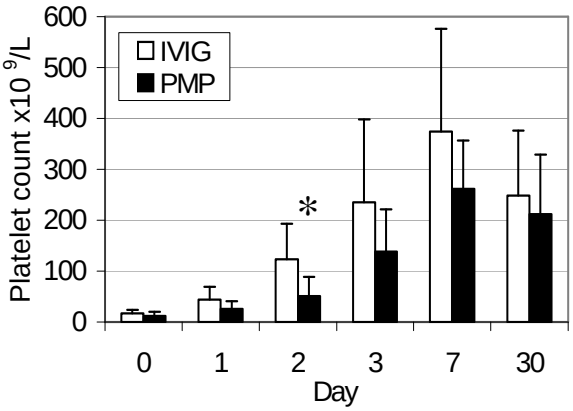

Figure 1. *Mean platelet counts on the treatment days. Mean platelet counts of IVIG and PMP groups on different treatment days compared by Mann Whitney U Test.*
***** *The platelet counts were significantly higher in IVIG group on day 2 (p=0.01).*
IVIG, intravenous immunglobulin; PMP, intravenous pulse-methylprednisolone.

Table 2. Coagulation and fibrinolysis parameters of the patients and control group before the treatment (mean ± SD).

Parameter	Patients (n=21)	Control (n=65)	Statistics (p value)
PC (%)	91.8 ± 25.3	93.3 ± 18.9	0.62
f-PS (%)	81.3 ± 14.0	65.4 ± 22.3	0.95
AT (%)	135.9 ± 40.1	104.0 ± 16.6	0.0001
DD (µg/ml)	0.231 ± 0.13	0.466 ± 0.18	0.0001
PF 1+2 (nmol/l)	7.86 ± 3.64	3.28 ± 3.21	0.0001
LA [#]	9	0	-

PC, protein C; f-PS, free protein S; AT, antithrombin; DD, D-dimer; PF 1+2, prothrombin fragment 1+2; LA, lupus anticoagulant.

[#] *Total number of patients with LA positivity.*

While there was no significant difference in PC and f-PS levels between the patients and the control group, AT levels were significantly higher (p=0.0001) in patients at

diagnosis. While DD levels of patients were significantly lower (p=0.0001) than the control group, PF 1+2 levels were significantly higher (p=0.0001) at diagnosis. At diagnosis, LA was positive in 9 (41 %) patients.

The results of two therapy groups before the treatment and on day 3 are given in table 3. On the third day of treatment PC, f-PS and AT levels were significantly higher in PMP group than IVIG group (p=0.004; 0.001; 0.017 respectively) Four of the 9 LA positive patients were treated with IVIG and LA positivity disappeared on the third day of treatment. Remaining 5 patients received PMP and LA positivity disappeared on third day in 3 patients and on day 30 in 2 patients. There was no significant difference in other parameters between two treatment groups.

Table 3. Coagulation and fibrinolysis parameters in two treatment groups before and after the treatment (mean ± SD).

Parameter	**IVIG** (n=11)		**PMP** (n=10)	
	Day 0	**Day 3**	**Day 0**	**Day 3**
PC (%)	92.4 ± 27.1	82.9 ± 21.8 *	92.3 ± 25.7	120.8 ± 40.1 *
f-PS (%)	77.0 ± 8.1	65.5 ± 9.9 **	88.1 ± 16.2	97.9 ± 18.4 **
AT (%)	136.5 ± 43.2	126.9 ± 21.6 ***	135.7 ± 41.0	159.8 ± 54.9 ***
DD (μg/ml)	0.195 ± 0.17	0.236 ± 0.24	0.234 ± 0.28	0.151 ± 0.14
PF 1+2 (nmol/l)	7.89 ± 4.01	9.33 ± 2.95	7.94 ± 3.64	8.96 ± 4.59
LA #	4	0	5	2

IVIG, intravenous immunglobulin; PMP, intravenous pulse-methylprednisolone; PC, protein C; f-PS, free protein S; AT, antithrombin; LA, lupus anticoagulant; DD, D-dimer; PF 1+2, prothrombin fragment 1+2.

 # Total number of patients with LA positivity.

** (p = 0.004), ** (p = 0.001), *** (p = 0.017).*

Discussion

The main purpose of ITP therapy is to increase platelet counts from <20 $x10^9$/L to >20 $x10^9$/L in first 24 - 48 hours and to prevent life-threatening bleedings (10-13). Main purpose of our study was not to compare the efficiency of IVIG and PMP therapies. However, we observed that safe platelet counts were obtained more rapidly with IVIG than PMP therapy. Although the platelet counts on the second day of treatment were significantly higher (p=0.01) in IVIG group than PMP group, this significance disappeared on the succeeding days.

It is known that the presence of antiphospholipid antibodies (APA) increases the tendency for thromboembolic events (14). Harris et al (15) reported high levels of anticardiolipin antibodies (ACA) in 30% of ITP patients. Subsequently, LA positivity was found in 36% of adult ITP patients at diagnosis (16). APA levels did not decrease significantly after low dose oral prednisone therapy in these patients. In another study (17) LA or ACA was present in 38 % of adult ITP patients. The ratio of response to methylprednisolone therapy was similar in APA (+) and APA (-) patients. Funauchi (18) reported 7 of 27 ITP patients to be LA or ACA positive. Among these 7 patients, 3 of them developed antiphospholipid syndrome (APS). Therefore it is suggested that screening of APA levels in ITP patients can be useful for the prediction of future thrombotic events. Dash also demonstrated LA positivity in approximately 28 % of the pediatric patients with acute ITP. After 6 months of follow up, LA positivity was persisting in 50 % of these patients. These ratios were similar to the adults. The authors concluded that APA persistence may be an important risk factor in

APS development in pediatric ITP patients as observed in adult patients (9). Bidot et all. indicate that APA are frequently observed in ITP and APS. However APA profile differs in these two conditions. In APS, antibodies were predominantly against β_2GP1 and 80 % had positive LA while in ITP the APA reacted most often with the phospholipids without LA. The authors suggest that this difference in APA may lead to the opposite clinical manifestations in two disorders (19). In our study 9 patients (41 %) had LA positivity at diagnosis. This result is consistent with previous observations. LA positivity of all 4 IVIG patients disappeared on day 3 and 3 of 5 PMP patients on day 3, the remaining 2 of PMP patients on day 30. This result is different from Stasi's (16) patients who received low dose oral prednisone therapy. These observations suggest that different therapy models may affect LA positivity differently. El-Bostany et all. showed a significant correlation between increased IgG concentrations (anti- β_2-GP1 and ACA-IgG) and steroid therapy resistance in ITP (20). We still do not know the role of high APA levels during thrombocytopenia. Previous studies have only discussed whether or not APA positivity has a role in pathogenesis of ITP. However, APA positivity may be a compensation against thrombocytopenia in ITP. Increased platelet activation (21) and an increased tendency for thrombotic events (17) in patients with APA are consistent with this hypothesis. In our study, the disappearance of LA positivity after the increase in platelet counts by the therapy also supports this idea. However, the disappearance of LA also may be related to the immunosuppressive treatment and this subject needs further investigation.

PF 1+2 is the most sensitive indicators of hemostatic system activation (22). This parameter has not been investigated in patients with acute ITP. In our study, mean PF 1+2 was significantly higher (p=0.0001) in patients than in the control group at diagnosis. PF 1+2 values of both groups increased on day 3. However, this increase was not significant. At diagnosis, PF 1+2, the most sensitive indicator of endogenous thrombin production, increased significantly in the patients. Increased level of PF 1+2 suggests that thrombocytopenia with its resulting loss of endothelial integrity has stimulated intravascular thrombin generation and increased levels of the prothrombin fragment. DD, an important indicator of fibrinolysis, was significantly lower (p=0.0001) in the patients than in the control group at diagnosis. These findings indicate a tendency for hypercoagulation when platelet count is low in acute ITP.

In the study performed by Oner, at diagnosis PC and PS levels were significantly lower in pediatric ITP patients than the control group (8). However in our study there was not a significant difference between two groups. Additionally, while there was no significant difference in AT levels at diagnosis between two groups in the study performed by Oner et al, our study indicated a significantly higher AT levels in patient group. However, PC, PS and AT levels increased significantly after PMP treatment both in our and Oner's studies. This increase observed in AT levels may develop secondary to the increased endogenous thrombin production. PC, f-PS and AT levels were significantly higher in PMP than IVIG group on day 3 (p= 0.004, 0.001 and 0,017 respectively). These data show that most of the natural anticoagulant levels are normal in ITP patients at diagnosis and their levels increase prominently with steroid therapy.

Conclusion

We can conclude that there are some reactive changes in the coagulation and fibrinolytic systems besides thrombocytopenia which is the major hematological finding in acute ITP. The reason for the changes observed in PF 1+2, DD and LA remains unclear. In addition, different therapy modalities can affect the balance between coagulation and fibrinolysis in different ways, as shown by the levels of the natural anticoagulants. Further studies are necessary to clarify these changes in patients with acute ITP.

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