Effect of Blood Transfusion on Platelet-Related Antibodies in ITP Patients

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Abstract: Objective: To detect the expression of platclet associated immunoglobulin (PAIg) on the ITP patients' platelet surface and study the effect and correlation of blood transfusion therapy on PAIg. Methods: The 173 patients confirmed as ITP selected form the outpatients and inpatients of the Department of Hematology and Rheumatology in the Third Affiliated Hospital of Guizhou Medical University, were divided into the blood transfusion group and the non-blood transfusion group, FCM fluorescence immunolabeling was used to measure expression quantity of PAIg, the positive expression rate and fluorescence intensity of PAIgG, PAIgM and PAIgA expressed on the platelet surface of ITP patients and the control group respectively. The softwave SPSS12.0 was applied to analyze the correlation. Results: (1) PAIgG (2.96 + 8.31) %, PAIgM (2.27 + 1.78) %, PAIgA (4.48 + 3.88) % in the non-transfusion group were significantly lower than those in the transfusion group (11.44 + 20.04) %, (9.72 + 15.24) %, (10.13 + 9.53) %, P<0.001. (2) The number of platelets in the non-transfusion group was significantly negatively correlated with PAIgM, r=-0.457, P<0.05. There was no significant correlation with PAIgG and PAIgA, all *P* values were >0.05. Conclusion: (1) It can increase platelet antibody after blood transfusion, and patients with ITP are more likely to produce platelet antibody after blood transfusion. (2) There is a certain degree of correlation between PAIg and blood transfusion in ITP patients.

Keywords: Idiopathic Thrombocytopenic Purpura, Platelet Related Antibodies, Blood Transfusion

1. Introduction

Idiopathic thrombocytopical purpura (ITP) is a clinically common hemorrhagic disease that destroys platelets by generating autoantibodies [1]. The morbidity in the pediatric population is approximately 0.5/1 million [2]. Studies have suggested that ITP is related to decreased immunity, the occurrence of infection, spleen function and genetic factors [3]. The relevant pathomechanisms include: (I) The production of autoantibodies [4]. ITP is characterized by antiplatelet autoantibodies. The antibodies combine with autoantigens to form compounds. Megakaryocytes cause serious damage to the compounds and reduce the number of platelets, as in platelet membrane glycoproteins (glycoprotein, GP) IIb/IIIa, Ib/IX, Ia/IIa, immunoglobulin (platelet associated immunity globin, PAIg) antibodies, after immunoglobulin antibodies combined with platelets and adsorbed on the surface of platelet antigen promoting the mononuclear macrophage system of platelet phagocytosis and clear [5]. (II) Impaired immune tolerance [6]. Autoimmune tolerance is impaired, and platelet membrane glycoprotein antigens are often maladjusted. Thereby reducing platelet count and causing ITP. (III) T and B cell-mediated humoral immune abnormalities [7]. B cell activating factor is involved in the pathophysiology of various diseases. Incremental B cytokines lead to decreasing levels of platelet autoantibodies and accelerate thrombocytopenia. (IV) Abnormal expression of Bone marrow megakaryocyte [8]. The reduction of megakaryocytes affects the production of normal platelets. (V) Th1/Th2 malfunction [9]. When the Th2/Th1 ratio decreases, IL-10/IL-4 production is increased and platelets are phagocytosed, Resulting in ITP.

There was study confirmed that production of platelet autoantibodies plays an important role in thrombocytopenia [10]. Donald M et al reported that 45% of non-splenectomized patients with ITP had anti-GPIIbIIIa or anti-GPIbIX autoantibodies at baseline [11]. Kuwana [12] also reported in the early years, ITP mainly exist GPII/IIIa and bIb /IX antibodies, those are beneficial to the damage of platelets. Changes in platelet autoantibody levels provide important information in assessing patient responses to treatments and disease prognosis. The assay of PAIg is a widespread method in detection of platelet antibodies. PAIG is a kind of autoantibodies that produced in pathological immune responses to platelet membrane glycoprotein GPII/IIIa and bIb/IX antigens. These antibodies bind to their corresponding antigens on the platelet membrane, subsequently, platelets are largely destroyed by the mononuclear - phagocytic system. Thereby shortening the life span of platelets, reducing the number, causing bleeding, resulting in ITP [13]. In this study, we examined the changes of platelet-associated antibodies in 173 patients with and without ITP transfusions, and 20 healthy subjects were selected as the control group to explore the influence of blood transfusion on platelet antibodies in ITP patients.

2. Clinical Resources

ITP patients were selected from August 2010 to November 2012, outpatients and inpatients of the Department of Hematology and Rheumatology in the Third Affiliated Hospital of Guizhou Medical University. All patients were up to standard «Blood Diagnostic and Curative Standard» (Second Edition, 1999), edited by ZhiNan Zhang [14], except other thrombocytopenic diseases. The case group was composed by 69 males and 104 females. Aged range 2 to 86 and the average was 41. The control group was selected from Hospital Physical Examination Center health check-up 20 cases, male 8 cases and female 12, aged range 21 to 65, an average of 39 years old. Except for tumor and autoimmune disease, in the number of platelet in $100 \sim 300 \times 10^9$ /L.

ITP patients were divided into blood transfusion group and non-blood transfusion group according to whether received blood transfusion (such as platelet suspension) within 3 months after PAIg was detected. There were 37 patients in the blood transfusion group, including 13 males and 24 females, range from 24 to 75 years old, with an average age of 42 years old. There were 136 cases in the non-transfusion group, men 55 and women 81, aged range 2 to 85, average of 40 years old.

3. Experimental Reagents and Instruments

FITC labeled sheep F (ab) 2 resistant IgG monoclonal antibody (Coulter company); Sheep (ab) 2 F anti-human IgG (gamma); FITC monoclonal antibody (Coulter company); Sheep (ab) 2 F resistance people IgM (mu) FITC monoclonal antibody (Coulter company); Sheep (ab) 2 F resistance of IgA (Alpha); FITC monoclonal antibody (Coulter company); PE tag CD41 (GPIIb) PE monoclonal antibody (Coulter company); EPICS ELITE ESP type flow cytometry instrument (Beckman Coulter Companies, USA); Fully automatic blood cell analyzer, high-speed refrigerated centrifuge Eppendorf (Germany Company).

4. Experimental Procedures

4.1. Samples Collection

2 ml of elbow-venous blood was taken from fasting subjects and transferred to sodium citrate anticoagulant tube. And shake it well in proportion of 9 to 1. In order to reduce the influence of external factors on specimen quality, the specimen shall be specially treated within 1 hour by a special person [15, 16].

4.2. Experimental Steps

The samples were centrifuged at 4°C with 800 rpm/min for 5 min. Platelet-rich plasma (PRP) was taken from the upper layer; 100ul PRP were transferred to a special Falcom tube, add 500 ul PBS buffer, mixed well, centrifuged at 2000 rpm for 3 min, discarded the supernatants, added the PBS buffer of equal volume for three times, and discarded the supernatants. Take four Falcom tubes, each tube was put in 50 ul suspended platelets and 5 ul CD41-PE, then add 10 ul FITC - sheep F (ab) 2 IgG, FITC - sheep F (ab) 2 anti human IgG, FITC - sheep F (ab) 2 people IgM, FITC - sheep F (ab) 2 IgA antibodies against people respectively, fully blending, incubation 15 min at room temperature away from light; Washed by PBS buffer, discard the supernatant, and resuspend it with PBS buffer of 500ul for detection within 2 hours [15, 16].

4.3. FCM Detection: Double Color Labeling Method

The excited light source was argon-ion, and the control group was set as the negative group, with the coefficient of variation < 2%, 10^4 platelet tests were collected [15, 16]. EXPO 321.2b Analysis software was used for data processing and analysis. Results can be expressed with positive percentages of PAIgG, PAIgM and PAIgA of platelet surface expression. Peripheral blood platelet count was detected with automatic blood cell analyzer.

4.4. Statistical Methods

SPSS12.0 software was used for statistical analysis. Use $\bar{x}$ $\pm$ s. First, normality and homogeneity of variance were tested for each group of data, and then T test was used for group comparison. If the data does not conform to the normal distribution and the variance is inconsistent, the square root inverse sine conversion was required before verification. Spearman's rank correlation analysis was performed for clinical indicators and interlaboratory correlation. *P*<0.05 was considered as a statistic difference.

5. Results

5.1. Comparison on PAIgG, PAIgM and PAIgA Between the Blood Transfusion Group of ITP Patients and the Non-Transfusion Group

A blood transfusion group is the one in which there is one or more transfusions before detection of PAIg. Non-transfusion group is the one in which patients have not received any blood transfusion within 3 months before detection of PAIg, and patients received less than two blood transfusion in life.

PAIgG, PAIgM and PAIgA in the non-transfusion group were significantly lower than those in the transfusion group, $P<0.001$, Blood transfusion group refers to one or more blood transfusions before testing PAIg; Non-transfusion group refers to those who have not received blood transfusion within 3 months prior to the detection of PAIg and have received blood transfusion for life < 2 times. PAIgG, PAIgM and PAIgA in the non-transfusion group were significantly lower than those in the transfusion group, $P<0.001$. As Table 1.

Table 1. Comparison on PAIgG, PAIgM and PAIgA between the ITP transfusion group and the non-transfusion group（$\overline{x} \pm s$).

Group set	cases	Platelet count ($\times10^9$/L)	PAIgG (%)	PAIgM (%)	PAIgA (%)
Non-blood transfusion group	136	46.44±28.98	2.96±8.31*	2.27±1.78*	4.48±3.88*
blood transfusion group	37	33.73±25.68	11.44±20.04	9.72±15.24	10.13±9.53
T value	-	2.596	-3.860	-5.564	-5.450
P value	-	0.010	0.000	0.000	0.000

Note: * $P<0.001$ compared with non-transfusion group.

5.2 Correlation Analysis on PAIgG, PAIgM and PAIgA Between the ITP Patients in the Blood Transfusion Group and Those in the Non-Transfusion Group

The number of platelets in the non-transfusion group was significantly negatively correlated with PAIgM, $P<0.05$. It had no significant correlation with PAIgG, PAIgA, $P>0.05$. Platelet count in the blood transfusion group was not correlated with PAIgG, PAIgM and PAIgA, $P>0.05$. As shown in Table 2

Table 2. Correlation analysis on PAIgG, PAIgM and PAIgA between the ITP transfusion group and the non-transfusion group.

Group set		PAIgG (%)	PAIgM (%)	PAIgA (%)
Non-blood transfusion group	r	-0.310	-0.457*	-0.320
	P	0.062	0.005	0.053
Blood transfusion group	r	-0.160	-0.011	-0.095
	P	0.063	0.899	0.270

Note: * $P<0.05$.

6. Discussions

ITP is a clinical syndrome induced by the increase in the number of platelet destruction caused by the deficiency of human immune mechanism, and is a hemorrhagic disease mainly occurring in children, young adults and women [17]. At the early years, Harrington [18] injected the blood or plasma of ITP patients into normal people and found that some normal people had significantly reduced platelets and induced bleeding, which was the first time to proposed and confirmed the existence of anti-platelet antibodies in the blood of ITP patients. The immune dysfunction of the patient results in abnormal platelet antibodies, which further bind to antigens on the platelet membrane. The immune reaction mediated by antibodies prompts platelets to be destroyed via mononuclear macrophages, and thus the number of platelets is sharply reduced [19]. Therefore, platelet-associated antigens and antibodies play an important role in the development of ITP.

Broadly speaking, PAIg consists of circulating immune complex, non-specific adsorbed plasma Ig on the platelet surface and strict PAIg [20]. The current study mainly focused on PAIg antibodies of PAIgA, G and M, and the results showed that: PAIg expression level increased in most ITP patients, which caused accelerated platelet destruction, and

showed clinical thrombocytopenia, prolonged clotting time, and finally purpura [21]. Other studies have reported that there is a significant negative correlation between the three antibodies PAIgA, G and M and the platelet count in ITP patients. When patients are recovering from the disease, the levels of the three antibodies are significantly reduced, while those patients with slow recovery of platelet count have higher levels of antibodies [22]. For patients receiving blood transfusion, ITP is also closely related to the increase of anti-platelet-associated antibodies. As the report goes [23], the serum or plasma of ITP patients was injected into normal people, and the number of platelets in the subjects was significantly reduced. When normal human platelets were transfused to ITP patients, the platelets were damaged within 12-24 hours in the patients, indicating that PAIg has guided significance in clinical practice for patients with blood transfusion.

It is a common treatment in clinical therapy to transfuse platelet to patients with high-risk bleeding caused by ITP in order to increase the number of platelets immediately. However, PAIg generated inside the patients with ITP after many platelets infusion may result in the infusion ineffectivity. Studies found that the increase of PAIgG in a considerable number of patients was undeniably associated with blood

transfusion, and the positive rate of PAIgG went up with-blood transfusion time [24, 25]. How to effectively transfuse platelet has been a research hot trend, especially in recent years, the ineffective transfusion caused by platelet antibodies. In this study, we found that the number of platelets in non-transfusion group was obviously higher than that of blood transfusion group, while PAIgG, PAIgM, PAIgA was lower. It certified that blood transfusions can produce higher level of platelet antibody. Blood transfusion result in ITP patients' likeliness to form the platelet antibody. No increase in platelet antibody was detected in the non-transfusion group due to the low number of blood transfusion or the long interval between blood transfusion and experiment. PAIg test is necessary for patients with clinically insufficient platelet and in need of continuous infusion, which has a certain effect on further understanding the effect of platelet transfusion.

7. Conclusions

To sum up, it can increase platelet antibody after blood transfusion, and patients with ITP are more likely to produce platelet antibody after blood transfusion; There is a certain degree of correlation between PAIg and blood transfusion in ITP patients. The close correlation between PAIgG, PAIgM, PAIgA and the effectivity or ineffectivity of platelet injection is one of the reasons why platelet infusion is ineffective. The detection of PAIgG, PAIgM and PAIgA can be used as a judgment basis for whether platelet transfusion is needed to fully optimize the use of blood products. Meanwhile, other treatment schemes can be adopted for patients with ineffective platelet transfusion as soon as possible, it has certain effects in guiding clinical treatment.

Acknowledgements

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