ORIGINAL ARTICLE

Pre and post apheresis platelet CD markers' evaluation using flow cytometry

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Abstract

Objective: To evaluate the effect of apheresis procedure on platelet's activation dependent glycoproteins' expression on their surface.

Methods: This study was conducted between June 2012 and June 2014, and comprised blood and platelet samples. Two samples were collected i.e. venous blood sample and apheresed platelet sample from the same donor. Platelet cluster of differentiation markers (41, 61, 62p and 63) were analysed within 2 hours of sample collection using flow cytometry. SPSS 20 was used for data analysis.

Results: A total of 100 donors were recruited in this study. Cluster of differentiation (CD) markers' expression of 100 pre-apheresis and 100 platelet apheresis samples was compared after the completion of platelet apheresis procedure. CD 41 and 61 showed no significant difference between pre- and post-apheresis platelets; (p=0.447 and 0.712, respectively). CD 62p positivity of pre-apheresis platelets (9.57±5.88%), and post-apheresis platelets (55.57±24.59%) showed statistically highly significant difference (p<0.001). CD 63 expression of pre- and post-apheresis platelets was 14.19±11.84% and 40.77±16.08%, respectively (p=0.04). Moderate correlation existed between post-apheresis platelets' CD 62p and 63 (r=0.62).

Conclusion: Platelet CD markers 41 and 61 did not show any change in pre- and post-apheresis samples while expression of 62p and 63 increased during the apheresis.

Keywords: Platelet, CD markers, Plateletapheresis, Flowcytometry. (JPMA 66: 1440; 2016)

Introduction

Platelets play a cardinal role in haemostasis.[1] Platelets are transfused in order to prevent or treat bleeding in various clinical conditions.[2] Platelet transfusion improves the management of patients with haemostatic or platelet disorders, reducing haemorrhagic complications and mortality.[3] Prophylactic and supportive use of platelet transfusion has significantly increased in the last few decades.

Platelets are obtained from a healthy volunteer donor by two methods i.e. classical whole blood preparation method and platelet apheresis technique. Classically whole blood platelet concentrates are prepared either from platelet rich plasma (PRP) or buffy coat. Single-donor platelet concentrates (platelet mega-unit) is the preferred transfusion therapy for most of the patients with bleeding complications due to decreased platelet count and to resolve the logistic problems of platelet supply.

Platelet receptors are important for normal haemostatic functions. These receptors either activate platelet or they act as adhesion molecules which interact with the damaged endothelium, other platelets and leukocytes.[4] Platelets are unable to perform these functions in the absence of their receptors. Cluster of differentiation (CD)-41 (glycoprotein (GP) IIb; molecular weight 140 kilo dalton [kDa]) is a glycoprotein present on platelets and megakaryocytes. CD61, also known as GPIIIa, is a 110 kDa glycoprotein that belongs to integrin family of proteins. These glycoproteins act as receptors for fibrinogen, von Willebrand factor (vWf), fibronectin and vitronectin. CD62p, also known as P-selectin or granule membrane protein 140 (GMP 140), is a glycoprotein of 140 kDa present in alpha (α)-granules of resting platelets and is translocated to the plasma membrane after activation. Expression of CD62p on circulating platelets indicates in vivo activation of platelets.[5] CD63 or lysosomal membrane-associated glycoprotein-3 (LAMP-3) is a 53kDa lysosomal membrane protein that appears on the surface of the activated platelets after release reaction.CD63 modulates platelet spreading and platelet tyrosine phosphorylation on immobilised fibrinogen. CD63 is an extremely reliable marker for in vivo platelet activation.[6] Increased expression of neoepitopes e.g. CD63or CD62p, on the surface of platelets is associated with diabetes mellitus (DM), acute coronary syndromes, peripheral vascular disease (PVD), acute cerebrovascular ischaemia

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and pre-eclampsia. Flow cytometry can best analyse the platelet functional status i.e. activation, aggregation and platelet leukocyte interaction.[1,7]

The current study was planned to evaluate the effect of apheresis procedure on platelet's activation dependent glycoproteins' expression on their surface.

Materials and Methods

This study was conducted between June 2012 and June 2014 at two centres. Donors' selection, recruitment and platelet apheresis procedures were performed at Hussaini Blood Bank and Oncology centre Karachi, Sindh. Platelet flow cytometric analysis was performed at Child Aid Association, National Institute of Child Health Karachi, Sindh. Two types of samples were analysed, i.e. an anti-coagulated venous blood sample from the donor (pre-apheresis samples) and platelet mega unit sample after apheresis (platelet- apheresis samples) of the same individual. This study was approved by the ethical institutional review board. Informed written consent was obtained from each donor. Donors for platelet apheresis were recruited according to donor's selection criteria. Donors with the mean platelet count of 358,000±35,000 /µl were included. Donors with low platelet count or not fulfilling the donor selection criteria were excluded. Donors' pre-apheresis blood samples were collected from the antecubital vein under aseptic conditions. Tourniquet was applied with low pressure and a syringe with 21-gauge needle was used. Tourniquet was released as the blood started to enter the syringe. Then 2.7ml blood was added to a vacutainer tube containing 0.3ml sodium citrate (3.8%). Two ml of apheresis sample was collected in polystyrene non-anticoagulated vacutainer tube from the mega-unit bag.

Platelets were analysed by flow cytometry {FACS Calibur (BD Biosciences, Qume Drive San Jose, California, United States)}using four monoclonal antibodies i.e. anti-CD41, anti CD63, anti CD61 and anti CD62p (BD Biosciences). All samples were analysed within two hours of collection. Total percentage for CD marker on x-axis was calculated by adding both upper right and lower right % gated antibody. For the antibody selected on the y-axis, the total percentage was calculated by adding the upper left and upper right % gated antibody.

Data was analysed for descriptive and inferential statistics using SPSS 20. Data was tested for normality using Kolmogorov-Smirnov test. Results were presented as mean± standard deviation (SD) and p-value was calculated using paired-sample t-test. P<0.05 was considered statistically significant. Pearson's correlation was calculated between CD markers CD62p and CD63 of post apheresis platelets.

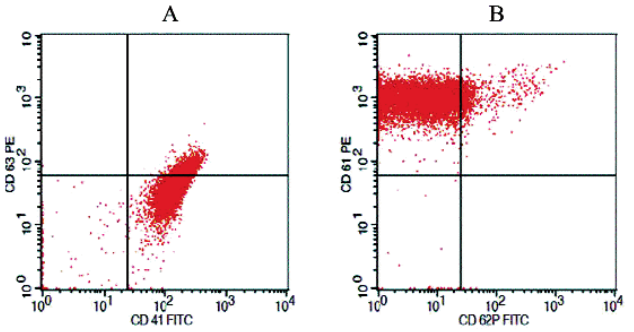
Results

A total of 100 donors were recruited in this study. CD markers' expression of 100 pre-apheresis and 100 platelet apheresis samples was compared after the completion of platelet apheresis procedure. Flow cytometry analysis of CD41 showed no statistically significant difference (p=0.447) (Table; Figure-1). CD61 positivity also showed no statistically significant change between pre-apheresis

Table: Mean ± SD of CD markers (41, 61, 62p and 63) of pre- and post-apheresis platelets.

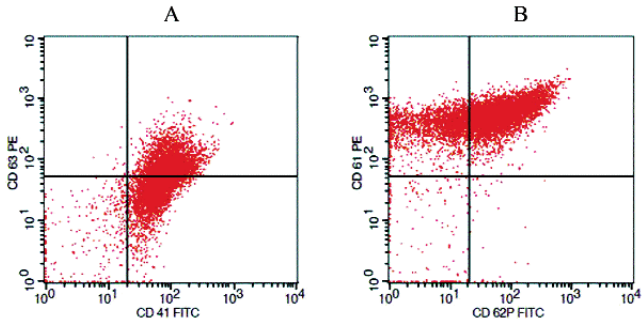
Platelet CD markers	Pre-apheresis	Post-apheresis	P
41	75.65±24.70	62.20±18.86	0.447
61	78.72±29.33	71.42±21.50	0.712
62p	9.57±5.88	55.57±24.59	0.001
63	14.19±11.84	40.77±16.08	0.043

SD: Standard deviation.



FITC: Fluorescein isothiocyanate; PE: Phycoerythrin

Figure-1: Platelets' histogram showing % positivity of CD markers of pre-apheresisplatelets.



FITC: Fluorescein isothiocyanate; PE: Phycoerythrin

Figure-2: Platelets' histogram showing % positivity of CD markers of post-apheresis platelet samples.

and post-apheresis, respectively. CD62p and CD63 percent positivity of pre- and post-apheresis platelets showed significant changes and statistically significant difference (p=0.001) was observed. CD62p and CD63 showed moderate correlation (r=0.62). Percent positivity of platelets' CD markers 41, 61, 62p and 63, and histogram of pre- and post-apheresis were noted (Figure-1-2).

Discussion

Platelet transfusion has significantly increased in the last few decades. Apheresis platelet concentrates or single-donor platelet concentrates are required for adequate platelet transfusion regimen. This study was conducted in order to evaluate the resting and activated state of pre- and post-apheresis platelets. Four CD markers i.e. CD 41, 61, 62p and 63, were evaluated using flow cytometry. CD41 and 61 were used for discrimination and identification of platelets during its gating in the resting and activated state of platelets. CD62p and 63 were used as markers of activated state of platelets.

Expression of CD41 of pre- and post-apheresis samples showed no statistically significant difference (p=0.447). This finding is consistent with those of Gutensohn 1997 et al. who also reported no change in the expression of CD41 before and after apheresis.[8]

Expression of CD41 on platelet surface is time dependent. Gutensohn et al. observed increased expression of CD41 on the surface of platelets during and after 5 minutes of apheresis, but after 60 minutes the expression of CD41 was reduced. He also noted a similar change for CD42.[8]

In vitro studies have shown no change in the expression of CD41 in resting and activated state. Furthermore, it was also observed that the presence of adenosine diphosphate (ADP) does not affect the expression of CD41 after 20 minutes, 2 hours and three hours of incubation.[9]

Wang et al. found that platelets prepared from platelet rich plasma (PRP) showed increased levels of CD41/CD61 complex (GP IIb/IIIa) on the first day; however, no further increased expression was noted on storage for upto fifth day.[10] The use of anti-PAC antibody, i.e. anti CD41/CD61 (GP IIb/IIIa), has shown increased expression of this complex.[10] However, when CD41 alone was used as a marker of platelet activation state, it did not show any change in its expression.

Comparison of expression of CD61 between pre- and post-apheresis platelet samples showed no statistical difference (p=0.712). CD61 was used as a primary discriminator between red blood cells (RBCs) and platelets. In vitro studies of apheresed and buffy coat derived platelet showed no increased expression of CD61 during storage.[11]

In vitro studies showed no change in the expression of CD61 in resting and activated state. Furthermore, platelet agonist did not affect the expression of CD61 on platelets membrane.[9]

CD62p was used as a marker of platelet activation. CD62p is stored in the α-granules of resting platelets. It is translocated and expressed on the surface of the platelets very early after platelet activation.[12]

A significant increase in the expression of CD62p was noted between pre- and post-apheresis samples (p=0.001). This finding correlates well with that of Wang et al.,[10] Krailadsiri and Seghatchian[13] who also reported the increased expression of CD62p.

CD62p is expressed on the surface of the platelets during extracorporeal circulation in a time dependent manner. Increased expression of CD62p was observed in most donors within 10 minutes of platelet harvesting.[14] Increased expression of CD62p was also observed in vivo by Rinder et al. (1992) during cardiopulmonary bypass[15] and by Gawaz and Ward[16] during haemodialysis.

CD63 expression was also statistically different between pre-apheresis and post-apheresis platelets (p=0.043). This finding was consistent with those of Hagberg and Akkok, who also observed increased expression of CD63 during platelet collection procedure and after 2 hours of storage on the agitator.[17]

Variation in the expression of CD markers 62p and 63 was assessed through Pearson's correlation. It was observed that these markers showed moderate correlation i.e. both markers showed similar increase in their expression in post-apheresis samples. This finding is consistent with that of Dijikstra et al.[18]

Mechanism of platelets activation during collection is not fully understood.[19] Various theories have been put forward to explain the platelet activation during extracorporeal circulation. Exposure of platelets to physical forces and artificial surfaces of the collection set during extracorporeal circulation may result in platelet activation.[20] Extracorporeal circulation, i.e. haemodialysis, cardiopulmonary bypass, haemoperfusion, and plasmapheresis, leads to platelet activation, expression of platelet activation CD markers and altered platelet function.[21,22] Non-endothelial contact during apheresis enhances the thrombogenic potential and expression of surface-associated receptors on the platelets.[23]

Conclusion

Increased positivity of CD62p and 63 showed the activation of platelets during apheresis procedure.

Clinical studies may also be conducted for the evaluation of the in vivo survival of platelets and any correlation of platelet activation with their in vivo haemostatic functions. In vivo studies are needed to correlate platelet CD62p and leukocytes interactions effects.

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Conflict of Interest: None.

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