Original Article

In Vitro Assessment of Platelet Lesions during 5-day Storage in Iranian Blood Transfusion Organization (IBTO) Centers

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Abstract

Background and Objectives: Platelet concentrates (PC) are used in thrombocytopenia and inherited or acquired platelet dysfunction disorders. Thus, retaining the platelets quality and function during storage will lead to desirable outcomes in treatment of such patients.

Methods: In this study, we evaluated 40 PC bags, prepared by PRP method in IBTO centers. We applied an array of assays, on first, third and fifth days of storage for PC quality control, including swirling, cell counting, bacterial contamination, measurement of CD62P, pH, and platelet aggregation test, to evaluate platelet lesion during storage.

Results: All units were negative for bacterial contamination. Swirling was positive for all units on various days; platelet count was in the acceptable range. Measurement of CD62P on fifth day was not significantly higher than third or first day ($P > 0.15$) ($P > 0.05$). pH on fifth day was significantly lower than first day ($P < 0.01$) ($P < 0.05$). Platelet aggregation with arachidonic acid and ristocetin showed significant decrease on fifth day compared to third day ($P < 0.01$) ($P < 0.05$).

Conclusions: CD62P associated with other platelet function tests can be used as an activation marker in evaluation of PC functions during storage.

Keywords: CD62P, platelet lesion, platelet storage

Cite this article as: Ghezelbash B, Amini kafiabad S, Hojjati MT, Hamidpoor M, Vaeli S, Tabtabae MR, Gharehbaghian A. *In Vitro* Assessment of Platelet Lesions during 5-day Storage in Iranian Blood Transfusion Organization (IBTO) Centers. *Arch Iran Med*. 2015; **18(2):** 114 – 116.

Introduction

Although the ability to produce a platelet concentrate (PC) for transfusion purposes has existed for more than 50 years, this product still presents one of the major challenges to the blood banks, because of its limitations of storing under standard blood bank conditions. Depending on the jurisdiction, platelets have a maximum storage time of 3 to 7 days.[1] During PC storage, a variety of changes occurs in platelets, including the expression of GPIb, GPIIb/IIIa, producing platelet activation markers (CD40L, CD63, and CD62P), membrane phospholipids inversion and exposure of phospholipids with negative charge, and platelet derived micro-particles formation.[2,3] CD62P (P-selectin), a member of lectin family, is an adhesive molecules that is stored in platelet alpha granules and Weible-palade granules in endothelial cells. After exposure to platelet activation stimulators, these molecules are expressed on the cell surface.[4,5] Increasing membrane expression of CD62P, as well as increased levels in plasma, is therefore indicative of platelet activation.[6] Evaluation of platelet activation in response to agonists is also another factor in evaluation of platelet activation.[7] After encountering these agonists, platelets initiate secreting granules contents to the outside which consequently leads to aggregation.[8] In this study, we evaluated the main biochemical and activation markers changes in PC during storage in IBTO centers for a period of six months.

Material and Methods

This was a cross-sectional study and the samples were obtained from IBTO centers. In this study, 40 samples were collected from IBTO centers. According to IBTO protocols, Quality Control (QC) must be performed in one percent of products of each center. The sample is randomly selected and QC is done according to IBTO standard operating procedure (SOP). Moreover, measuring CD62P, response to agonists and platelet aggregation tests is done on each sample. During the tests, all samples are kept in platelet shaker at the authorized temperature (20–24ºC) and in a closed system. All PC exanimations took place on the first day after preparation for volume measurement, Platelet count, pH measurement, swirling, CD62P and aggregation tests. On the third and fifth days, we repeated swirling, pH, CD62P and aggregation test for all bags. Evaluation of microbial contamination was also performed for each sample in tioglycolate medium, and monitored for five days after preparation. For microbial culture, 1 mL of PC was added to 10 mL of tioglycolate medium and incubated at 37ºC for 7 days. During this time, the media was monitored on a daily basis for turbid appearance (indication of positive culture). PC volume was estimated by multiplying each bag`s weight by 1.03. Swirling was induced by gently rotating or tapping a platelet bag in front

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Accepted for publication: 31 December 2014

of a light source. CD62P was measured by flowcytometry according to the manufacturer's procedure (Immunocytometry System, Becton Dickinson, Belgium). Approximately 2 mL of PRP samples was collected from each bag. Platelet samples studied were diluted to a volume of PRP + three volumes phosphate buffer solution (PBS). Then, three tubes were prepared for testing. The first tube was added 5 µL antibody (Becton Dickinson BD) negative control isotype, the second tube 5 µL CD41 antibody conjugated with PE ((F0803 Dako) mouse monoclonal anti-CD41 human platelets and the third tube 5 µL CD62P antibody conjugated with FITC (BD PHarminogen FITC) mouse anti-human CD62P. Up to 50 µL prepared samples were added to tubes containing antibodies. The three tubes were reacted for 30 min away from light, and then mixed with 1 mL of 1% paraformaldehyde at 4°C. After sample preparation procedures, testing with flow cytometry assay (Becton-Dickinson) was performed. For each sample run, data were acquired for 10,000 events gated on forward and side-angle light scatter with gains adjusted to include the platelet population. The percentage of CD62P gate activated platelets was determined and the results were reported as the percentage. Aggregometry was also performed by Helena Paks-4 according to the procedure. Platelet aggregation assays at the selected storage intervals were performed using the classical turbidimetric technique. Briefly, platelet samples were stimulated in aggregometer cuvettes with agonists and the aggregation response was monitored optically on an aggregometer (Aggregometer Helena Paks-4). The results are reported as the maximum change in light transmission (%) for a total time of 5 minutes, using the initial platelet suspension as the baseline and platelet-poor plasma as 100%. The agonists were arachidonic acid (Sigma-Chemical), ristocetin (Sigma-Chemical). Statistical analyses were performed using SPSS 17. The results are expressed as mean and standard deviation (SD) of the mean, and minimum and maximum values when appropriate. Changes of platelet parameters through storage were analyzed by means of a one-way ANOVA test. Values of parameters were compared by a Student's *t* test for paired data. Confidence intervals used in the analysis of experiments are in the range of 95% and P value of ≤0.05 was considered statistically significant.

Result

The mean volume of PC units was 57 mL. Microbial cultures for all samples were negative during 5-day storage. In addition, all samples had optimal swirling on the first, third and fifth days of storage for each unit. Mean platelet count in three IBTO regional center were 5.9×10^{10}. The results for pH, CD62P and aggregometry are presented in Table 1. According to the results, pH had significant change ($P < 0.01$) and decreased during the storage time, but it was in the authorized range. CD62P measurement had no significant changes between day one, day three and day five ($P > 0.15$) and only had a mild elevation.

In aggregation test, response to arachidonic acid was significant on day three and day five, compared to day one. In response to Ristocetin, on days one and three, the value was not significant ($P > 0.17$), but it was significant on day five compared to day three ($P < 0.01$) and had mild elevation.

Discussion

During preparation and storage, PC undergoes metabolic and morphologic changes that leave undesirable effect on platelet structure and functions, causing poor outcomes after transfusion.[9,10] The aim of this study was to evaluate morphological and biochemical alternations in PC during storage at 22°C, as a program of QC in IBTO centers. According to international standards, CPD-A1 is used as anticoagulant in bags, and PC is prepared by PRP method, the commonest PC preparation method in the world. The mean volume of PC per bag in the three centers was 57 mL and the mean platelet count was 5.8×10^{10}. Although smaller volume does not affect platelet functions, reduced plasma volume in PC, causing decrease in platelets movements in bags, increases the incidence of direct contacts of platelets to each other. These contacts leads to activation and aggregation of platelets before transfusion, and consequently aggregated platelets filtrate by transfusion sets and undesirable platelet count will achieve in patients after transfusion. This condition increases the demand for more PC, and increases the incidence of adverse effect of platelet transfusion like being refractory to platelet transfusion. The swirling score, done as a rapid inexpensive quality control measure, was assessed in our study. Swirling has a direct correlation with pH and function of platelets. Functional platelets have a discoid morphology, which allows them in this form appearance waves or hurricane can be seen with the naked eye.[11] For evaluation of temperature effects on swirling of platelets in PC, Gabriele and colleagues compared swirling at 4°C and 22°C. Their results showed that swirling at 4°C was negative, but it was positive at 22°C.[12] In our study, swirling was positive in PC during storage that indicates its preparation and storage was done in a good condition in these centers. Over the years, improvements in donor screening has nearly eliminated hepatitis B virus, hepatitis C virus and HIV from many countries' blood supply. As the transfusion-transmission rates of these agents decreased, platelet bacterial contamination assumed a new noticeable role as the most frequent infectious risk of transfusion. Unlike other products such as red cell and FFP, while room temperature storage allows transfused platelets to circulate *in vivo*, it has the negative side of promoting bacterial growth. Because of this risk, platelet storage is ordinarily limited to only 5 days, making platelet

Table 1. Results of changes in pH, CD62P and platelet aggregation test in five-day storage of PC in IBTO centers.

	Day one	*P*-value	Day three	*P*-value	Day five
pH	0.35 ± 7.46			($P < 0.01$)	0.3* ± 7.34
CD62P	7.94 ± 19.07	($P > 0.19$)	8.26 ± 21.93	($P > 0.15$)	8.99 ± 28.09
% aggregation by arachidonic acid	22.6 ± 67.6	($P < 0.01$)	18.9* ± 54.9	($P < 0.01$)	23.7* ± 41
% aggregation by ristocetin	6.44 ± 83.2	($P > 0.17$)	16.6 ± 70.29	($P < 0.01$)	21.4* ± 55.37

t-test was applied to obtain the average of both groups. $P<0.05$ was considered statistically significant. *Comparison between days, $P < 0.05$

inventory management extremely challenging.[13,14] In our study, microbial culture was negative during storage. Whereas, potential sources of contamination transfer from the venopuncture site into bags, the proper education of staff engaged in PC production has a crucial role in safety of blood. Platelet aggregation responses to a number of agonists drop significantly during storage. In our study, a significant decrease was observed in response to agonists on day five compared to day three and day one. In similar studies, Kocazeybec, et al.[15] and Holger, et al.[16] reported similar results to our study. Their results showed a significant decline in response to agonist on day five. One of the most important changes in PC during storage is platelet activation, whereby the function and survilance of platelets will decline. One of useful platelet activation markers is CD62P.[9] Platelet surface CD62P is used as a marker for the detection of circulating degranulated platelets in clinical settings. The expression of CD62P has been shown to be inversely correlated with recovery of platelets and platelet count increment, because of triggering fast CD62-mediated platelet clearance. In our study, there was no significant change in CD62P value between three times of evaluation during storage time.[17] Michelson et al. demonstrated that circulating degranulated platelets rapidly lose surface CD62P to the plasma pool, indicating that platelet surface CD62P is not an ideal marker for the detection of circulating degranulated platelets although it may still be a useful marker of platelet degranulation if there is continuous activation of platelets.[18] Previous studies suggested that the plasma concentration of soluble CD62P could be used as a marker of platelet activation in clinical settings, although an increase in the plasma concentration may also reflect the release of CD62P from activated and/or damaged endothelial cells.[19] Although these studies indicated the relative role of CD62P as an indicator of platelet activation *in vivo*, it should not be neglected that we are measuring this marker *in vitro*, where no mechanism of elimination of this marker is available and the system is closed. Thus, CD62P can be a good predictor of activation in PC during storage. Similar to our study, gentle elevation of CD62P in PC was reported by Meltem et al.[20] In another study, Nie and colleagues.[21] reported no significant changes in CD62P value in PC in the first 72 hr after preparation with a mild elevation occurring after day three, and these are similar to our findings. As expected, according to the results of other related tests in our study, none of the PC under study underwent platelet activation and their transfusion can be safe and effective to patient to achieve the ideal CCI.

Acknowledgment

We are thankful to flowcytometry, coagulation and QC Laboratory of IBTO for their continued support and collaborations.

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