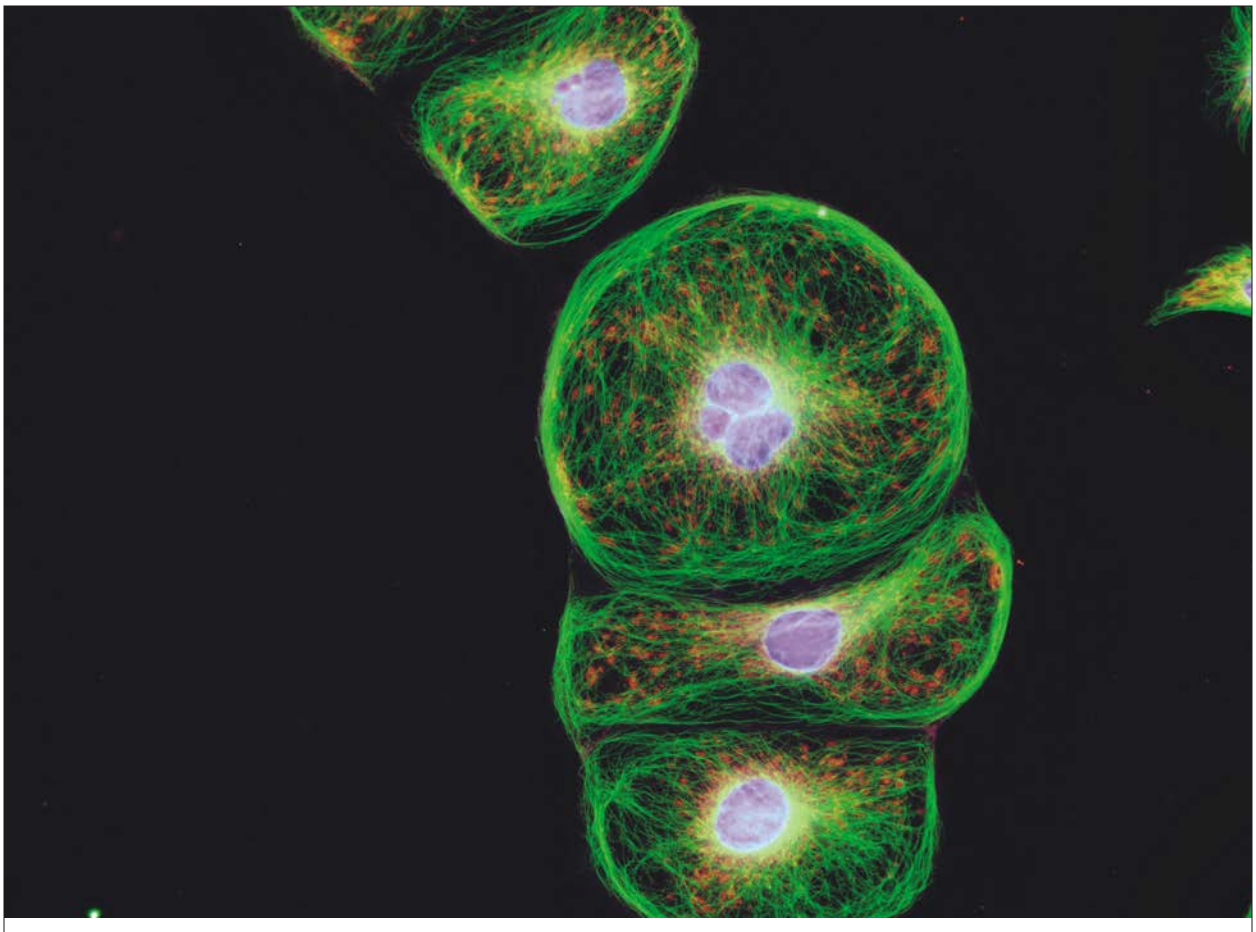

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Effect of Preparation and Storage Methods of Platelet Products on Complement Activity

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ABSTRACT

Platelet transfusion is considered a vital therapeutic approach for several diseases. However, like other blood products, it can cause transfusion reactions mainly due to various plasma proteins. With regard to provision of platelet products by a variety of methods including random donors and platelet apheresis, the risk of adverse reactions is assumed to be different in products produced by different methods. The aim of this study was to evaluate the difference in the level of C3 and its derivatives (C3a and C3b anaphylatoxins) as a plasma protein causing reactions in random and platelet apheresis methods. The study was conducted on healthy volunteer blood donors referred to Shiraz Blood Transfusion Organization. Platelet products were prepared by the two mentioned methods based on standard operating methods approved by the Iranian Blood Transfusion Organization. The C3 level was evaluated in three time points using immunoturbidimetric test: immediately after processing, one day and three days later. The data were analyzed using SPSS 16 software in terms of student t test and Pearson correlation. The $p<0.05$ was considered statistically significant. The C3 level was in maximum level in both method of platelet preparation immediately after preparation and was in minimum in the first day. In addition, statistically significant difference was observed between C3 level in each point of measurement: immediately after preparation ($p = 00$), the first day ($p = 00$) and on the third day ($p = 0.45$). A significant correlation was also observed between C3 level and type of platelet storage bag. Our findings show that the method of platelet preparation and storage plays an important role in the level of accumulated C3 in these products. The C3 concentration, the resulting complement activity and accumulation of anaphylatoxin factors was higher in this product. Therefore, further investigation might prove which method of platelet preparation bears a lower risk of reactions related to the injection of this product.

Key words: Platelet, apheresis, random donor, platelet product, C3b concentration

INTRODUCTION

Platelets, the cell fragments derived from megakaryocytes, play an important role in normal hemostasis. Platelet transfusion is an accepted vital treatment method in patients with bleeding disorders associated with severe thrombocytopenia or ineffective platelet production (Refaai *et al*., 2011). Every year, about 2.2 million platelet units are consumed in the United States, the majority

of which are used as prophylaxis to reduce the risk of spontaneous bleeding in patients undergoing chemotherapy and Hematopoietic Progenitor Cell Transplantation (HPCT) (Kaufman *et al*., 2015). There are three methods for preparation of platelet products. In the first and second methods, randomly donated whole blood is used to prepare the platelet product. Platelet-Rich Plasma (PRP) and buffy coat are used to prepare the platelet product in the first and second methods, respectively. Apheresis is used in the third method to prepare platelets in which only one donor is used. After preparation, the platelet concentrate is stored in bags for 3-5 days. The product concentration is different in these methods. In addition, several other factors can affect the quality of final product in the processes of preparation and storage of platelet product (Pietersz, 2009). These products are injected to patients as a treatment or prophylaxis approach to save their lives.

Although, platelet transfusion can save patient's life, it bears potential risks like other blood products, so that in some cases transfusion reactions are more common in platelet transfusion than red blood cell transfusion (Spiess *et al*., 2004). These reactions occur in a wide range, from an allergic reaction (the most common one) or mild fever to acute hemolytic reacts, Transfusion-Related Acute Lung Injury (TRALI), anaphylaxis or sepsis (Tormey *et al*., 2009).

Complement system is involved in allergic reactions and is activated by alternative, classic and lectin pathways and plays a role in effective immune surveillance (Ricklin *et al*., 2010). The C3 is an important component of complement system, cleavage of which to C3b can activate all the three pathways and cause anaphylactic reactions via conversion to C3a. This molecule is normally found in plasma (Nilsson and Ekdahl, 2012). As a result, C3 can be assumed as a protein that can accumulate in supernatant of platelet products and cause reactions due to platelet injection, so that a number of studies have shown that washing platelets with saline to remove the supernatant accumulated in the bag before transfusion reduces febrile reactions to less than 0.1% and can also reduce allergic reactions due to blood transfusion (Vo *et al*., 2001).

Given the importance of injection efficacy of platelet products in prophylaxis and treatment of patients as well as evaluation of potential effects of complement system activation on patient and quality of product, this study investigated the effect of preparation and storage methods of platelets on complement activation level in these products.

MATERIALS AND METHODS

Design and study population: This study was conducted on healthy volunteer blood donors referred to Shiraz Blood Transfusion Organization. The sample size was 102, sampling was done by census and all the healthy individuals who donated blood were randomly divided in two groups. From whole blood of group A, the platelets were prepared and apheresis was used to obtain platelet from group B. This study was approved by the ethics committee of the Iranian Blood Transfusion Organization. The consent form was filled out by participants in the study and they voluntarily participated in the study.

Inclusion criteria: Informed consent and signing the informed consent form before donating blood or platelet, negative screening tests for all routine tests and no use of aspirin or nonsteroidal anti-inflammatory drugs or medication affecting the platelets.

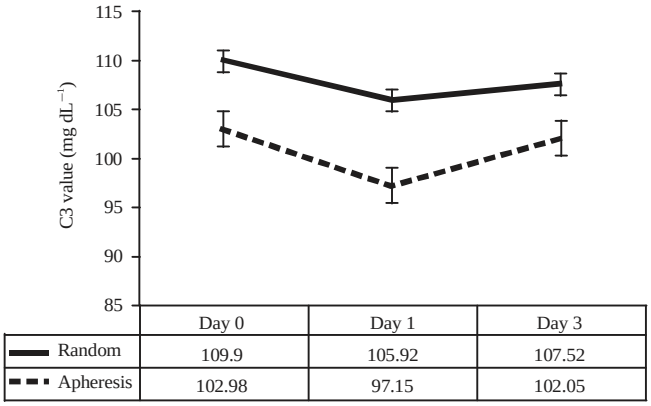
Exclusion criteria: Positive screening test or a history of consuming any of the drugs affecting platelet function or complement system. These criteria were examined by a physician in blood transfusion organization.

Methods: Platelet products were prepared by both methods according to standard operating procedures approved by the Iranian Blood Transfusion Organization. In the whole blood method, platelets were isolated after drawing blood in room temperature within 8 h. In the first round of platelet preparation, blood bags (JMS blood bags from Singapore, Macopharma from France) were centrifuged in low revolution to collect platelet-rich plasma. In the second round, PRP was centrifuged in high revolution to precipitate the platelets. After centrifuge, samples were taken from various stages of platelet product preparation and in the third day after preparation (expiration date). In platelet apheresis method, after selection and medical examination of the donor, preparation of platelet apheresis instrument (Trima Excel and Hemontics from USA) and connection of needle tip of Trima and Hemontics platelets apheresis set to vessels of donor's arm and taking laboratory samples, a K3EDTA pilot was prepared and a unique label was attached to it with a specific number to identify and monitor it. After finishing platelet apheresis operations and dispatch of bags containing the platelet product to storage unit and K3EDTA to laboratory, the pilot was centrifuged for 10 min at 3000 rpm. The samples taken from both random and platelets apheresis groups, including K3EDTA samples and samples taken from the cord (ACD or CPDA1 plasma samples), were immediately stored in 63°C after the end of sampling operations. The samples were stored under controlled conditions until testing and were dispatched to laboratory by maintaining the cold chain to be tested. The level of C3 in plasma added to the product was measured by immunoturbidimetric method using Cobas Mira S autoanalyzer (USA) and test kits (Pars Azmoon, Iran) with minimum C3 measurable concentration of 1 mg dL^{-1}.

Statistical analysis: The obtained data was collected and analyzed by SPSS 16 software. For comparison between groups, student t test and Pearson correlation were used with significance level of $p<0.05$.

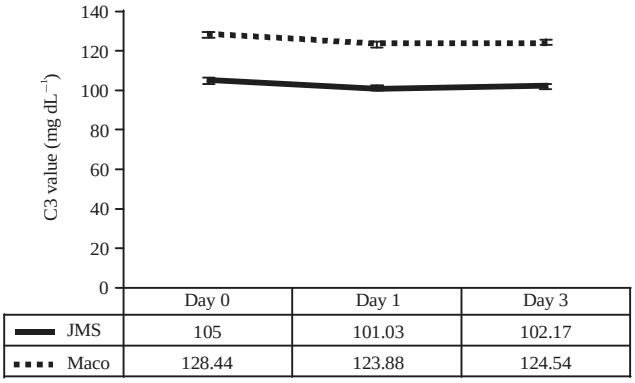
RESULTS

This study included 102 blood donors (90 men and 12 women), who were divided into two groups of 51. In Fig. 1, C3 level was compared immediately after sampling, the first day and the third day in both methods of platelet preparation. The C3 level was maximum immediately after sampling in both methods of platelet preparation and was minimum in the first day. In addition,



	Day 0	Day 1	Day 3
Random	109.9	105.92	107.52
Apheresis	102.98	97.15	102.05

Fig. 1: Comparison of C3 level in both platelet preparation methods in three different time points



	Day 0	Day 1	Day 3
JMS	105	101.03	102.17
Maco	128.44	123.88	124.54

Fig. 2: Comparison between Macopharma and JMS platelet storage bags in random method

statistically significant difference was observed between C3 level in each point of measurement, immediately after sampling ($p<0.05$), the first day ($p<0.05$) and the third day ($p = 0.45$) in apheresis and random methods.

Figure 2 shows a comparison with regard to C3 level during platelet storage in which C3 level has indicated significant reduction in JMS bags. Moreover, Pearson analysis showed that the relationship between the blood bag and C3 level was statistically significant immediately after collection ($p = 0.54$), one day ($p = 0.48$) and three days after storage ($p = 0.51$).

DISCUSSION

Platelet concentrate is prepared using three different methods in which some plasma is added to platelets. This plasma contains biologically active agents that can cause platelet transfusion reactions, particularly allergic reactions (Heddle *et al*., 1994). Complement protein C3 is among these agents in plasma, which plays a critical role in complement activation and generation of anaphylatoxin factors (Nilsson and Ekdahl, 2012). It's believed that the platelet stimulation and expression of P-selectin and other platelet contents and granules cause the complement activation (Del Conde *et al*., 2005; Hamad *et al*., 2008; Peerschke *et al*., 2006). Although, in other study the C3a of complement degradation can mediate platelet activation (Polley and Nachman, 1983).

Our findings show that the level of C3 in platelet product from apheresis is significantly lower than whole blood method. Burnouf *et al*. (2003) in a study showed very low levels of complement products in various apheresis methods, which may be due to consumption of plasma proteins during apheresis method whereas, Bock *et al*. (2002) showed the lower C3 degradation products in apheresis platelet than platelet concentrate from buffy coat. Nadiah *et al*. (2013) in their study found that the level of some plasma proteins is reduced after platelet apheresis. Andreu *et al*. (2007) study indicated that leuco-reduced Platelet Rich Plasma Concentrate (PRPC) versus leuco- reduced Apheresis Platelet Concentrate (APC) was same in febrile non hemolytic transfusion reactions and bacteria contamination but the incidence of allergic reactions with APC was four times more than PRPC. The allergic reactions in platelet components prepared in additive solution instead of plasma was 10 times less than APC. Heddle *et al*. (1994) showed that the transfusion reactions were more induced by plasma than cells and in older platelet components was more frequent. In contrast with previous studies, Chambers and Herman (1999) have reported that apheresis platelet associated with less allergic reactions than whole blood platelet components and not correlated with storage age and leucocyte (Muylle *et al*., 1996). Considering the fact that donor

blood is processed in cell separator machine, platelets are separated into a separate bag, the remaining cells and plasma are returned to donor, consumption of these compounds can be due to constant contact of blood with different surfaces of platelet apheresis instrument during the preparation of platelet concentrate (Burgstaler, 2006; Chambers and Herman, 1999). However, further studies are required on the absorption property of the materials used in these instruments. The complement C3 level in platelet concentrate prepared by apheresis method is reduced one day after storage, which could be due to activation of the complement system as a result of C3 conversion to C3a and C3b. In study of Metcalfe *et al*. (1997), the platelet activation was higher in PRP single donor platelet concentrates in comparison with apheresis platelet concentrates.

In platelet, preparation from whole blood, donated blood is subjected to low and high speed or heavy centrifuge after collection and then the isolated platelets enter into separate bags together with some plasma (Seghatchian and Krailadsiri, 1997; Tynngard, 2009). The period between whole blood collection and processing sometimes reaches over 8 h, which can cause several changes in blood bag, including activation of the complement system and thus reduced C3 level. Hyllner *et al*. (1997) support this hypothesis, as they showed that the complement system is activated during the storage of whole blood and not blood-derived products such as RBC. Schleuning *et al*. (1992) also showed similar results.

Finally, this significant difference in C3 level can indicate that the use of platelet from apheresis method can most likely increase platelet transfusion reactions due to increased level of anaphylatoxins.

Another important factor is the choice of bags for storage of platelet products. Our study showed a significant correlation between the type of bag and complement C3 level in platelet concentrate (Fig. 2). However, we found no study on this subject in our search. Although, McLeod *et al*. (1983) in a study have shown that some plastic surfaces have the ability to activate the complement system during plasmapheresis. Therefore, different plastic compounds are used in platelet storage bags, it can be concluded that this difference is due to the material used in the bags (Prowse *et al*., 2014; Seghatchian and Krailadsiri, 1997).

The C3 concentration was increased from the second to the third day in both platelet products (Fig. 1). Other studies were similar and confirmed our findings (Gyongyossy-Issa *et al*., 1994; Schleuning *et al*., 1994). Two hypotheses can be addressed in this regard. Given the secretion of many complement components by leukocytes (Hogasen *et al*., 1995), the first assumption is secretion of C3 by leukocytes remaining in the platelet concentrate. The second assumption can be considered in connection with the method of C3 measurement, so that excessive accumulation of some materials in the bag might have caused false positive error.

In overall, although the complement activation can led to platelet storage lesions and reduce the quality of platelet concentrates, that not means that the complement degradation products were the only factor affect the quality of them (Bradley *et al*., 2008) and other factors should take in account.

In summary, this study showed that the method of platelet preparation and storage affects C3 concentration in the final product and whole blood method seems to be better for platelet production. It is suggested to examine the bag type for storage of platelet concentrate and choose the bag with minimum effect upon the complement systems.

CONCLUSION

Platelet transfusion is a vital therapeutic measure in patients with thrombocytopenia associated with life-threatening bleeding. However, platelet preparation for injection involves

various microparticles as inflammatory mediators that can cause allergic reactions due to platelet transfusion. We have shown that platelet preparation and storage method plays a role in the level of these agents. As a result, further studies can show the role of these agents in reactions caused by blood transfusions.

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