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QUALITY ASSESSMENT OF PLATELET CONCENTRATES PREPARED BY DIFFERENT METHODS

Pathology

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ABSTRACT

Background: Role of any transfusion services is to provide blood and blood products which are safe, pure, potent and effective. So quality control is the most important parameter to assess the efficacy of blood and its component. The present study was undertaken to assess the ex-vivo quality of platelet concentrates prepared by three different methods and compare the efficacies of all three methods on the basis of various parameters.

Method: The study evaluated a total of 200 randomly selected samples of platelet concentrates, of which 150 were Random Donor Platelets (RDPs) and 50 were platelet Apheresis/ Single Donor platelets (SDPs). Out of 150 RDP samples, 100 were prepared by platelet rich plasma-platelet concentrate (PRP-PC) and 50 by buffy coat-platelet concentrate (BC-PC).In-vitro quality of platelet concentrates were assessed by observing volume, platelet yield, WBC and RBC contamination, pH changes, swirling, culture study, and compared amongst three methods.

Results: Comparing all three methods, Apheresis had significantly highest platelet yield, PH value and least WBC contamination.Variation in volume was more in PRP-PC. All three methods were comparable in terms of RBC contamination and swirling. PC prepared by all the three methods was negative for any microbiological growth.

Conclusion: Apheresis-PC units showed superior platelet yield, improved other quality control parameters and reduced the risk of infection, allo-immunization to the recipients. Hence it should be considered as a best method amongst all the three methods.

KEYWORDS

Quality control, Platelet yield, Buffy coat, Platelet rich plasma, Apheresis, Swirling, Contamination

INTRODUCTION

Platelets or thrombocytes are the cells circulating in the blood that are involved in cellular mechanisms of primary hemostasis leading to the formation of blood clots. Dysfunction of platelets (thrombasthenia) or low levels of platelets (thrombocytopenia) predisposes to bleeding. Until 1970, thrombocytopenic bleeding was the major cause of serious morbidity and mortality in patients undergoing intensive chemotherapy for malignancy [1].Platelet concentrate (PC) are an important resource to treat patients with either qualitative or quantitative defects. Platelet concentrate (PC) is defined as the component derived from fresh whole blood that containsthe majority of the original platelet content in a therapeutically effectiveform [2].

There are two types of platelet concentrates available for transfusion; one which is the co-product of normal blood donation i.e. random donor platelets (RDP), (platelet rich plasma-platelet concentrate (PRP-PC) and buffy coat poor-platelet concentrate (BC-PC) and the other is single donor platelets (SDPs), (apheresis-PC,) collected from voluntary thrombocytapheresis donors with the help of an automated cell separator [3].The basic principle behind preparation of components from whole blood is that each component has its specific gravity and by applying centrifugation, each component is separated and removed, thus allowing the transfusion of desired component according to the need of the patient. The recommended shelf life of platelet concentrates in presently available platelet storage bags is 5 days at 22±2°C with continuous agitation. The platelets undergo various storage changes starting from collection, processing to storage and the underlying conditions within the patients, which may affect the therapeutic benefit to the recipient [4].

The quality control is the most important parameter to assess the benefit of each blood component specially the PC.Therefore it is required to study the ex-vivo quality of PCs prepared by different method to establish the maximum therapeutic benefits to the patients. In vitro platelet quality can be assessed by using certain parameters (swirling, volume, platelet count and WBC count per bag and pH changes) [3].Both national and international quality requirements have been implemented and are a part of the health quality management. Several approaches to perform QC of blood components have been promulgated by the American Association of Blood Banks (AABB), the Council of Europe (CE), and Director General of Health Services (DGHS) India. These are among the few that provide benchmarks for meeting the quality of blood components [5].

In present study, three different methods of platelet preparations are used, namely, single donor derived apheresis product, whole blood-derived platelets prepared by platelet-rich plasma (PRP-PC) method, and buffy coat (BC-PC)method. This study was undertaken with the goal to assess the ex-vivo quality of PCs prepared by above three methods and compare the efficacies of all three methods on the basis of various parameters.

MATERIALS AND METHODS

Atotalof 200 randomly selected samples of platelet concentrates were studied, of which 150 were Random Donor Platelets (RDPs) and 50 were platelet Apheresis/ Single Donor platelets (SDPs). Out of 150 RDP samples, 100 were prepared by platelet rich plasma method (PRP-PC) and 50 by buffy-coat method (BC-PC). The study was done mainly from the segments of these routine, already prepared platelet-concentrates. These concentrates were prepared during the period of eighteen months in the blood bank of tertiary care hospital.

(A)For Random Donor Platelets (PRP-PC and BC-PC)

Routine blood collection was done by outdoor blood donation camps and indoor collection with all the prerequisites and consent from the donor. Timely component separation was done as per departmental standard operating procedures by trained FDA approved technicians. Platelet concentrates (PC) were prepared. PC was kept at 22^0 C under constant agitation in platelet incubator with agitator meant to store untested platelets. Serology was done, if serology (HIV,HBsAg, HCV, malaria, VDRL) was negative, it was taken on stock, then stored in platelet incubator with agitator to store tested platelets. After thorough mixing, PC bag was inspected for swirling against light for motion of platelets and any gross RBC contamination. PC volume checked on electronic digital weighing scale. After thorough squeezing and stripping of PC segment, around 2-3cms of segment obtained. Platelet count, WBC and RBC count done in an electronic blood cell counter. Same samples were used for pH analysis. Few of the above segments were labeled and sent to Microbiology Department for culture studies. Statistical analysis was performed.

(B)For Single Donor Platelets (SDP)

The donor is explained about the procedure and informed consent was taken from them.Serology (HIV, HBsAg,etc.) and blood group testing was done. If serology was negative, the donor can donate. Apheresis procedure performed under aseptic conditions. Small amount of platelet quantity was also collected in the attached platelet sample pouch in a closed system after thorough mixing. Platelet concentrates kept at 22^0 C under constant agitation in platelet incubator with agitator meant to store untested platelets. After thorough mixing, PC bag was inspected for swirling against light for motion of platelets and any gross RBC contamination. PC volume checked on electronic digital

weighing scale. Platelet count, WBC and RBC count done in an electronic blood cell counter. Same samples were used for pH analysis. Few of the above segments were labeled and sent to Microbiology Department for culture studies. Statistical analysis was performed.

(C) Quality assessment of platelets

The quality assessment of apheresis-PC and RDPs [platelet richplasma-platelet concentrate (PRP-PC) and buffy coat-plateletconcentrate (BC-PC)] were done. A proposed totalof 200 platelet units, (PRP-PC, BC-PC and apheresis-PC:100, 50 and 50 units respectively) were selected randomlyand tested for the following parameters:

i)Platelet yield: It was evaluated on an automated hematology cell counter from platelet concentrate segment (A). Platelet yield per bag or per unit was calculated as:
Platelet yield/unit= platelet count (A) $\times 10^6 \times$volume of platelet unit in ml.

ii)WBC contamination: It was evaluated on an automated hematology cell counter from platelet concentrate segment (B). WBC Contamination per bag or per unit was calculated as:
WBC Contamination/Unit=WBC count (B) $\times 10^6 \times$volume of platelet unit in ml.

iii)RBC contamination: It was evaluated on an automated hematology cell counter from platelet concentrate segment ©.

RBC Contamination per ml was calculated as: RBC Contamination/ml=RBC count © $\times 10^9$.

iv)pH: It was evaluated with the help of pH meter.

v)Volume of the platelet concentrate: Measured by electronic scale and dividing by specific gravity of the platelets (1.058).

vi)Swirling: After thorough mixing, the individual units of platelet-concentrates were inspected against light for the swirling movements of platelets and for the presence of platelet-clumps, if any.If there was no swirling movement of the platelets, that unit was considered swirling – negative and if swirling was seen then it was classified as swirling-good for good swirling properly of that unit, swirling-intermediate for lesser swirling and if platelet clumps were observed then swirling-positive with platelet clumps.

vii)Microbiological study: Another 2-3cm of the sealed platelet segment was sent to the microbiology department for culture-assay to check for sterility.

All these parameters were assessed as per the requirements of Directorate General of Health Services, India (DGHS).

Data Analysis

The parameters were analyzed using ANOVA test. 3 methods of platelet concentratei.e. PRP-PC, BC-PC and Apheresis/SDP were compared using Post-hoc Tukey's test. The parameter of swirling of the platelet-concentrates was analyzed using Chi Square Test. A probability of $p<0.05$ was considered to be significant.

Observations and Results

The quality parameters of 200 platelet concentrates (50 for BC-PC, 100 for PRP-PC and 50 for apheresis-PC) were studied for checking the quality control of platelet concentrate prepared by different methods. Table 1 shows the platelet yield, WBC contamination, RBC contamination, pH and volume by above three methods as expressed in Mean±SD (SD = standard deviation) format.

Table 1: Comparison of quality control parameters amongst three methods

Parameters	Random Donor Platelets (RDP)		Single Donor Platelets (SDP)
	PRP-PC	BC-PC	Apheresis
Platelet Yield	$4.912\times10^{10}\pm1.36$	$6.253\times10^{10}\pm1.9$	$4.440\times10^{11}\pm0.93$
WBC contamination	$0.404\times10^{8}\pm0.32$	$0.473\times10^{8}\pm0.33$	$0.123\times10^{8}\pm0.12$
RBC contamination	0.056×10^{9}/ml±0.02	0.041×10^{9}/ml±0.03	0.056×10^{9}/ml±0.04
pH	6.942±0.20	6.770±0.18	7.096±0.17
Volume	51.54±2.95	55.6±2.66	309.32±31.35

Amongst the RDPs, BC-PC shows higher platelet yield than PRP-PC, ($p<0.001$) but comparing all three methods, Apheresis has the highest platelet yield, ($p<0.0001$). All the three methods showed >75% of the PC bags to have platelet count of $>3.5\times10^{10}$/unit for RDPs> 3.0×10^{11}/unit for SDPs. Thus, 100% of BC-PC, 98% of PRP-PC and100% of Apheresis-PC satisfied the standardized quality control criteria.

PRP-PC shows lesser WBC contamination than BC-PC but difference was not statistically significant, ($p>0.05$) whilecomparing all three methods, Apheresis has the least WBC contamination ($p<0.0001$).All three methods showed >75% of the PC bags to have WBC contamination of $<5.5\times10^{7}$-5×10^{8} for RDPs and $<5\times10^{6}$/unit for SDPs. Thus, 100% of all the PC methods satisfied this standardized quality control criteria.

BC-PC shows lesser RBC contamination than PRP-PC and Apheresis-PC showed RBC contamination similar to PRP-PC.However, difference was not statistically significant, ($p>0.05$). Thus, none of the method is superior in terms of RBC contamination.All the three methods showed >75% of the PC bags to have RBC contamination of <0.5 ml. Thus, 100% of all the PC methods satisfied this standardized quality control criteria.

Amongst the RDPs, PRP-PC has higher pH value than BC-PC but the difference was statistically significant. Comparing all three methods, apheresis has the highest pH value and the difference was statistically significant.Variation in volume was more in PRP-PC. All the three methods showed good swirling activity and all the PCs were comparable in terms of swirling, (Table 2). PC prepared by all the three methods were negative for any microbiological growth.

Table 2: Comparison of swirling of PC by different methods

Swirling		Random Donor Platelets (RDP)		Single Donor Platelets (SDP)
		PRP-PC	BC-PC	Apheresis
Positive for swirling	Positive	76	80	86
	Intermediate	11	12	10
	Positive with visible PLT clumps	7	4	2
Negative		6	4	2

DISCUSSION

In the present study commonly used and standardize ex-vivo parameters were correlated and compared those for three methods of PC preparation i.e. PRP, BC and apheresis method.We have selected 350ml bags as they are frequently used and thus easily available in our blood bank. The self-life of platelets is for 5 days but our storage of these PC was only for 24 to 48 hours due to their increased needas compared to limited availability. If the platelets are stored beyond their maximum storage capacity, it will result in immediate negative effects on platelet function, activation and disintegration [6].

Platelet yield is the most important quality control parameter which will compare the efficacy of PC. Higher platelet count responds to higher yield [7] and the higher the platelet yield, better is the method of preparation. Platelet yields reflected in transfused platelet dose, influences platelet recovery in the patients and allows prolonging interval between transfusions [7,8]. In present study amongst the RDP, BC-PC showed significantly higher platelet count than PRP-PC, this finding is agreement with the previous studies [9-11].However on comparing Apheresis/SDP with the RDP, SDP platelet-concentrates showed significantly higher platelet count which is similar to the study done by Singh et al [3]. Previous studies [3,12,13] had platelet values well above the quality control guidelines of greater than 3.0×10^{11}/unit. Existing study was conducted on the platelet concentrates being prepared from 350ml bags than other studies [3,12,13] which were done from 450ml bags therefore our platelet counts were lower than those of other studies [3,12,13]but >75% compliance to the quality guideline parameters suggests good platelet concentrate preparation.

Platelet-apheresis is considered to yield a superior quality of platelets than the Random-donor platelet concentrates as this technique is designed to only collect the platelets. However, if pooling of the random donor platelet concentrates is done in an appropriate manner, its platelet yield can be comparable to that of apheresis –platelet concentrates. But, as pooling of the PCs is not practiced in our institution, this comparison study could not be done. Thus PC by

Apheresis should be preferred method due to its significantly superior platelet-yield and its added advantages, but adequately cautious approach in preparing them by Random-Donor method may be preferred as a cost effective approach according to the catered needs of this product.

WBC contamination is an important parameter for assessing the quality of PC preparation. It has long been a principle of blood component production that they should contain as few white cells as possible, since leucocytes increase the risk of untoward complications. WBC's in platelet concentrates have a detrimental effect on them with significant drop in pH, increase in glucose consumption, lactic acid production and LDH release during storage. In current study, amongst RDP, BC-PC showed WBC contamination greater than the PRP-PC. Both the methods were well above the set quality control of >75% of the PC bags to have WBC contamination of less than of 5.5×10^{7}-5×10^{8}/unit, 100% of both methods satisfied this criteria, this is in agreement with the study done by Racz et al [11]. On comparing SDP with RDP, SDP-PC showed significantly lower WBC contamination than RDP, this is comparable with the results found in the other studies [3,14].

RBC contamination should be minimum in a given platelet concentrate. The lesser the contamination, better is the quality of the PC. Amongst the RDPs, BC-PC showed lesser RBC contamination than the PRP-PC.The reason could be that initial soft spin of PRP-PC could lead to incomplete separation of RBCs from the platelet rich plasma above. Also slight errors in the manual clamping of the tubing as soon as RBC layer reaches the top of the bag can also led to its contamination.Folds created due to inappropriate placement of the bags during centrifugation in RDPs can lead to RBC trapping, thus preventing its sedimentation and causing increased contamination. Both the methods (BC-PC, PRP-PC) were well above the set quality control of >75% of the PC bags to have RBC contamination of less than of 0.5ml, 100% of both methods satisfied this criteria. On comparing SDP with RDP, SDP –PC showed RBC contamination similar to PRP-PC. In a study done by Evaldo [15], red cell contamination is more in PRP-PC than BC-PC which is in agreement with current study.

Extremes of pH have detrimental effect on the viability of platelets is a known fact. Recommended quality control guidelines are pHof greater than 6.0 at the end of its maximal storage period. However pHvalues of greater than 7.4 are also harmful to the quality of platelets. Amongst the RDP, PRP-PC had higher pHthan BC-PC and the difference was significant statistically and on comparing them with SDP, the latter showed a significantly higher pH than RDPs.Both BC-PC and PRP-PC method were well above the set quality control of >75% of the PC bags to have pHof >6.0, 100% of both methods satisfied this criteria. The findings of present study were comparable with the other studies [3,14, 15].

Platelets prepared from whole blood or by platelet-apheresis, are stored in the donor plasma. This serves as a good buffering agent for the platelets and also helps to maintain their pH. PC's prepared from RDP are stored in 40-70 ml of plasma. The platelet-suspending volume should be such that it should prevent volume overload at the same time also maintain the viability, buffering capacity and the pHof PCs. With the advent of new second generation highly oxygen permeable containers, there is a very small risk of exhausting the plasma-buffering capacity as the platelet energy metabolism is predominantly aerobic. Under these conditions, there is relatively little information on how much plasma is required to maintain the viability. In current study, amongst the RDP, BC-PC showed greater volume than the PRP-PC.Both the methods (BC-PC, PRP-PC) were well above the set quality control of >75% of the PC bags to have volume of >50ml, 77% of PRP-PC and 100% of BC-PC satisfied this criteria. Apheresis/SDP platelet concentrates had volume with an average of 309.32±31.35 ml/unit. 100% of these units satisfied the quality control of >200 ml. in a study done by Singh et al [3], the Apheresis-PC had volume of 214±9ml/unit. Thus, there is a lot of variation in the volumes of the platelet concentrates in the above mentioned study as many of them had been prepared from 450 ml bags as compared to our study being done from 350ml bags. Standardization is required considering a wide variation range in the volumes of the platelet concentrates.

Evaluation of swirling is a simple noninvasive procedure that can perform by visual inspection and is useful for routine quality control of each individual PC on a large scale [3]. Visual inspection of swirling correlates with platelet morphology; the presence of swirling indicates discoid morphology and its absence correlates well with platelet disc-to-sphere transformation. This morphological feature has been associated with poor platelet viability [16].All the three methods of our study showed good swirling activity and found no difference among the three methods of platelet concentrates. Absence of platelet swirling may be used as one of the surrogate markers for bacterial contamination of the PC, though its sensitivity is less than microscopy with Gram's stain [17]. However in present study, absence of platelet swirling did not correlate with the bacterial contamination as all the platelet segments were negative for bacterial contamination.

Bacterial contamination is considered the second most common cause of death overall from transfusion with mortality rates for platelet-related sepsis ranging from 1:20,000 to 1:85,000 donor exposures. Recent studies of platelets have suggested a bacterial contamination rate of about 1 per 1-3,000 units, clinical sepsis in about 1 per 20,000 transfusions and related fatality in about 1 per 60,000 transfusions as compared to bacterial contaminated RBCs which is about 1 per 500,000. Bacterial contamination can occur due to bacteremia, contamination during the whole blood collection procedure, contamination of the collection pack and contamination during the blood processing procedure. Thus, care and asepsis has to be ensured at each and every step from blood collection/platelet-collection to its separation to storage and its use in the recipient. Thus, various measures should be aimed at reducing the risk of the transfusion of cellular blood product units that are contaminated with bacteria by: 1) reducing recipient exposure; 2) avoiding contamination (i.e. improved donor screening or better skin disinfection); 3) optimizing blood component processing and storage; and 4) implementing tests and procedures that can be used to detect the presence of bacteria in blood product units.In present study, selected platelet segments were sent to microbiology department for culture on day 2 of the platelet preparation to check for growth of micro-organisms 20 segments each of all three methods were tested. All the tested samples were negative for any bacterial growth.

CONCLUSION:

Considering the significant superior yield of the platelet-concentrates prepared by single-donor platelet method/Platelet-Apheresis method, improvedvarious quality control parameters and many other advantages including reduced risk of infection, allo-immunisation to the recipients, it should be considered the best method amongst all the three methods compared in current study. Hence, increase use of this method for platelet concentrates preparation is advocated.

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