Evaluation of Random Donor Platelets Produced from Buffy Coat Stored for 24 hrs at Ambient Temperature. A Better Alternative to 8 hr Limitation

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Abstract:

Introduction-*Whole blood derived platelets are made from platelet rich plasma (PRP) method or buffy coat method. In India majority of random donor platelets are prepared by PRP method. However, Buffy coat method offers the advantage of less platelet activation and fewer WBC contamination. Presently in India RDPs are prepared within 8 hour of whole blood collection, whereas, in Europe this time limit is up to 24 hours.*

Aim-*Our aim was to evaluate the platelet count, WBC contamination, platelet CD 62 P expression, and biochemical parameters of RDPs prepared from buffy coat within 8 hours and within 24 hours of collection.*

Materials and method-*We prepared 40 units of RDP by the buffy coat method from whole blood stored at room temperature within 8 hours of collection (Fresh BC),& another 40 units from buffy coat stored at 22°C for less than 24 hours (Stored BC). We analyzed the platelet counts, CD62P expression, WBC counts, glucose levels, pH, PO_2, PCO_2 in both the groups of RDPs, 24 hours after respective preparation.*

Results-*The platelet count from stored BC was higher in fresh BC. CD 62P expression was low in stored BC compared to fresh BC. There were no differences pH, pO_2, pCO_2 and glucose levels between fresh BC and stored BC. WBC contamination was more in fresh BC.*

Conclusion-*Our study stored BC contained higher platelet counts, less WBC contamination and less platelet activation. We conclude that RDP prepared from stored BC is the better method for RDP production*

Keywords: Buffy coat, Platelet count, WBC contamination, Platelet activation, CD 62P expression.

I. INTRODUCTION

Buffy coat (BC) preparation of platelet is the most popular method of preparation of Random Donor Platelets (RDP) in countries such as Canada, UK and Europe. In Europe, there is an approximately 50:50 split between the use of BC- and apheresis-derived platelet concentrates (PC). [1] In Canada (Quebec exempted), 70% of platelets are derived from whole blood donation. Denmark, Finland, and the Netherlands prepare 85% to 95% of their concentrates by the BC method [2]. These countries demonstrate that a national platelet supply can be derived almost entirely from collected whole blood rather than relying upon apheresis PC production. [1, 2] A special kind of bag which has top and bottom outlets are used to prepare the platelet concentrate in this technique. Whole blood collected into the main bag is subjected to heavy spin and semi-automated expresser is used to expel out platelet poor plasma from the top port and red blood cells from the bottom port. Thirty mL of RBC and thirty mL of plasma remains in the main bag with majority of platelets and WBCs. The main bag is subjected to re-centrifuge in soft spin and platelet rich plasma is expelled out and collected from the top port, leaving buffy coat and red blood cells in the main bag.

In the United Kingdom and continental Europe, four to six buffy coats are combined, re-suspended in plasma from a male donor or in a platelet additive solution, and soft centrifuged to remove red cells and white cells. [3] Platelets can be prepared using this method from WB stored at

room temperature (not less than 20°C) for up to 24 hours [4]. Advantages of the buffy-coat method are that there is less activation of platelets than in the PRP method because in the buffy-coat method, platelets are cushioned against red cells during the hard spin. Plasma units prepared from buffy-coat-depleted units have approximately 41 mL more plasma than plasma units prepared by the PRP method. Platelets prepared by the PRP method result in 21% of the plasma and 19% of the platelets remaining with the red cells. Therefore, the hematocrit of the packed RBCs produced with the PRP method is lower (51%) than in the buffy-coat method (60%). Buffy coat depletion also results in a 13% loss of the donated red cells.[5] In PRP method of platelet preparation PRP is subjected to hard spin leading to formation of platelet pellet. Pelleting make close contact of platelets which make them activate temporarily. In contrast buffy coat method gives the luxury of cushion to platelets among RBC during the hard spin. Therefore platelets are less activated than PRP method. [6]

Platelet activation during processing and throughout storage is accompanied by surface expression of sequestered granular membrane proteins (P-selectin and CD63) and conformational changes of the fibrinogen receptor, GP-IIb/IIIa. [7] P-selectin (also known as CD62P, GMP-140, and PADGEM) is an integral membrane protein found in α-granules that becomes expressed on the surface of activated platelets after granule release. [8]

In India when RDP is prepared by the buffy coat method, it is done so, almost entirely from the whole blood stored at room temperature, for less than 8 hrs as per DGHS guidelines. [9]

The aim of our study was to evaluate the platelet count, WBC contamination, platelet activation and other biochemical parameters of platelet concentrate prepared from buffy coat within 8 hrs and within 24 hrs of collection, respectively, in order to assess and compare the two methods

II. MATERIALS AND METHOD

Forty units of RDP prepared from fresh buffy coat separated from whole blood within 8 hrs of collection (Fresh BC) and forty units of RDP prepared from buffy coat stored at 22° C, overnight () were studied in an unpaired study design.

Eighty units of whole blood were collected in to quadruple bag (Fenwal, Lake Zurich, IL 60047, United States) and subjected to high spin separation within 8 hrs of collection. Semi-automated plasma expresser (Optipress – Fenwal, Lake Zurich, IL 60047, United States) was used to express out the platelet poor plasma from the top port of the bag and red blood cells were expressed out from the bottom port leaving buffy coat and platelets in the primary collection bag. Out of them 40 units were subjected to rest for one hour and thensubjected to soft spin centrifugation. Platelets were expressed out from the top port using semi-automated plasma expresser, leaving the buffy coat and RBC in the primary collection bag. The other 40 units were left undisturbed overnight at 22° C and RDPs were prepared after soft spin centrifugation on the following day. Samples were taken after 24 hrs of preparation of platelets and day 5 of blood collection in both the groups.

All in vitro assays were undertaken according to the established validated method. Platelet counts and WBC counts were determined by using automated cell counter (Sysmax, Wakinohama-Kaigandori, Chuo-ku, Kobe, 651-0073, Japan) in the blood bank. In vitro glucose values were determined by the biochemistry laboratory of the hospital. [National accreditation board of laboratories (NABL) accredited lab]. pO2, pCO2 and PH were determined by using blood gas analyzer (Roshe, Carolina Inc.Old Marion Highway, Florence, SC, United States). Platelet surface CD 62P expression were measured by using flow cytometry (FACS Calibur, BD Biosciences, Qume Drive,San Jose, California, USA). Two samples from each platelet units (50 uL) were taken and one sample was stained by 10 uL monoclonal mouse antibodies and phycoerythrin according to the manufacture's instruction. Second sample was analyzed without staining. First unstained sample was analyzed, followed by the stained sample. 6,000 events per second were counted in both samples by the flowcytometer. Result graphs of both samples were superimposed and results were obtained as a percentage of activation.

Statistical analysis were done by using IBM SPSS statistic software version 21 and $p < -0.05$ were considered as statistically significant.

Randomly selected platelet samples were sent for bacteriological culture to the microbiology department of the hospital.

III. OBJECTIVES

Our primary objective was platelet count in the RDP and secondary objectives were WBC count, pH, glucose, pO2, pCO2 and platelet surface CD 62P expression

IV. RESULTS

At 24 hrs after preparation:

Fresh BC and Stored BC showed mean platelet counts of 5.7 x 10^{10} ± 1.57 and 6.32 x 10^{10} ± 1.18 per unit, respectively. Among the fresh BC, 31 (77.5%) out of 40 units contained more than 6 x 10^{10} platelets per unit whereas in stored BC only 36 (90%) units out of 40 units contained more than 6 x 10^{10} platelets per unit. Mean WBC contamination in fresh BC was 5.88 x 10^6 ± 2.85 and stored BC was 5.55 x 10^6 ± 1.53 per unit respectively.(Fig 1) Fresh BC, 31 units and stored BC 35 units out of 40 units each, contained less than 5.5 x 10^6 WBC respectively. Mean CD 62 P expression in fresh BC and stored BC showed 33.35% ± 13.75 and 19.5% ± 14.45 respectively(Fig 2). Range CD 62 P expression of platelet in fresh BC was 14% to 55% and in stored BC, it was 4% to 44%. Mean plasma glucose levels in fresh BC was 389 mmol/L ± 67.91 and stored BC was 360 mmol/L ± 62.18. Mean pH of the fresh BC was 6.99 ± 0.23 where as in stored BC was 6.91 ± 0.21. Mean pCO_2 of both fresh BC and stored BC was 7.7 kPa ± 3. Mean pO_2 of the fresh BC was 17.12kPa ± 4.1 and stored BC 16.5kPa ± 3.12. (Table 1)

At 5th day of storage:

Fresh BC and Stored BC showed that mean platelet counts at the 5th day of storage were 5.5 x 10^{10} ± 1.4 and 5.83 x 10^{10} ± 1.05 per unit, respectively. Mean WBC contamination in fresh BC after 5 days of storage was 5.33 x 10^6 ± 2.69 and stored BC was 4.93 x 10^6 ± 0.59 per unit respectively. CD 62 P expression in fresh BC and stored BC at the end of the 5 day storage showed that 38.47% ± 13.18 and 22.3% ± 13.86 respectively. Mean plasma glucose levels in fresh BC was 379.5mmol/L ± 56.48 and stored BC was 348.35 mmol/L ± 53.13. Mean pH of the fresh BC was 6.93 ± 0.27 where as in stored BC was 6.85 ± 0.25. Mean pCO_2 of fresh BC and stored BC were 8.55 kPa ± 2.72 and 8.4 ± 2.58 respectively. Mean pO_2 of the fresh BC was 16.6 kPa ± 3.46 and stored BC 15.37 kPa ± 2.82. Microbiological cultures of randomly selected samples of both fresh BC and stored BC were found to be sterile.(Table 2)

Table 1: 1 Mean values of each variables of platelet concentrate of 24hrs after of preparation.

Variables	Fresh BC	Stored BC
Platelets x 10^{10} per unit	5.7 ± 1.57	6.32 ± 1.18
WBC x 10^6 per unit	5.88 ± 2.85	5.55 ± 1.53
CD 62P expression %	33.35 ± 13.75	19.5 ± 14.45
Glucose mmol/L	389 ± 67.91	360 ± 62.18
pO_2 (kPa)	17.12 ± 4.1	16.5 ± 3.12
pCO_2 (kPa)	7.7 ± 3.07	7.7 ± 3.07
pH	6.99 ± 0.23	6.91 ± 0.21

Table 2: 1 Mean values of each variables of platelet concentrate of 5 days after of preparation

Variables	Fresh BC	Stored BC
Platelets x 10^{10} per unit	5.5 ± 1.4	5.83 ± 1.05
WBC x 10^6 per unit	5.33 ± 2.69	4.93 ± 0.59
CD 62P expression %	38.47 ± 13.18	22.3 ± 13.68
Glucose mmol/L	379.5 ±56.48	348.35±53.13
pO_2 (kPa)	16.6 ± 3.46	15.37 ± 2.82
pCO_2 (kPa)	8.55 ± 2.72	8.4 ± 2.58
pH	6.93 ± 0.27	6.85 ± 0.25

V. DISCUSSION

In our study we studied the effect of ambient temperature on platelet concentrate which was prepared from overnight hold buffy coat, and results were compared with platelet concentrate prepared from fresh buffy coat within 8 hrs of collection. It was found that the stored BC contained comparatively higher platelet counts, and were less activated compared to the fresh buffy coat prepared platelet concentrate.

Lower platelet count in the Fresh BC may be due to relatively less resting period of buffy coat before preparation of platelets. Platelets within the 8 hrs of collection, are still in a relative stage of aggregation and activation due to the centrifugation they were subjected to, while being prepared. Due to these possibilities platelets were trapped among the concentrated WBCs and could not come to the plasma layer during soft centrifugation. On the other hand stored BC, have the advantage of relatively more resting period during which period the aggregation and activation of platelets would reduce, which will eventually lead to more and more platelet release into the plasma layer in soft second centrifugation of platelet production. In our study 10.87% more platelets were concentrated in stored BC compared to fresh BC. Margriet J, Dijkstra-Tiekstra,, Willeke Kuipers, at el explained in their study that the overnight platelet concentrate (PC) showed higher PLT count (approx. 460 × 10^9/PC vs. approx. 310 × 10^9/PC) compared to that of fresh buffy coat derived PC.[10]. This finding was comparable with our results.

Mean WBC contamination of fresh BC and stored BC was 5.88 ± 2.85 and 5.55 ± 1.53 WBC x 10^6 per unit respectively. There was more contamination with WBC (5.94%) more WBC s in fresh BC compared to stored BC. Overnight exposure to room temperature (20 – 24°C) lead to lysis of the WBC s, and, overnight undisturbed standing may have settled down the WBCs in the stored buffy coat. Therefore stored BC contained less WBC s compared to fresh BC. Fig 1 CD62 P expression in the 24 hrs after the production, was 33.35 ± 13.74% in fresh BC and 19.5 ± 14.44 % in stored BC respectively. Thus in fresh BC, 13.85% more platelets were activated, compared to that of stored BC. However this difference was not statistically significant. At the end of the 5th day of storage both fresh and stored BC platelets expressed CD 62 P more than their respective 1st day values. In fresh BC 5.13% more platelets expressed CD 62 P after 5 days of storage when compared to that of fresh BC in 1st day. This difference was statistically significant. In stored BC 2.8 % more platelets were activated when 1st day and 5th day were compare but was not statistically significant. Comparison of both fresh and stored BC at the 5th day of storage has shown that fresh BC contained 11.18 % CD 62 P expressed platelets than in the stored BC.(Table 2) High expression of CD 62 P of platelets in fresh BC appear to be due to shorter resting period compared to stored BC. Longer resting period would result in disaggregation as well as less activation of the platelets. However the platelets expressed CD 62 P more and more during the storage due to reduced pH.

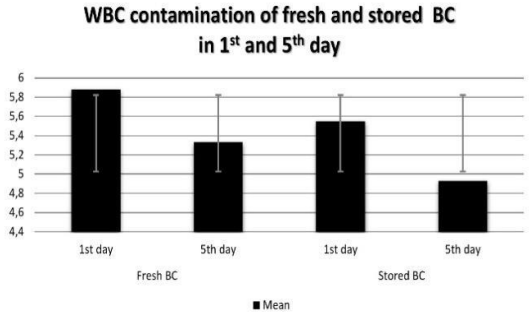

Figure 1:

We analyzed metabolic parameters of the platelets, such as glucose pO_2 pCO_2 and pH after 24 hrs and 5th day of storage. Amount of glucose (mmol/L) in fresh BC was 389 ± 67.91 and in stored BC was 360 ± 62.18. WBC s and platelets stored overnight in room temperature, metabolized glucose and produced lactic acid as a byproduct. This was the reason of reduced amount of glucose in stored BC. Due to increased amount of lactic acid production in stored BC, pH has reduced to 6.91 compared to fresh BC (6.99). However pH was within the quality control parameters of buffy coat platelets. The pO_2 and pCO_2 values of both products were almost similar. However none of the metabolic parameters between two groups were statistically significant even after 5 days of storage. Margriet J, Dijkstra-Tiekstra,, Willeke Kuipers, at el found that the overnight PC higher pCO2, and lactate concentration and lower pH, pO2, glucose concentration, CD62P expression (until Day 5). We did not measure the lactate concentration but rests of the findings are comparable with our results [10]. In another study M.J. Dijkstra-Tiekstra, P.F. van der Meer, R. Cardigan, at el studied the lactate, pCO2, and hypotonic shock response pH, glucose, pO2, and CD62P expression swirling effect, white blood cell count, annexin V binding, oand aggregation between overnight and fresh buffycoat derived PC. They found significant difference in lactate, pCO2, hypotonic shock response, pH, glucose, pO2, and CD62P expression between the fresh and overnight held PC. In our study we found

that findings were not statistically significant [11]. Another similar study done by Fa Qiang Lu, Wei Kang, Yu Peng, Wei Ming Wang revealed that the platelet yield in PCs prepared from an overnight-hold WB sample higher, while CD62P expression and annexin V binding were lower ($p < 0.05$). These findings were comparable with our results [12].

Platelets experience a progressive decline in function accompanied by characteristic morphologic changes. Studies document up to 20% loss of platelet recovery through 5 days of storage. In our study 12.75 % loss of platelet was observed in fresh BC at the 5th day of storage, whereas the amount lost in the stored BC at the end of storage was 6.82 %. [8] Activation of platelets was the main reason for platelet loss during storage. Less activated platelet in stored BC showed a much lesser loss during storage in our study; 15% in stored BC versus 30% after 5 days of storage. Exposure to room temperature for longer time in stored BC resulted in lysis of WBC more thanin fresh BC. This has an additional advantage of less WBC contamination in stored BC. However the effects of cytokines, released in to the platelet concentrate due to lysis of

WBC has to be studied further. An added advantage of stored BC is that when WBC s are in contact with prospective bacterial contaminants for a longer period of time, as is the case with stored BC, then the chance of increased pathogen destruction due to phagocytosis is higher .[13].

However keeping buffy coat for 24 hrs before preparation of platelets, reduce the storage time of platelets for 24 hrs is a drawback of the study.

VI. CONCLUSION

Our study concluded that stored BC contained higher platelet counts, less WBC contamination and less platelet activation. Platelets can be prepared from stored BC, during business hours of following day. This would provide for better supervision, ensuring a better product. This would benefit blood banks where the preparation of components are delayed due to longer transportation time either from satellite blood banks or blood donation camps, which is a common problem in India. Also fewer RDPs are discarded due to low platelet counts, as this method recovers comparatively more platelets. Also less number of units are required to be transfused to patients, since the quality per unit is better, which eventually leads to patient exposure to fewer donors. We conclude that RDP prepared from buffy coat method stored at room temperature for 24 hrs, is a better method for production of platelet concentrate.

	Fresh BC		Stored BC		
	Mean	**Range**	**Mean**	**Range**	**p**
Day 1- (%)	33.35±13.74	13 - 60	19.5±14.44	4 - 44	**16.17**
Day 5- (%)	38.48±13.18	17 - 73	22.3±13.86	5 - 49	**13.35**
p	**0.04**		**0.19**		

Table 2: CD 62 P expression (%) in fresh BC and stored BC in day 1 and day 5 of storage.

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