Effect of Separation, Transport and Storage on the Quality of Platelets Concentrates

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ABSTRACT

For patients with thrombocytopenia or impaired platelet function, platelets transfusion can be of significant value in preventing or treating hemorrhage. Platelets transfusion should provide platelets with good functionality.

The quality of platelet concentrates (PCs) is affected by the preparation method, transportation of blood and the storage conditions including duration of storage, type of storage container, and storage temperature. Different in vivo and in vitro techniques can be used to analyze PCs. The aim of this study is to study the effect of separation technique, transport and storage conditions on the quality of platelets concentrate.

Methods: This is a cross-sectional descriptive and analytical study, conducted in the National Transfusion Center (Khartoum) from April to July 2011. A total of 100 bags of platelets concentrates, that were prepared from random donor whole blood collected locally in the blood
bank (62 bags) and from mobile sessions (38 bags), were investigated at day one and 75 bags of them were stored five days and the same investigations repeated at day five.

All donors were interviewed directly at the time of donation about aspirin ingestion within the last 72 hours and a base line platelets count was done. Donors with a negative history of aspirin ingestion and a normal range base line platelet count were included in this study.

These platelets concentrate bags were investigated for viability by doing platelets count, platelet volume, pH, and Swirling test at day one and repeated at day five on the stored bags.

**Results:** Different centrifugation speeds showed a significant finding; also storage conditions adversely affected the platelets quality parameters while the transport conditions had no effect on platelets’ quality. Of the 100 bags of platelets concentrate bags investigated at day one 50% had a platelets count >5.5x10^{10} per micro liter, 100% had a pH >6.2, 87% had a swirling test of +3 and 54% had a volume range of 50-70ml.

**Conclusion:** Platelets concentrate that fulfilled the desired quality control criteria in terms of volume, count, pH, and Swirling test. This suggested that further standardization of platelet concentrates preparation and storage is required. A quality control program need should be adopted to ensure availability of better quality platelets concentrates.

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**INTRODUCTION**

Blood components are those products derived from whole blood (or platelet-rich plasma) collected from normal donors by phlebotomy using the technique of differential centrifugation. These products are prepared in blood centers or hospital laboratories and should be distinguished from plasma derivatives, which are fractionated from large volumes (thousands of liters) of plasma in large, industrial manufacturing sites (1).

These components include; plasma, cryoprecipitate, red cells, lymphocytes, granulocytes and platelets have different requirements for optimum storage and preservation of function.
left in units of red cells at refrigerator temperatures clump and interact with red cells and plasma proteins and rapidly lose their haemostatic effectiveness and/or are removed by filtration during transfusion. Coagulation factors, especially factors V and VIII, are somewhat labile even at 4°C.

Thus, to get the maximum benefit from a unit of donated whole blood, plasma, red cells, and platelets must be separated from each other and stored at different temperatures and under different conditions. As a result, red cells can be stored for up to 42 days at refrigerator temperatures, plasma or cryoprecipitate can be stored frozen for one year and platelets remain viable and active for up to five days when stored at room temperature

Blood can be collected into a single, double, triple, or quadruple blood pack so that the components can be prepared by differential centrifugation within an integrated system. For some pediatric uses, multiple smaller volume packs are integrally attached, primarily for use in newborns. Thus, a single whole blood donation can provide one unit each of red cells, platelets, cryoprecipitate, and frozen cryoprecipitate-poor plasma for transfusion or for further manufacturing into plasma derivatives. Most blood is collected into double (red cells plus plasma) or triple (red cells, platelets, plasma) bags. Whole blood is almost never used unmodified

**Blood components preparation:**

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 major blood components are red cell, platelet concentrates, fresh frozen plasma and cryoprecipitate

**Preparation from whole blood:**

The most important advance in component preparation and therapy occurred with the development of plastic blood bags and citrate-based anticoagulant solutions, which could be
interconnected to several satellite bags. This permitted separation of blood into its various components within a closed system, thereby reducing the chance of bacterial contamination. With the advent of special gas-permeable plastic bags, it became possible to safely store the platelet concentrates for up to five days at room temperature under constant agitation. In addition, techniques were developed to prevent or disperse platelet clumps when preparing the platelet concentrates.  

Platelets concentrate: is defined as a preparation containing less than 50 ml of plasma and platelets representing at least 50 percent of the total number in the original fresh whole blood. It is prepared from platelet-rich plasma by centrifugation at a force and for a time optimal for platelet sedimentation and the removal of a maximal amount of supernatant plasma under conditions known to maintain sterility and ensure platelet function.

Types of platelet concentrates prepared using different methods of preparations:

1. Random donor platelets (RDP), which is the co-product of normal blood donation, has two different preparation methods \(^4\)
   
   a. Platelet rich plasma-platelet concentrates (PRP-PC)
   
   b. Buffy coat poor-platelet concentrates (BC-PC)

2. Single donor platelets (SDPs), (aphaeresis-PC,) collected from voluntary donors with the help of an automated cell separator. \(^4\)

Platelet transfusion dose:

Platelet concentrates from whole blood donors consist of individual units, each containing 0.55 to 0.8 \(\times 10^{11}\) platelets. A standard dose of platelets for adults is approximately one unit per 10 kg of body weight. This translates into one aphaeresis concentrate or pools of five to eight units of whole blood-derived platelet concentrates for prophylactic therapy in adults. Higher doses may be needed for patients with thrombocytopenia who are bleeding, undergoing invasive procedures or surgery, or are victims of trauma. \(^2\)
Determining the optimal dose:

Infusion of one aphaeresis unit or six units of whole blood-derived platelets to an adult with a body surface area of 2.0 square meters raises the platelet count by approximately 30,000/micro Liter at one hour after the infusion. There is no specific guidance on the optimal dose of platelets that should be transfused.

Following transfusion of platelets, the expected response is an immediate increase in the platelet count that is maximal at about 10 minutes to one hour post-transfusion, there is a steady linear decrease in the platelet count, which usually returns to baseline at about 72 hours post-transfusion (2)

A more quantitative, but also more cumbersome way to determine whether the response to platelet transfusion is adequate is by calculating an index known as the corrected count increment (CCI). The CCI can be determined by using the following formula:

\[ \text{CCI} = \frac{\text{platelet count increment} \times (\text{Body surface area, in sq. meters}) \times (10^{11})}{\text{number of platelets transfused}} \]

The theoretically expected value of the CCI is approximately 20,000/micro Liter. However, in practice the observed increase in platelet count following transfusion in many patients is often less than expected, and the CCI value is closer to 10,000/micro Liter as calculated from the above formula. A widely accepted definition of refractoriness, or poor response to platelet transfusions is when two consecutive platelet transfusions lead to 10-minute to one-hour post transfusion CCI values of less than 5000/micro Liter (2)

Indications for platelet transfusion:

Historically, platelet transfusions were given to thrombocytopenic patients undergoing chemotherapy for leukemia and to those with thrombocytopenia due to aplastic anemia.
More recently, platelets have been utilized in treating patients with accelerated platelet consumption or destruction as in acquired immunodeficiency syndrome, sepsis, disseminated intravascular coagulation, and for various surgical indications; cardiopulmonary bypass surgery or surgery in patients treated with drugs which impair platelet function.\(^{(1)}\)

**Factors that affect platelets quality:**

**Preparation methods and centrifuge:**

Preparation of platelets concentrate from whole blood occurs in two separation steps: primary and secondary separation using cold centrifuge. There is no consensus regarding optimal centrifugation speed, any lab should adjust its own speed.

The first step in preparing the three major blood components, RBCs, platelets, and fresh frozen plasma is centrifugation of whole blood (primary separation). The three major variables that affect the recovery of cells from whole blood by differential centrifugation are rotor size, centrifuge speed, and duration of centrifugation, more than one combination of these affect platelets in platelet concentrates.\(^{(5)}\)

PRP is centrifuged using higher g-forces (hard spin) to separate platelets poor plasma and platelets concentrates, supernatant platelet-poor plasma (PPP) is transferred to another container, leaving approximately 40 to 70 mls of PPP with the platelets which is allowed to remain undisturbed for a minimum of 1 hour and then is resuspended in the plasma (secondary separation). Once platelets are resuspended, they are stored at room temperature (between 20 to 24 C\(^{0}\)) with continuous agitation for a maximum of five days storage period.\(^{(5)}\)

On the day of preparation, some units may contain clumps that are composed of platelet aggregates. Most of the clumps seen on day 0 disappear on day 1 of storage with continuous agitation, particularly those showing light to moderate clumping. The temperature at which platelets are prepared may influence clumping; those platelets prepared at 24 C\(^{0}\) appear to show the least amount of clumping when compared to those prepared at less than 24 C\(^{0}\).
Visual inspections of the platelet concentrate to ensure absence of visible red cells in the vast majority of units is needed, which implies that the units contain fewer than 0.4 times $10^9$ red cells.

Generally, the number of red cells should not exceed 1.0 in a unit of platelets. \[4\]

**Transportation of whole blood:**

Whole blood must be transported as soon after collection as possible to the central component preparation laboratory. Immediately after collection, blood is placed in a storage environment designed to cool the blood toward $10 \, \text{C}^0$. Units from which platelets will be made are allowed to cool toward room temperature $20-24 \, \text{C}^0$. By the time phlebotomy is completed, blood is air cooled to about $20\, \text{C}^0$. If such units are left at the ambient temperature, the cooling rate is quite slow and about 6 hours are needed for units to reach $25 \, \text{C}^0$. \[5\]

**Storage of Platelets Concentrate:**

Platelets must be continuously agitated during storage, and the storage temperature must be between $20\, \text{C}^0$ and $24 \, \text{C}^0$. Platelets may need to be transported to distant regions during cold weather and may therefore be exposed to temperatures lower than $20 \, \text{C}^0$. According to these data, proper steps should be taken to maintain the required range of temperature during storage at the blood center and during transport. \[5\]

**Objectives:**

**General objective:**

This study seeks to assess the validity of platelets concentrates prepared from random donor whole blood collected both locally in the blood bank and from mobile sessions.

**Specific objectives:**

1. To investigate the effect of separation procedures on the platelets count, function and volume.
2. To examine the effect of transport condition on the platelets validity.
3. To study the effect of storage on platelets quality.
MATERIALS AND METHODS

This is a cross sectional descriptive and analytical study conducted in the period April to June 2011 at the National Blood Transfusion Center in Khartoum. Platelets concentrate bags were collected both locally in the blood bank and from mobile session.

A total of 10104 bags of whole blood were collected during this period both locally in the blood bank and from mobile sessions and 6967 bags of these were processed into blood components. From these 6967 platelets concentrates bags, 100 bags that fulfill the inclusion criteria were selected randomly and investigated at day one to study the effect of separation procedure on the platelets concentrates and at day five to assess the effect of storage conditions.

Different centrifugation speeds were used to separate the platelets concentrates using four programs.

Program one used speed 2900 rpm for 3.5 min. with acceleration 9 and deceleration 2 and then high spin 4000 rpm for 7 min- seven units were selected randomly according to the availability of the blood bags and separated using this program.

Program two used speed 1700 rpm for 7 min. with acceleration 9 and deceleration 2 and then high spin 3100 rpm for 5 min- seven units were separated using this program.

Programs three used speed 1500 rpm for 7 min. and high spin 3100 rpm for 5 min. five units were separated using this program.

Program four used 2800 rpm (8-B5), for 3.5 min. and high spin 3200 rpm (6-B3) for 10min. (braking allow prompt deceleration with minimal resuspension of centrifuged elements), eighty one bags of platelets concentrates were separated using this program.

The accepted donors were interviewed directly using design questionnaire including drug history if the patient receiving non steroidal ant-inflammatory drugs within 72 hours and if not a sample of whole blood taken from him in EDTA container for doing a baseline platelet count. If the platelet count was found to be within normal range, then this bag of whole blood will be followed during preparation, transportation, and storage and investigated. After platelets

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concentrates were labeled as virally screened (non reactive), they were investigated at day one; the volume, pH, platelets count and swirling test were done and some of the bags were stored and the same investigation were repeated at day five.

The specific gravity of the platelets concentrate was and its value used to calculate the volume of platelets concentrates.

The volume was determined by subtracting the weight of the empty bag from that of full bag. To convert weight to volume, the resultant weight is divided by 1.03 the specific gravity of PRP-PC

Volume of the concentrate (ml) = Wt. of the full bag – Wt. of the empty bag

Specific gravity

The accepted volume is 50-60 ml, 75% of units examined should meet this criteria. (5)

The swirling is evaluated by examining the units against light and the pattern of movement when pressing the bag should reflect the function of platelets and scored as followed:

- Score 0: Homogen turbid and was not changed with pressure.
- Score 1: Homogen swirling only in some part of the bag and was not clear.
- Score 2: Clear homogenic swirling in all parts of the bag.
- Score 3: Very clear homogen swirling in all parts of the bag.
- All units should have score 3 at any time during storage period (3)

The platelet count in the bag was done using an automated cell counter. The platelet count per bag was calculated by the following formula:

Platelet count per bag = N x 10^6 x platelet concentrate volume per bag

N (platelets count per microliter, accepted count) was (>5.5X10^10), 75% of units examined should meet this criteria. [3]
The pH was evaluated at the end of the maximum day of storage and a pH meter was used for pH assessment. Accepted pH is not < 6.2 at any given day of storage. (3)

Scoring was done on the basis of parameters (i.e., swirling, volume, platelet count, and pH) used for quality control evaluation of platelet concentrates and score 4 or 3/4 is accepted.

RESULTS

The quality parameters of a 100 bags of platelets concentrates investigated at day one showed volume ranges between 43-120ml with a mean of 71ml +/- 13.9 (54% have a volume 50-70ml) and a platelets count range between 0.4 -11.6X10$^{10}$ with a mean of 5.5 +/-2.5 (50% have a count>5.5X10$^{10}$). The swirling score ranged between (+1)-(+3) with a mean of +3 (87% have score +3) and the pH ranged of 6.9-8.2 with a mean of 7.5 +/-0.3 (100% have a ph >6.2) as shown in table (1).

The different centrifugation speeds used to separate the platelets concentrates were found to have a significant effect on the platelets concentrates quality:

Program one showed a mean volume of 81.5ml, a mean count of 0.7x10$^{10}$, a swirling of + 1, and a pH of 7.3.

Program two showed a mean volume of 84.2ml, a mean count of 4.6 x10$^{10}$, a swirling of +2 and a pH of 7.3.

Program three showed a mean volume of 86.0ml, a mean count of 6.0x10$^{10}$, a swirling of +3 a pH of 7.3.

Program four showed a mean volume of 68ml, a mean count of 6.2x10$^{10}$, a swirling of +3 and a pH of 7.5 as seen in table (2).

The eighty one bags that were separated using program four were investigated to assess their viability. The volume ranged between 43 to 120ml with a mean of 68ml, the platelets count ranged of 1.4 to 11.6x10$^{10}$ with a mean of 6.2X10$^{10}$ the swirling score ranged between 2 to 3 with a mean of 3, the pH ranged of 6.9 to 8.2 with a mean of 7.5, as showed in table (3).
25 bags (31%) from a total of 81 bags fulfilled the desired quality criteria from the bags that were separated using program four, the volume ranged from 50 to 70 ml, platelets count >5.5x10^10 pH>6.2 and swirling score +3, at day one.

There was no single bag that fulfilled the desired quality criteria, from a total of 19 bags that were separated using program one, two, or three.

When comparing the 43 bags collected locally in the blood bank and the 38 bags collected from mobile sessions, no significant differences were found between them in terms of volume, count, pH and swirling test, as sowed in table (4). A total of 75 bags were stored for five days and investigated at day one and day five to study the effect of storage on platelets concentrates, showed the following results:

The volume decreased slightly from a mean volume of 68.5ml at day one to 67.6ml at day five, as seen in fig (1). The calculated platelets count also decreased from a mean of 6.0x10^10 at day one to a mean of 5.6x10^10 at day five, figure (2). The pH decreased from a mean of 7.6 at day one to a mean of 7.2 at day five, figure (3) and the swirling score decreased from 93% of the bags having a swirling score of +3 at day one to only 32 % of them having a swirling score of +3 at day five, figure (4 ).

The P value of the difference in the swirling between day one and five was 0.000 which is highly significant statistically and clinically (any bag should have score +3 at any time during the storage period) ,while the difference in the volume ,count, pH between day one and day five statistically significant but still accepted clinically and within normal range.

Out of the 22 bags that met the desired quality criteria at day one, only 5 bags at day five were found to have acceptable quality parameters.
Table (1): The means and ranges of volume, PH, count, and swirling of 100 platelets concentrate bags at day one

<table>
<thead>
<tr>
<th></th>
<th>Volume</th>
<th>pH</th>
<th>Platelets count</th>
<th>Swirling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>71.6 ml</td>
<td>7.5</td>
<td>5.5x10^{10}</td>
<td>2.8</td>
</tr>
<tr>
<td>Range</td>
<td>43- 120 ml</td>
<td>6.9 -8.2</td>
<td>0.4 -11.6x10^{10}</td>
<td>1 – 3</td>
</tr>
<tr>
<td>% of accepted results</td>
<td>54%</td>
<td>100%</td>
<td>50%</td>
<td>87%</td>
</tr>
</tbody>
</table>

Table (2): Different centrifugation programs and the mean volume, count, swirling and pH of platelets concentrates bags.

<table>
<thead>
<tr>
<th>Program ( rpm)</th>
<th>Mean volume(ml)</th>
<th>Mean Platelets countx10^{10}</th>
<th>Swirling test</th>
<th>Mean pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1)1500,3100</td>
<td>86</td>
<td>6.0</td>
<td>+3</td>
<td>7.3</td>
</tr>
<tr>
<td>2)1700,3100</td>
<td>82</td>
<td>4.6</td>
<td>+2</td>
<td>7.3</td>
</tr>
<tr>
<td>3)2800,3200</td>
<td>68</td>
<td>6.2</td>
<td>+3</td>
<td>7.5</td>
</tr>
<tr>
<td>4)2900,4000</td>
<td>81.5</td>
<td>0.7</td>
<td>+1</td>
<td>7.3</td>
</tr>
</tbody>
</table>
Table (3): The means and ranges of volume, pH, count, swirling of 81 bags of platelets concentrates separated using the adjusted program(2800,3200) at day one

<table>
<thead>
<tr>
<th>Volume (ml)</th>
<th>pH</th>
<th>Plt.countx10^{10}</th>
<th>Swirling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>68.6</td>
<td>7.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Range</td>
<td>43 – 120</td>
<td>6.9 - 8.2</td>
<td>1.4 -11.6</td>
</tr>
<tr>
<td>% of accepted results</td>
<td>63%</td>
<td>100%</td>
<td>54%</td>
</tr>
</tbody>
</table>

Table (4): Difference between the bags collected locally in the blood bank and those collected from mobile sessions in the mean volume, pH, count, swirling at day one.

<table>
<thead>
<tr>
<th>Platelets volume (ml)</th>
<th>N</th>
<th>Mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locally</td>
<td>43</td>
<td>70.09</td>
<td>43</td>
<td>120</td>
</tr>
<tr>
<td>Mobile</td>
<td>38</td>
<td>67.03</td>
<td>50</td>
<td>92</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locally</td>
<td>43</td>
<td>7.549</td>
<td>7.1</td>
<td>8.1</td>
</tr>
<tr>
<td>Mobile</td>
<td>38</td>
<td>7.737</td>
<td>6.9</td>
<td>8.2</td>
</tr>
<tr>
<td>Platelets Count x10^{10}</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Locally</td>
<td>43</td>
<td>6.002</td>
<td>2.2</td>
<td>11.6</td>
</tr>
<tr>
<td>Mobile</td>
<td>38</td>
<td>6.055</td>
<td>1.4</td>
<td>10.2</td>
</tr>
</tbody>
</table>
DISCUSSION

The ability of transfused platelets to circulate and function is dependent on both the effect of the in vitro quality that undermines platelet functionality and the in vivo status of the transfused individual. In hospitalized thrombocytopenic patients factors affecting platelet consumption may be so strong in their influence on platelet recovery and survival that may outweigh the effects of in vitro platelet concentrates. Nevertheless it has long been recognized that changes in platelets that occur during preparation and storage can contribute to poor platelet function and decreased post transfusion survival.

Quality assessment of platelet concentrates is an important step to evaluate ex-vivo functional viability of platelet concentrates and post transfusion recovery and survival in recipients. Various parameters are used for routine in-vitro quality assessment of platelet concentrates such as swirling, volume, platelet count, pH and WBC count. Although other parameters are also used such as, measurements of ATP, membrane glycoprotein levels (P-selectin, GP Ib, GP IIb-IIIa) etc., these tests are cumbersome, not well standardized and difficult to perform on every PC unit in a routine setting. It is, as yet, not possible to select a fully reliable standard in vitro procedure to accurately predict the in vivo behavior of transfused platelets. (3)

The in vivo viability of a transfused platelet product is determined by the percentage of the transfused platelets recovered in the recipient's circulation immediately after transfusion (% recovery) and by the life span in circulation of these recovered platelets (survival). (6) The main variables during blood collection, processing, and component preparations that affect product quality are centrifugation conditions, major factors include temperature, duration of centrifugation, maximum g-force achieved, balancing of centrifuge cups, and degree of braking. (5)

In our study, platelet concentrates which were prepared by centrifugation of random donor whole blood and then a platelet rich plasma-platelet concentrate (PRP-PC) were
investigated to assess the effect of separation, transport and storage conditions on the in vitro quality of platelet concentrates, by observing swirling, volume of PC, platelet count/unit and pH. A total of 100 PCs (PRP-PC) were enrolled randomly and investigated at day one; 50% had a platelet count $>5.5 \times 10^{10}$, 100% had pH $>6.2$, 87% have swirling +3, 54% have volume of 50-70ml.

Different centrifugation speeds appeared as the most significant finding that affects platelets concentrates quality during this study. The program that was adjusted in the centrifuge at the beginning of data collection, (1700 for 7 min then 3100 for 5 min), gave unacceptable preliminary results. Another program was adjusted and the speed increased to (2900 rpm for 3.5 min. then 4000rpm for 7 min) with this program7 bags were investigated and all of them were out of the recommended levels of platelets count and no bag found to have swirling +3.then a slower speed with longer first time was tried in 5 bags (1500 rpm for 7 min then 3100 rpm for 5 min), this program gave good results in terms of platelets count, and all bags have swirling + 3 but the problem appeared with the volume, all have a volume $>70$ml, so the remaining volume of platelets poor plasma was not enough to be frozen as Fresh Frozen plasma this means we will end with two blood component instead of three which is a waste of resources.

With the above mentioned 3 programs all bags were found to have a pH within the recommended level i.e. $>6.2$, the main problems were with the count and swirling; which were below the accepted level, and the volume which was above the required level.

When QC data showed that the platelet counts and swirling test in the platelet concentrates are not satisfactory a process of functional calibration of the centrifuge, to allow maximizing platelet yield, became mandatory and when our blood bank engineer adjusted the manufacturer recommended speed that was written in the centrifuge manual and recalibrate the centrifuge, the quality parameters changed dramatically and the rest of our data were collected after calibration and adjusting the speed.

This is in keeping with what is mentioned in the American Association of Blood Banking (AABB) guidelines about the three major variables that affect the recovery of cells from whole blood by differential centrifugation, i.e. the rotor size, centrifuge speed, and duration of centrifugation.
Published papers often refer to relative centrifugation force (g-force) that is derived from the radius of the centrifuge rotor and the revolutions of the rotor. \(^{(5)}\)

More than one combination of these parameters can provide the optimal yield of platelets in platelet concentrates. For a given centrifuge, the rotor size is generally not variable. Therefore, the other two variables (centrifuge speed and duration) can be altered in a stepwise fashion in a Simplex strategy to determine the optimal conditions for preparing PRP\(^{[5]}\). Platelet loss during centrifugation does occur and studies show a 5-20% platelets loss in units centrifuged at 5000 g for 6 minutes or 2000g for 10 minutes as shown by many studies. There is no consensus regarding optimal centrifugation rate \(^{(7)}\).

In a study done by Singh RP, \textit{et al} \(^{(3)}\) for quality assessment of platelet concentrates prepared by platelet rich plasma-platelet concentrate which was separated from whole blood by light spin centrifugation by a Heraeus 6000i, Germany refrigerated centrifuge at 1750 rpm for 11 minutes at 21°C, with acceleration and deceleration curves of 5 and 4 respectively and the platelets were concentrated by heavy spin centrifugation at 3940 rpm for 5 minute at 21°C, with acceleration and deceleration curves of 9 and 5 respectively with subsequent removal of supernatant plasma.

Swirling with score 3 was observed in 79.7%, score 2 swirling was noticed in 20.3% of PRP-PC. No unit had score 1 swirling. The mean pH was 6.7± 0.26 (mean ± SD) and ranged from 6.5-7.0. The mean volume of the PRP-PC unit was 62.30±22.68 (mean ± SD) and ranged from 22-135 ml. The platelet count of individual units was calculated and analyzed. Sixty-four PRP-PC units were assessed and the mean platelet count was 7.60± 2.97 x 10^{10} (mean ± SD) per unit and ranged from 3.2-16.2 x 10^{10} per unit.

The number of units and (%) of PRP-PC meeting the desired quality control criteria of platelet count per unit were analyzed. 78.2% of PRP-PC units had platelet counts >5.5 x 10^{10} per unit. This in comparison with our study we investigate 81 bags of platelets rich plasma separated using Hettich Zentrifuge;2800 rpm (8-B5) for 3.5 min. and high spin 3200 rpm (6-B3) for 10 min, the number of platelets concentrates bags that fulfill the desired quality criteria was (25/81) 31% bags. These comparable results seems to be due to the proper adjustment of centrifuge and
following manufacturer guidelines rather than same speed used because as we see the result of
our PRP-PC that their mean volume was 84.2ml, mean platelets count was 4.6 x10^{10}, swirling
scored +2 and pH was 7.3, although here we use a near speed but our result are far away from
their accepted levels. This may be due to the different centrifuges used in each lab and different
programs that needs to be followed so as to give optimal results.

Platelets must be continuously agitated during storage, and the storage temperature must
be between 20 and 24 C. In-vitro studies showed that platelets are neither damaged when stored
without agitation for 24 hours, nor when stored at 37 C for 6 hours followed by room
temperature storage without agitation for an additional 18 hours. Platelets may need to be
transported to distant regions during cold weather and may therefore be exposed to temperatures
lower than 20 C. Studies have shown that storage at 18.5 C for 3 days is associated with
decreased platelet survival when compared to storage at 21.5 C. Platelets stored at 12 C for 17
hours resulted in a decrease in the in-vivo recovery from 48% to 38%. Similarly, platelets stored
at 16 C for 17 hours resulted in a decrease in recovery from 49% to 42%. Also, platelet survival
was decreased to 2 days at 12 C and to 3.5 days at 16 C. According to these data, proper steps
should be taken to maintain the required range of temperature during storage at the blood center
and during transport.\(^{8}\)

In Sudan high ambient room temperature is the main problem which we faced when we
store platelet concentrates rather than the cold temperature and regular checking and adjusting of
the room temperature is mandatory, an agitator which is well maintained and checked for the
number of movement and if necessary adjusted regularly (60+/-3 movements /minute).\(^{9}\)

Another factor seems to affect our stored platelet is the quality of prepared platelets from the
start if not accepted unlikely to preserve any function till the end of the storage period. The
swirling is the only non-invasive test that can predict good morphology and therefore good
survival. A study by Bertolini\(^{(1)}\) reported that fresh PCs have positive swirling in 83% of units
and negative in only 2%, the rest having intermediate swirling. After 5 days of storage, the
proportion of PCs with positive swirling decreased to 65% and Bertolini concluded that this drop
of swirling could be due to lesions that are known to occur during platelet preservation.
The results of swirling in the present study were comparable to reported data where 93% of the bags have +3 swirling at day one decreased to 32% at day five. B. G. Solheim said A ‘swirling test’ is mandatory for all platelets issued for clinical use and only platelets demonstrating ‘swirling’ are transfused. (10)

Platelets prepared from whole blood are stored in donor plasma, which serves as a buffering agent. PCs from RDPs are typically suspended in 40 to 70 ml plasma to maintain pH. The major reason for using this volume range was based on early studies with PCs stored in first generation containers. Because of the insufficient permeability of these containers to oxygen there was a risk of a drop in the pH in the PCs from anaerobic conditions and elevated lactic acid production. The platelet-suspending volume was, therefore, maximized to increase buffering capacity while maintaining as little volume as possible, to minimize the risk of volume overload of the recipient's circulatory system. (11) With the advent of new second generation highly oxygen permeable containers, there is little risk of exhausting the plasma-buffering capacity, since platelet energy metabolism is predominantly aerobic. Under these conditions, there is relatively little information on how much plasma is required to maintain viability.

The pH decreases during storage depending on the stabilizer in plastic platelet storage bags and storage conditions used. Increased platelet glycolysis resulting in a fall in pH to levels approaching 6.0 in PC stored in plasma is associated with substantial loss of viability. (14) Gulliksson said that: the majority of fresh, un-stimulated platelets are discoid with few projections. In the early observations of PCs stored at 20-24 °C, a gradual disc-to-sphere transformation was seen during storage. Some of these changes are reversible with incubation at 37 °C in fresh plasma. Qualitatively similar changes occur during PC storage, but in first generation containers a major additional variable is pH fall. If pH does not fall to less than 6.8, platelet volume decreases by approximately 10% during three days.

However, if pH falls below this level, there is a progressive rise in platelet volume and decrease in density suggesting swelling due to influx of extracellular fluid. The swelling begins at pH of 6.8 and reaches its maximum at a pH of 6.0, at which point platelet volume is increased almost two-fold. At the same time, there is an accelerated rate of disc-to-sphere transformation.
so that only swollen spheres are seen if pH reaches 5.7 to 5.9. These changes are almost entirely reversible if pH stays above 6.1, but they are not reversible if pH falls below 6.1

In the present study a total of 75 units were analyzed for pH changes between day one and day five and a decrease in pH was observed in all bags but only six bags found to have a pH <6.8 at day five, this drop also occurred in their volume at day five. This is in contradistinction to what mentioned in Gulliksson’s study about the increase in volume with the dropping of the pH to < 6.8 with the storage.

The swirling also decreased to +1 in all these six bags and the observed volume in these bags at day one was around or below 60 ml and this may be the cause of their dropping in the pH as the volume of plasma preserved their pH during storage. Adams et al. have suggested the PCs may be stored for 5 days with a volume as low as 30 ml without significant changes of the in vitro platelet characteristics which are believed to reflect platelet viability and haemostatic function.

In our study the mean volume decreased from 68.5ml at day one to 67.6ml at day five. 36% of the units had volumes more than desired for quality control of volume but a higher volume does not have any deleterious effect on platelet function and maintains the pH throughout the storage period by its buffering action but increasing the risk of volume overload to the patients. PCs that have been gently prepared and then immediately transfused without a significant storage interval (within 24-48 hours of donation) have uniformly high recovery, good survival and preserved function. The storage of platelets was found to be associated with higher levels of platelet activation (i.e. about 10% of the release was associated with the preparation and about 30% with the subsequent storage period) This is in agreement with our study, as the mean platelets count at day one was 6x10^10, it became 5. 6x10^10 at day five this may be of no clinical significance since still these counts are acceptable. But most of these bags with accepted counts had relatively a large volume (>70ml) which may give acceptable counts because we calculate the count in a given volume.

75 platelets bag investigated at day one and stored for five days; Out of 22 bags that met the desired quality criteria at day one, only 5 bags at day five found to have acceptable quality parameters.
This storage problem may be related to the procedure of sampling collection from the bags. Whole blood collected at mobile blood drives or at fixed collection sites must be transported as soon as possible after collection to the central laboratory for component preparation. The time between collection and the separation of components must not exceed 8 hours at room temperature. Immediately after collection, blood is placed in a storage environment designed to cool the blood towards 10°C. Units from which platelets will be made are allowed to cool toward room temperature 20-24°C.

Shipping containers used for transportation should be validated to ensure that proper temperature is maintained. Validation is performed with various quantities of units in the transport container and with a fixed amount of ice or gel pack to determine the number of units that can be stored and transported while maintaining the desired temperature. Portable temperature monitors should be available to record the temperature continuously.

Conclusions:

During preparation of PCs there is deterioration of platelet quality manifested by abnormal shape changes (poor swirling), low platelets count per bag and out of range volume.

The main cause of deterioration of platelet function during preparation is lesions associated with the preparative manipulation.

The centrifuge used for preparation of platelet concentrate plays the main role in product viability; cleaning, calibration and adjusting manufacturer program for separation of each component are important precautions to get satisfactory results.

The most practical way to evaluate the preparation steps is by regular checking of the components quality and if the results are inconsistent, the variables to be monitored include; calibration of the centrifuge, initial platelet count of the donor, sampling technique and counting method.

Blood collected in mobile session does not affect the platelet quality in comparison with PCs prepared from blood collected locally in blood bank.
It should be emphasized that only in vivo studies (platelet recovery and survival after transfusion) will provide the final proof that the product is adequate for therapeutic use in patients.

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