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Quality Control Assessment of Platelet Concentrates in Blood Bank

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Abstract:

Background: Quality assessment of platelet concentrates is an important step to evaluate ex-vivo functional viability of platelet concentrates. This study aimed to assess the in vitro viability and to evaluate the quality and activation of platelets during storage. **Patients and Methods:** The study was conducted on 60 platelet concentrate bags at different days of storage, 15 were single donor platelets and the rest were random donor platelets. All the PCs were subjected to quality control parameters including: pH, platelet count, WBC count, swirling, glucose level, LDH level and assessment of CD62P (P-selectin) by flowcytometry. **Results**: Both preparations fulfilled the desired quality control criteria of swirling and pH levels in which it should be more than 6.0 at the end of maximum days of storage in all the studied bags. The results fulfilled the quality requirement for platelet count in SD-PC which is for minimum $2x10^{11}$ per unit and also in WBC count of SD -PC which is preferred to be $<0.3x10^{9}$ per unit and in RD-PC $<1x10^{9}$ per unit in all the studied bags. Activation of platelets was higher in RDP than SDP as CD62P level higher in RDP. **Conclusions:** SDP is better for transfusion than RDP as it fulfilled the quality control criteria regarding the platelet count, TLC, pH and swirling, while RDP fulfilled the criteria in TLC, pH and swirling but not as regards platelet count**.**

Keywords: Platelet Transfusion, Quality Control, Blood Platelets.

INTRODUCTION

Platelets play an important role in the haemostatic process by sealing damaged blood vessels, forming a platelet plug and prevent blood loss. Once the damage to the blood vessel wall has been covered, the platelets retract the coagulum, to allow the blood flow freely in the vessel[(1)]**.**

The successful treatment of malignant haematological diseases is dependant on transfusion of blood components as these patients have a lack of functional blood cells that may be caused by their disease or chemotherapy treatment [(2)]**.**

Platelets are transfused to patients who are severely thrombocytopenic or to patients with platelet dysfunction to prevent bleeding or induce haemostasis to ensure good haemostatic function in the recipient it is important that the functionality of the platelets used for transfusion is well preserved [(3)]**.**

Many factors influence the quality of platelets during storage. These include the preparation methods of the platelets, the plastic material of the storage bag and the ability of bags to exchange gas across its surface. Other important factors that affect the quality are the storage temperature, the type the anticoagulant used, the platelet concentration in the bag and the agitation[(2)]**.**

The quality of platelets during storage can be evaluated by determining the recovery and survival of the transfused platelets in thrombocytopenic patients[(4)]**.** In the present study, we aimed to evaluate the quality of platelets during different days of storage by determining the in vitro viability and the activation of the platelet concentrates.

PATIENTS AND METHODS

This study was conducted at the main Blood Bank of Ain Shams university hospitals on 60 platelet concentrate bags. All the platelet concentrates in the study were selected randomly at different dates of storage. They were 15 bags of single donor platelets (aphersis platelets) and the rest were random donor platelets (platelet rich plasma). The samples were taken from the same bags of random donor platelets at days 1, 3 and 5 of storage. As regard single donor platelets the samples were taken from the same bags at days 1 and 3.

All the platelet concentrates in the study were subjected to swirling**,** Platelet count, WBC count, pH changes, Metabolic parameters (concentration of glucose and LDH), Platelet activation by assessment of P-selectin (CD62P) using flowcytometry[(4)]**.**

Before sampling, the bag was examined for the swirling and then three milliliters of platelet concentrates were collected and divided into three plain test tubes without anticoagulant. One tube was used for the measurement of the pH, the second tube was for platelet count and WBC count, then was centrifuged (at 3000 x g for 5 minutes) and the separated platelet concentrate used for the measurement of concentration of glucose and LDH and the third tube used for the assessment of P-selectin (CD62P). Storage in the refrigerator, freezing and thawing were avoided.

Methods:

The bag of the platelet concentrate was held horizontally against white light source and gently moved, so that the platelets were in motion in front of the light. In the thin areas of the bag, the appearance of swirling was observed.

Glucose was assayed spectrophotometrically on StarDust MC15* automated photometer by glucose oxidase method. The photometer measures the absorbance at 500 nanometer which is directly proportional to the concentration of glucose in the sample. The analysis occurs according to the following reaction:

Glucose oxidase

$$\text{Glucose} + O_2 + H_2O \longrightarrow \text{Gluconic acid} + H_2O_2$$

Peroxidase

$$2\ H_2O_2 + \text{4-aminophenazone} + \text{phenol} \longrightarrow \text{quinoneimine} + 4\ H_2O$$

LDH was assayed spectrophotometrically on StarDust MC15* automated photometer by the backward reaction method measuring the NADH consumption. The photometer measures the rate of absorbance decrease at 340 nanometer. The analysis occurs according to the following reaction:

L.D.H

$$\text{Pyruvate} + NADH + H^+ \longleftrightarrow \text{Lactate} + NAD^+$$

* DiaSys Diagnostic Systems GmbH.

pH was measured using pH meter model 350, Jenway.

Platelet count and WBC count were measured using Sysmex KX-21 automated hematology analyser and number of platelets and WBC count was calculated per unit platelet preparation (RDP unit contain 50 ml and SDP unit contain 200ml).

Determination of CD62P percentage was measured using EPICS XL Coulter Flowcytometer.

Statistic analysis

Statistical analysis of the data was performed by using SPSS 15 software package under Windows 7® operating system. Central tendency of quantitative data parameters was presented in the form of mean and median; and measure of spread was presented as standard deviation, 25th and 75th percentiles. Testing the normality of data distribution was performed by using both Shapiro Wilk test and Kolmogrov Smirnov test. Comparative analysis was performed by using Mann-Whitney U test (Z value) for comparisons between two independent samples with non-parametric distribution and Wilcoxon Signed Ranks Test

for paired comparisons. Probability level (P value) was assumed significant if less than 0.05 and highly significant if P value was less than 0.001. P value was considered non-significant if greater than or equal to 0.05. Graphic presentation of data was done by using EXCEL ® 2010 software.

RESULTS:-

The results of this study showed that RD-PC was characterized by lower glucose level in the 3 studied days (337.5±34.5, 259.3±38.1, 199.0±36.4 respectively) compared to SD-PC (404.3±54.7, 357.9±41.1 respectively). Regarding the platelet count, it was lower in RD-PC in the studied 3 days (742±72, 654±75, 578±89 respectively) compared to SD-PC(1068±125, 1097±248 respectively) (table 1).

However, LDH level was higher in RD-PC in the 3 studied days (462.7±80.8, 610.5±49.0, 741.8±72.6 respectively) compared to SD-PC (353.8±73.0, 450.5±70.9 respectively). In addition in TLC, it was higher in RD-PC in the studied 3 days (1.07±0.36, 1.04±0.32, 0.87±0.28 respectively) compared to SD-PC (0.69±0.27, 0.35±0.25 respectively) (table 1).

Regarding CD62P level, it was higher in RD-PC in the studied days (35.4±8.0, 47.0±9.6, 29.3±4.4 respectively) compared to SD-PC (23.7±11.9, 43.3±6.2 respectively) (table 1).

The mean pH value in the studied 3 days was (7.0±0.2, 6.8±0.1, 6.8±0.1 respectively) in RD-PC and its values in SD-PC was (7.0±0.1, 6.9±0.2 respectively) (table 1). Statistical comparison between the two types of platelet preparation revealed that there was no statistical significant difference between RD-PC and SD-PC within the same day either day 1 or day 3 (p=0.548, p=0.506 respectively) (table 2).

However, on comparison of pH value in RD-PC between different days, there was a statistical significant decrease in day 3 compared to day 1 (p=0.013) and in day 5compared to day 1 (p=0.003). On the other hand, there was no statistical significant difference between pH value of RD-PC between day 3 and day 5 or pH value of SD-PC between day 1 and day 3(p=0.174, p=0.075 respectively) (table 3).

The mean glucose level in the studied 3 days was (337.5±34.5, 259.3±38.1, 199.0±36.4 respectively) in RD-PC and in SD-PC was (404±54.7, 357.9±41.1 respectively) (table 1).

On comparison of glucose level of RD-PC between different days, there was a high statistical significant decrease in glucose level in day 3 and 5 compared to day 1 and in day 5 compared to day3($p \leq 0.001$). On the other hand, there was a significant statistical decrease in glucose level of SD-PC in day 3 compared to day 1(p=0.030) (table 4).

The mean LDH level in the studied days in RD-PC was (462.7±80.8, 610.5±49.0, 741.8±72.6 respectively) and in SD-PC was (353.8±73.0, 450.5±70.9 respectively) (table 1).

In addition, there was a high statistical significant increase in RDP of LDH level in day 3 and day 5 compared to day 1($p \leq 0.001$) and in day 5 compared to day 3 ($p \leq 0.001$). There was also a high statistical significant increase in SD-PC in day 3 compared to day 1 ($p \leq 0.001$) (table 5).

The mean platelet count in the studied days in RD-PC was (742±72, 654±75, 578±89$\times 10^9$ per liter respectively) (table 1) and (per unit was 0.371±0.04, 0.327±0.03, 0.289±0.04$\times 10^{11}$ respectively) and in SD-PC was (1068±125, 1097±248$\times 10^9$ per liter respectively) (table 1) and (per unit was 2.136±0.25, 2.194±0.49$\times 10^{11}$ respectively). Statistical comparison between the two types of platelet preparation revealed that there was a high statistical significant decrease in platelet count of RD-PC compared to SD-PC within all the studied days either day 1 or day 3 ($p \leq 0.001$) (table 6).

Additionally, there was high statistical significant decrease of platelet count in RD-PC in day 3 and day 5 compared to day 1($p \leq 0.001$) and in day 5 compared to day 3 ($p \leq 0.001$). However, there was no statistical significant difference of SD-PC between day 1 and day 3 (p=0.779) (table 7).

The mean TLC in the studied days in RD-PC was (1.07±0.36, 1.04±0.32, 0.87±0.28$\times 10^9$ per liter respectively) (table 1) and (per unit was 0.0535±0.02, 0.052±0.02, 0.0435±0.01$\times 10^9$ respectively) and it was in SD-PC (0.69±0.27, 0.35±0.25$\times 10^9$ per liter respectively) (table 1) and (per unit was 0.138±0.05, 0.07±0.05$\times 10^9$ respectively). Statistical comparison between the two types of platelet preparation revealed that there was a statistical significant and high significant decrease in TLC of SD-PC compared

to RD-PC within day 1 and day 3 (p=0.019, p≤0.001) (table 8).

On comparing TLC of RD-PC between different days, there was no statistical significant difference between day 1 and day 3 (p=0.386) but there was statistical significant decrease in day 5 compared to day 1(p≤0.018) and in day 5 compared to day 3(p=0.030). On the other hand, there was a statistical significant decrease in TLC of SD-PC in day 1 compared to day 3 (p=0.011) (table 9).

The mean CD62P level in the studied days was (35.4±8.0, 47.0±9.6, 29.3±4.4 respectively) in RD-PC and in SD-PC was (23.7±11.9, 43.3±6.2 respectively) (table 1). Statistical comparison between the two types of platelet preparation revealed that there was a statistical significant decrease in CD62P level of SD-PC compared to RD-PC in day 1 (p=0.011), however there was no statistical significant difference in CD62P level between RD-PC and SD-PC in day 3 (p=0.294) (table 10).

On comparing CD62P level of RD-PC in different days, there was a statistical significant increase in day 3 compared to day 1(p=0.005) and a statistical significant and high significant decrease of CD62P level in day 5 compared to day 1and day 3 (p=0.023, p≤0.001 respectively). On the other hand, there was a high statistical significant increase of CD62P level in SD-PC in day 3 compared to day 1 (p=0.017) (table 11).

Table (1): Comparisons between RD-PC and SD-PC in different days of storage

	RD-PC (45) **X±SD**			**SD-PC (15)** **X±SD**	
Days	**1**	**3**	**5**	**1**	**3**
pH	7.0±0.2	6.8±0.1	6.8±0.1	7.0±0.1	6.9±0.2
Glucose (mg/dl)	337±34.5	259.3±38.1	199.0±36.4	404.3±54.7	357.9±41.4
LDH (IU/L)	462.7±80.8	610.5±49.0	741.8±72.6	353.8±73.0	450.5±70.9
Platelet count x10^9/L	742±72	654±75	578±89	1068±125	1097±248
WBC count x10^9/L	1.07±0.36	1.04±0.32	0.87±0.28	0.69±0.27	0.35±0.25
CD62P %	35.4±8.0	47.0±9.6	29.3±4.4	23.7±11.9	43.3±6.2
Swirling	Positive in all the 60 bags				

X: mean.

SD: standard deviation.

Table (2): Comparison between RDP and SDP as regards pH

		RD-PC			**SD-PC**			**z**	**P**	**Sig**
pH (Day 1)	**X±SD**	7.0	±	0.2	7.0	±	0.1			
	Med		6.9			7.0		0.633	0.548	
	IQR	6.9	-	7.1	6.9	-	7.1			N.S
pH (Day 3)	**X±SD**	6.8	±	0.1	6.9	±	0.2			
	Med		6.8			6.9		0.740	0.506	N.S.
	IQR	6.8	-	6.9	6.8	-	7.0			

Med: median.

Sig: significance.

N.S.: non significant.

IQR: interquartile range.

Table (3): Comparison of pH levels in different studied days of both preparations

		X±SD	Med	IQR	z	P	Sig
RD-PC	Day1 versus Day3	7.0±0.2 6.8±0.1	6.9 6.8	6.9-7.1 6.8-6.9	2.492	0.013	S
	Day1 versus Day5	7.0±0.2 6.8±0.1	6.9 6.8	6.9-7.1 6.7-6.9	2.970	0.003	S
	Day3 versus Day5	6.8±0.1 6.8±0.1	6.8 6.8	6.8-6.9 6.7-6.9	1.358	0.174	N.S
SD-PC	Day1 versus Day3	7.0±0.1 6.9±0.2	7.0 6.9	6.9-7.1 6.8-7.0	1.781	0.075	N.S

s.: significant.

Table (4): Comparison of glucose levels (mg/dl) in different studied days of both preparations.

		X±SD	Med	IQR	z	P	Sig
RD-PC	Day1 versus Day 3	337.5±34.5 259.3±38.1	343.0 271.0	319.0-353.0 219.0-295.0	3.408	≤0.001	H.S.
	Day 1 versus Day 5	337.5±34.5 199.0±36.4	343.0 200.0	319.0-353.0 187.0-218.0	3.408	≤0.001	H.S.
	Day 3 versus Day5	295.3±38.1 199.0±36.4	271.0 200.0	219.0-295.0 187.0-218.0	3.294	≤0.001	H.S.
SD-PC	Day 1 versus	404.3±54.7	405.5	363.5-443.5	2.714	0.030	S

	Day 3	357.9±41.1	354.5	321.3-397.8			

Table (5): Comparison of LDH (IU/L) levels in different studied days of both preparations

		x±SD	**Med**	**IQR**	**z**	**P**	**Sig**
RD-PC	Day 1 versus Day 3	462.7±80.0 610.5±49	492.0 619.0	414.0-514.0 595.0-635.0	3.408	≤0.001	H.S.
	Day 1 versus Day 5	462.7±80.8 741.8±72.6	492.0 715.0	414.0-514.0 700.0-810.0	3.408	≤0.001	H.S.
	Day3 versus Day 5	610.5±49 741.8±72.6	619.0 715.0	595.0-635.0 700.0-810.0	3.408	≤0.001	H.S.
SD-PC	Day 1 versus Day 3	353.8±73.0 450.0±70.9	356.5 470.0	312.3-419.8 635-498.0	3.408	≤0.001	H.S.

Table (6): Comparison between RD-PC and SD-PC as regards Platelet count ×10^9/L

	RD-PC				**SD-PC**			**Z**	**P**	**Sig**
PLT (Day 1)	**X±SD**	742	±	72	1068	±	125			
	Med		720			1055		3.878	≤0.001	H.S.
	IQR	700	-	801	948	-	1200			
PLT (Day 3)	**X±SD**	654	±	75	1097	±	248			
	Med		633			1176		3.617	≤0.001	H.S.
	IQR	613	-	717	845	-	1306			

Table (7): Comparison of platelet count in different studied days of both preparations

		x±SD	**Med**	**IQR**	**Z**	**P**	**Sig**
RD-PC	Day 1 versus Day 3	742±72 654±75	720 633	700-801 613-717	3.351	≤0.001	H.S.
	Day 1	742±72	720	700-801			

	versus Day 5	578±89	583	543-636	3.408	≤0.001	H.S
	Day3 versus Day 5	654±75 578±89	633 583	613-717 543-636	3.408	≤0.001	H.S.
SD-PC	Day 1 versus Day 3	1068±125 1097±248	1055 1176	948-1200 845-1306	0.280	0.779	N.S.

Table (8): Comparison between RD-PC and SD-PC as regards TLC x10^9/L

		RD-PC			**SD-PC**			**z**	**P**	**Sig**
	X±SD	1.07		0.36	0.69		0.27			
TLC	**Med**		1.00			0.70		2.308	0.019	S
(Day 1)	**IQR**	0.80		1.40	0.40		0.98			
	X±SD	1.04		0.32	0.35		0.25			
TLC	**Med**		0.90			0.25		3.765	≤0.001	H.S.
(Day 3)	**IQR**	0.80		1.30	0.13		0.65			

Table (9): comparison of TLC in different studied days of both preparations

		x±SD	**Med**	**IQR**	**z**	**P**	**Sig**
	Day 1 versus Day 3	1.07±0.36 1.04±0.32	1.00 0.90	0.80-1.40 0.80-1.30	0.866	0.386	N.S.
RD-PC	Day 1 versus Day 5	1.07±0.36 0.87±0.28	1.00 0.80	0.80-1.40 0.70-1.00	2.362	0.018	S.
	Day3 versus Day 5	1.04±0.32 0.87±0.28	0.90 0.80	0.80-1.30 0.70-1.00	2.176	0.030	S
SD-PC	Day 1 versus Day 3	0.69±0.27 0.35±0.25	0.70 0.25	0.40-0.98 0.13-0.65	2.536	0.011	S

Table (10): Comparison between RD-PC and SD-PC as regards CD62P%

		RD-PC			SD-PC			z	P	Sig
	X±SD	35.4	±	8.0	23.7	±	11.9			
CD62P	Med		33.8			21.2		2.487	0.011	S
(Day 1)	IQR	28.0	-	39.9	15.5	-	28.9			
	X±SD	47.0	±	9.6	43.3	±	6.2			
CD62P	Med		46.4			44.5		1.098	0.294	N.S.
(Day 3)	IQR	43.3		49.4	38.5	-	46.8			

Table (11): comparison of TLC in different studied days of both preparations

		x±SD	Med	IQR	z	P	Sig
RD-PC	Day 1 versus Day 3	35.4±8.0 47.0±9.6	33.8 46.4	28.0-39.9 43.9-49.4	2.783	0.005	S
	Day 1 versus Day 5	35.4±8.0 29.3±4.4	33.8 30.4	28.0-39.9 24.5-32.5	2.272	0.023	S
	Day3 versus Day 5	47.0±9.6 29.3±4.4	46.4 30.4	43.9-49.4 24.5-32.5	3.408	≤0.001	H.S.
SD-PC	Day 1 versus Day 3	23.7±11.9 43.3±6.2	21.2 44.5	15.5-28.9 38.5-46.8	2.380	0.017	H.S.

DISCUSSION:

Regarding pH changes, its value decreased with increase the period of storage in both preparations. In several studies done by ***Tynngård***[(2)] and ***Ravindra et al.*** [(5)] the pH value decreased by increasing days of storage.

The pH level is an essential requirement for quality control of blood components. Increased platelet glycolysis results in a fall of pH to levels approaching 6.0 which is associated in loss of viability[(2)]**.** When the pH drops below 6.2, platelets become spherical, this change in shape becomes irreversible and below 6.0 the platelet metabolism ceases completely[(6)] **.**

Although the pH value was decreased by increasing days of storage in the present study, it didn't reach to this critical value that affects the viability as its level was ranged from 6.7-7.1 in all the storage days.

On the contrary, ***Jose et al.*** [(7)] found that there was an increase in the pH value between the first and the third day of storage which may have

occurred due to changes in gas concentrations, which are usually at high levels at the beginning of storage, then it stabilizes later[8].

Regarding the metabolic parameters, the glucose levels decreased and the LDH levels increased within days of storage in both preparations. In studies done by ***Tynngård*** [2], they found that the glucose levels decreased and the LDH levels increased with increase the period of storage. The concentration of glucose is commonly used as a quality parameter. Fall in glucose level showed its consumption and could be an indicator for the energy generation in the cells[9]. LDH is another parameter that was analyzed to show the extent of PC destruction during storage[10] .

Regarding the swirling phenomenon, it was positive in all the PCs in both preparations within days of storage. ***Tynngård*** [2]***, Jose et al.*** [7]***, and Ravindra et al.*** [5] found that it was present in all the units. Evaluation of swirling is a simple non invasive procedure that can be performed by visual inspection and is useful for routine quality control of each individual PC on a large scale. Visual inspection of swirling correlates with platelet morphology, the presence of swirling indicates discoid morphology and its absence is indicative of old platelets of spherical morphology, a shape that does not diffract light. After 5 days of storage, the proportion of PCs with positive swirling decreased to 65% and this drop of swirling could be due to lesions that are known to occur during platelet preservation[5].

Regarding the platelet count, it was decreased within days of storage in RD-PC only. The platelet count decreased with increasing the days of storage. A probable indicator of decreased platelet count is the increase in LDH level that reflects the platelet membrane damage [11].

In the analysis of TLC, it was decreased in both preparations within days of storage. ***Ferizhandy*** found that the TLC decreased with increase period of storage. WBCs in PCs have a negative effect on platelet viability resulting in a significant drop in pH, increase in glucose consumption, lactic acid production and release during storage. As a result, in the PCs with high content of leukocytes, the platelet condition up to 5 days of storage was also significantly affected, as reflected by a high excretion of β-TG, loss of platelet nucleotides, decreased ability to incorporate H-adenosine and poor platelet morphology. In addition to these, transfused leukocytes during platelet therapy may be associated with a variety of adverse effects[5].

According to the European Directorate for the Quality of Medicines and Health Care 2010, in both preparations the swirling phenomenon should be positive and the pH more than 6.4. In RDP, the platelet count and the WBC count per unit minimally 2×10^{11} and less than 1×10^{9} respectively. In SDP, the platelet count and the WBC count per unit 2×10^{11} and less than 0.3×10^{9} respectively.

CD62P (P-selectin) is a sensitive PC quality marker. Its expression measures platelet secretion and indicates the level of activation of the platelets and is extensively used as a platelet quality measure as well as in platelet function tests[12].

Increased P-selectin expression during storage has been reported by **Holme et al.** [13]**, Leytin et al.** [14] **Rinder et al.** [15] demonstrated that platelets with increased in vitro activity are rapidly cleared from the circulation in vivo. P-selectin is shown to be involved in regulating post-transfusion platelet clearance by mediating adhesive interactions of platelets with counter receptors on macrophages and endothelial cells. Significant negative correlations between P-selectin exposure and post-transfusion platelet recovery and survival have been reported[16].

In the present study, CD62P level was increased in RD-PC and SD-PC in day 3 compared to day 1 and returned to decrease in day 5 in RD-PC only. In several studies done by ***Tynngård***[2] and ***Elisabeth and Kate***[17] CD62P level showed increased expression progressively by storage time.

The basic principle in preparing RD-PC is sedimentation, therefore two-phase centrifugation may affect many activation markers on the surface membrane, the morphology and the function of the platelets. On the other hand, apheresis platelets are subjected to intermittent or continuous flow centrifugation during collection but not sedimentation. So, SDP are less likely to be activated during preparation[18] .

CONCLUSION :

SDP is better for transfusion than RDP as it fulfilled the quality control criteria regarding the platelet count, TLC, pH and swirling, while RDP fulfilled the criteria in TLC, pH and swirling but not as regards platelet count. CD62P was higher in RDP than in SDP and this reflects less platelet activation in SDP that will result in more clinical effectiveness.

We recommendation the need for further studies on a larger number of platelet activation markers like CD31, CD36, CD41, CD42a, CD42b, CD61, and CD36 for more comprehensive statistical analysis and conclusions. The study of the effect of increasing glucose concentration in the additive solution of the platelet concentrates for prolongation of the storage period and stabilizing the platelet viability. Further research studies for the in vivo viability (post transfusion) and its correlation to the in vitro viability of the platelet concentrates.

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