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Research Article

Importance of homogenization in platelet-rich plasma researches

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ABSTRACT

Aim: To achieve homogenization, one of the most important factors to overcome during platelet-rich plasma (PRP) is standardization phases.

Method*:* We used 10 cc 'BD vacutainer ACD-A' tubes which consisted of 1.5 cc ACD-A. 3 tubes blood was collected from healthy volunteers from 20 to 40 years. All tubes were centrifuged by 180g x 12 min. Then 0.01 ml PRP was collected from just the level of buffy coat.

Results*:* Platelet concentration decreased from buffy coat to top of PPP as expected. If any counting done before homogenization the results will be misleading.

Conclusion*:* When we sample 3 cc PRP homogenized with shaker at 3 point from 3 different point, we see that they are close to each other. It is important that the PRP to be used in the studies must be homogenized before any measurement.

Keywords: Platelet-rich plasma; platelet-poor plasma; homogenization.

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Introduction

During PRP preparation, it is generally known that PRP is confined to a small area immediately on the buffy coat [1]. Sampling from the platelets collected on the buffy coat determines the number of platelets in the PRP [1]. However, having similar platelet counting throughout the PRP is important for treatment. Because the main thing that provides treatment is related to the growth factors that are released from the platelets [2,3] this platelet counting is necessary after platelets in the PRP are homogeneously dispersed in the plasma. PRP is obtained in different amounts depending on the tubes used [3-6].

When preparing PRP, single spin or double spin can be used [2], single spin is generally used in most clinics. Its convenience is that the sterility is more reliable as there is no tube transfer. Different PRP volumes can be obtained according to the tubes used. Approximately 1 cc from the tube containing 8.5 cc of blood + 1.5 cc ACDA containing ACDA, about 0.25 cc of PRP from the 2.5 cc volume of Na citrated tube can be obtained [3-10]. In fact, the amount of PRP collected is

related to how much the amount is concentrated. If a concentrated PRP is desired, we collect platelets in less plasma. Of course, the problem here is how much of the platelets can be collected in the desired area while preparing the PRP, in other words how much of the platelets can be separated from the blood. Double spin is very effective for this reason [2,7,11]. When separating the blood and the plasma, we will lose least amount of platelet when we take the whole plasma. With the second spin, the thrombocytes are collected at the bottom and diluted in the plasma to the desired extent [11].

In this study, we compared platelet concentration with single spin and the subsequent PRP measurements after homogenization by the first PRP measurement.

Methods

The BD Vacutainer clot activator tube, NF 400 centrifuge, precision marking gauge, PSU-10i, orbital shaker and ABX Micros 60 devices were used in our study.

3 tubes blood was collected from healthy volunteers from 20 to 40 years. 25.5 cc blood taken from peripheral veins. We used 10 cc 'BD Vacutainer ACD-A' tubes which consisted of 1.5 cc ACD-A. All tubes were centrifuged by 180g x 12 min with 'NF 400'. Then 0.01 ml of PRP was collected from just the level of buffy coat with delicate marking gauge (special designed by M.I.O*). The platelet count in the blood was examined with 'ABX Micros 60'. The platelets at this level are counted and marked as tube 1, tube 2, and tube 3 PRP. Then from the first tubes we also counted the platelets at the middle of the plasma and the top of the plasma. These are marked as tube 1 middle and tube 1 Platelet-poor plasma (PPP) [Fig. 1A-D].

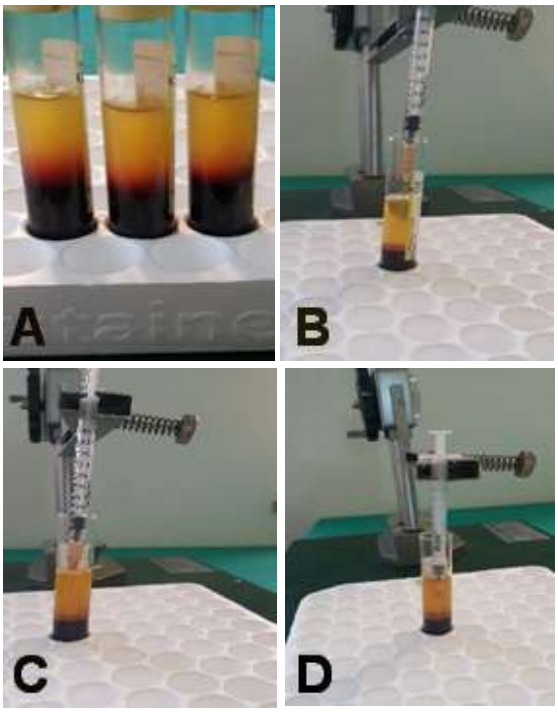

Fig. 1. A) Plasma separation after 180 g x 12 min centrifuge. **B)** Tube 1 PPP sampling. **C)** Tube 1 middle sampling. **D)** Tube 1 PRP sampling.

Homogenization

1 cc PRP was collected from each of the 3 tubes and in an empty tube combined. Then 3 cc PRP was homogenized by a PSU-10i, orbital shaker for 5 min and 300 rpm [Fig. 2]. The counting of the platelets were done from deep, middle and top of the homogenized PRP by collecting 0,01ml samples [Fig. 3A,B,C].

Fig. 2. Orbital shaker.

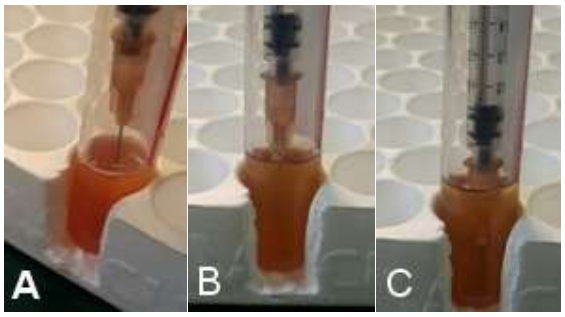

Fig. 3. Delicate sampling after homogenization A: top, B: Middle, C: Bottom.

Results

We calculated the differences between the groups for ABC separately for each volunteer, and for XYZ group separately for each volunteer, and also for groups ABC against XYZ. ANOVA analysis and t-test showed no significant difference between groups A, B, C, and X, Y, Z groups ($p > 0.05$).

Although there is no statistical important difference was shown, there was differences between the values of ABC samples, and also mean values of ABC against XYZ samples. The XYZ values were usually lower then ABC.

The values of homogenization were compared with the values obtained from 3 tubes of PRP. Independent sample test analysis showed no significant difference between groups.

Table 1. Platelet counts of PPP, PRP, homogenized samples

Patients	Blood Platelet	Tube 1 PPP	Tube 1 Middle	Tube 1 PRP	Tube 2 PRP	Tube 3 PRP	Homogenize deep	Homogenize middle	Homogenize top
UR	252	330	540	653	590	652	595	573	585
KL	287	167	288	832	962	1066	624	574	624
BA	235	173	261	454	463	477	458	395	435
EK	189	326	496	487	497	472	451	456	475
MM	223	223	390	421	417	373	364	367	372
NG	235	260	383	478	418	451	411	438	431
ZK	249	290	389	410	394	403	402	389	400
ZP	247	430	564	570	540	525	605	592	610
KM	173	322	395	674	681	665	548	551	562
LG	422	193	528	630	609	601	605	617	600

PPP: Platelet-poor plasma PRP: Platelet-rich plasma

Table 2. Platelet counts of PPP, PRP, homogenized samples and concentration rate

Patients	Blood Platelet	Tube 1 PRP	A Tube 1 PRP-C	Tube 2 PRP	B Tube 2 PRP-C	Tube 3 PRP	C Tube 3 PRP-C	H deep	X H deep C	H middle	Y H middle C	H top	Z H top C
UR	252	653	2,59	590	2,34	652	2,59	595	2,36	573	2,27	585	2,32
KL	287	832	2,90	962	3,35	1066	3,71	624	2,17	574	2,00	624	2,17
BA	235	454	1,93	463	1,97	477	2,03	458	1,95	395	1,68	435	1,85
EK	189	487	2,58	497	2,63	472	2,50	451	2,39	456	2,41	475	2,51
MM	223	421	1,89	417	1,87	373	1,67	364	1,63	367	1,65	372	1,67
NG	235	478	2,03	418	1,78	451	1,92	411	1,75	438	1,86	431	1,83
ZK	249	410	1,65	394	1,58	403	1,62	402	1,61	389	1,56	400	1,61
ZP	247	570	2,31	540	2,19	525	2,13	605	2,45	592	2,40	610	2,47
KM	173	674	3,90	681	3,94	665	3,84	548	3,17	551	3,18	562	3,25
LG	422	630	1,49	609	1,44	601	1,42	605	1,43	617	1,46	600	1,42

PPP: Platelet-poor plasma, PRP: Platelet-rich plasma, H: Homogenize, C: Concentration rate.

Discussion

Various companies have used their own tubes and some additives to prepare PRP s [10-12]. We used the ACD-A tubes to make homemade PRP s after evaluating the various protocols published in the literature [1-12]. There are many protocols for PRP and different concentrations are reported while using different spin times and different g forces. There have been many studies suggesting that 2-3 times more concentrated PRPs are sufficient for an essentially effective PRP to be applied [4]. It has also been suggested that in different studies the PRP concentration should be above 1,000,000/microliters [4].

When PRP is prepared it is known that the concentration measurements can be measured differently in the different levels of PRP prepared tubes. When we work in concert with the literature, it is seen that PRP concentration is different in measurements made from 3 different levels in our tubes 1 (Table 1,2). Although there is no statistical important difference was shown, there was differences between the values of PRP samples against homogenization samples (Table 1,2). In addition, three tubes taken from the same volunteer draw attention to differences in the measurements made at the buffy coat level.

The reason for these differences is that the dissociation of platelets during spin is not homogenous. Another reason may be that it is not possible to take samples from the same point in each tube while sampling for counting how careful it is. In addition, there may be differences when sampled from the right and left spots immediately above the buffy coat. For this reason, PRP or PPP samples must be homogenized before thrombocyte administration or pre-study counting.

Homogenization may be applied by using a mechanic shaker, but also manually hand shaking as well. We preferred shaking by machine for shaking standardization. We set shaker to 300 rpm 5-minute for homogenization (this protocol was clarified in a different research, not shown in this article).

In this study, we showed that the results of 3 different spots sampled from homogenized tubes are close to each other. The study shows that the PRP that will be used in studies needs to be homogenized before any measurement. The platelet concentration was decreased from the buffy coat to the PPP as expected. Also if any counting done before homogenization the results will be misleading.

Compliance with ethical statements

Conflicts of Interest: None.

Financial disclosure: None

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