Original Article

Different Professional Skill to Produce Platelet Rich Plasma May Effect the Clinical Results

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Background: Previous study of a Manual PRP preparation which stated clearly about the procedure of producing PRP that has platelet concentration higher than baseline. Since there are steps that require skills of performers that prepare the manual PRP, there should be a study of probability that the Manual PRP preparation can minimize human error.

Objective: To study the reproducibility of the PRP produced by the manual PRP preparation. The quality of PRP from manual PRP preparation performed by doctor and nurse, including platelet concentration with low levels of white and red blood cells, will be compared.

Materials and Methods: In the present study, the performers in this study were the orthopedic resident and the nurse who worked in the orthopaedic out-patient department at HRH Princess Maha Chakri Sirindhorn Medical Center as the representatives of different professions. 12 volunteers were participated. The samples of PRP obtained were then given to the laboratory for analysis to obtain platelet concentration.

Results: The PRP performed by doctor had a higher concentration of platelet and lower concentration of WBCs when compared with PRP performed by the nurse. The difference in platelet concentration of PRP from manual PRP preparation between samples performed by doctor and nurse was significant. The difference between the WBC count of PRP from manual PRP preparation carried out by doctor and nurse was significant but the difference of RBC count was not significant.

Conclusion: The different medical profession of the performers, which doctors and nurses were the representatives in the present study, gives a statistically significant difference in platelet concentration and the amount of WBCs and RBC.

Keywords: Platelet-rich plasma, PRP, Manual PRP preparation, Platelet concentration, Professional skill

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Platelet rich plasma (PRP) is plasma extracted from centrifuged blood that consists of a higher concentration of platelet than in the normal complete blood component. It contains many important components that help repair injured tissue[1-3]. Many previous studies showed significant improvement of injuries of ligaments, tendons and joints[4-8]. The key quality of the PRP is the concentration of the plasma, which varies depending on the methods in producing the PRP. In generally, PRP has platelet concentration of two to four times higher than the normal CBC does[9,10], which could be used for treatment. Nevertheless, there is no standard definition of how much platelet concentration needed comparing to the platelet in CBC baseline to consider as a PRP.

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Even though the use of PRP has been used in the current general practice, there are limitations. The PRP requires a high expense which cannot be refunded in any healthcare scheme. Moreover, small-sized and distant hospitals possibly be able to hardly access to the use of PRP. Therefore, manually producing the PRP might give the solution to those hospitals in those situations. There was a previous study of a Manual PRP preparation which stated clearly about the procedure of producing PRP, including the rate and duration of the centrifuge, that was the spinning of blood at 1,000 rpm for 3 minutes was the best protocol of manual PRP preparation[11] to gain plasma that has a high platelet concentration and low WBC contamination[11]. Since there are steps that require skills of performers that prepare the manual PRP, there should be a study of probability that the Manual PRP preparation can minimize human error.

The purpose of this study was to study the reproducibility of the PRP produced by the manual PRP preparation, which suspected to be influenced by human error. The quality of the PRP performed by different performers, using a doctors and a nurses as representatives,

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was compared. The basic knowledge and understanding of performing the manual PRP preparation is new to the performers in this study. The hypothesis was the doctor and nurse in the present study will produce a different outcome of platelet and WBC concentration using the manual PRP preparation.

Materials and Methods

The performers in the present study were the four orthopedic residents and the two nurses who worked in the orthopaedic out-patient department at HRH Princess Maha Chakri Sirindhorn Medical Center (MSMC). All of them had no experience in performing the manual PRP preparation.

According to previous study[11], we calculated that 10 sample size to detect a statistically significant difference between groups with an a of 0.05 and 80% power. In this study, 12 volunteers were used. All of them were healthy, aged over 20 years old, agreed with the informed consent, and had no hematological disease. Ethical approval for the study was granted by the review board and the ethics committee of HRH Princess Maha Chakri Sirindhorn Medical Center (MSMC), Srinakharinwirot University (SWUEC-058/59E).

The instruction of how to perform the manual PRP preparation under sterile technique would be given in a protocol sheet and also in the oral teaching session.

Firstly, 12 ml of blood was taken from each volunteer. The blood sample was separated into 3 portions: 2 ml for CBC, then two syringes of 5 ml mixed with few drops of heparin. The tips of the two 5-ml syringes were closed with a lock tip cap and the plungers were cut in the finger grip level. For further secure, the cut plungers were wrapped with adhesive tape to prevent leakage of the fluid while centrifuging (Figure 1). They were then put into the centrifuge machine (Digisystem Model DSC-200T-voltage 220V) (Figure 2) with the speed of 1000 RPM for 3 minutes to obtain the separated layers of plasma and red cell.

After the blood sample were separated into two layers of plasma above and red cells below, each of the two syringes was given to the orthopedic resident and the nurse. The plasma in the post-centrifuged blood were carefully drawn using a pipette, in which both the orthopedic resident and the nurse would try not to draw the red cell layer, giving the PRP of the volunteers (Figure 3).

Finally, the PRP pipetted by doctor and by nurse was taken to analyzed, focusing on the platelet concentration and the amount of WBCs and RBCs. The data was analyzed by SPSS software before it was analyzed with Mean and standard deviation. The *p*-value of ≤ 0.05 was used to determine the significance.

The possible difference between doctors and nurses could be the basic knowledge in preparing the PRP. The important step was pipetting the layer of PRP from the post-centrifuge blood. Normally, after centrifuging the blood, the blood will be separated into 3 layers: RBC in the bottom layer, then a thin layer of PRP above that, then the low platelet plasma on the most top. They should pipet from the

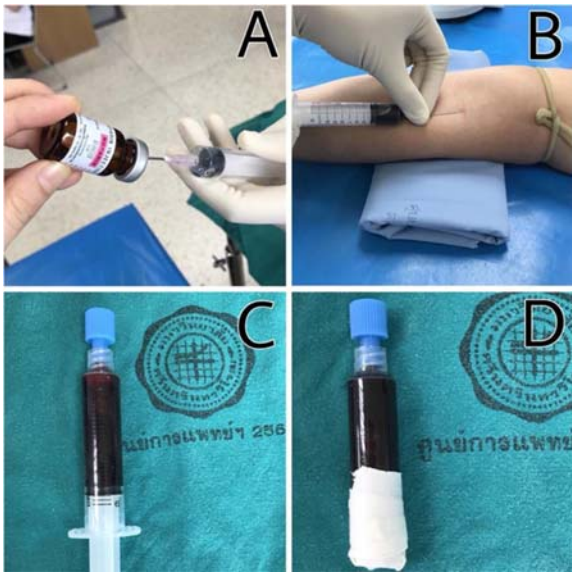

Figure 1. Manual PRP preparation (A): 5-ml syringe was coated with heparin. (B) 12-ml of blood was taken from a volunteer. (C) 5-ml of blood in syringe was locked with cap at the tip (D) The plunger was cut and wrapped with adhesive tape preventing the plunger from moving.

Figure 2. Centrifuge machine Digisystem Model DSC-200T.

thin layer of PRP and not the RBC layer.

Results

The platelet concentration and the amount of WBCs and RBCs from PRP produced by the doctor and nurse were compared with those from CBC baseline of the 12 volunteers, 10 men and 2 women (Figure 4 to 6). Mean platelets of PRP using the Manual PRP Preparation performed by doctors and nurses were 441.667±113.2056x10^3/mm^3 (mean ± SD) and 410.75±118.1156x10^3/mm^3 (mean ± SD), respectively (Figure 7). Mean platelets concentrations of PRP from Manual PRP Preparation were 1.69 and 1.58 times higher than that of CBC baseline, performed by orthopedic residents and nurses, respectively (Table 1). Furthermore, the mean RBC of PRP from the Manual PRP Preparation by the residents and nurses were 0.0208±0.009x106/mm^3 and 0.08±0.10812x106/mm^3 (Mean±SD), respectively. The mean WBC of PRP from manual PRP preparation by the resident and nurse were 132.5±154.692/mm^3 and 693.33±776.640/mm^3, respectively (Figure 8,9).

Using the Manual PRP Preparation, there was a statistically significant difference between the platelet concentration of PRP done by the doctor and done by the nurse, 30.9167x10^3/mm^3 (p = 0.019) (Figure 8). The difference between the WBC count of PRP from manual PRP preparation carried out by doctor and by nurse was also statistically significant (p = 0.21), while the difference of RBC is not statistically significant (p = 0.076) (Table 2).

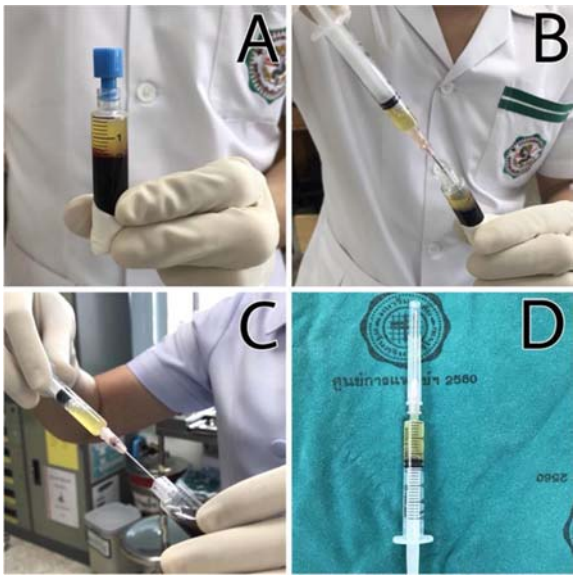

Figure 3. (A) Two layers of plasma and red cells after the centrifugation (B), (C) The plasma was pipetted from the syringe and then was put into a new syringe by an orthopedic resident and a nurse, respectively (D) PRP.

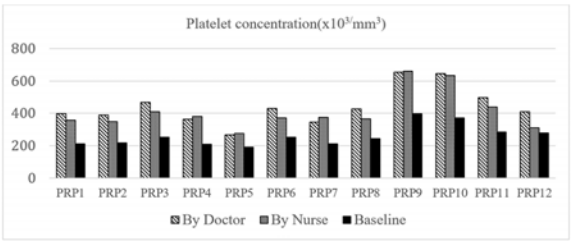

Figure 4. Comparison of platelet concentration from CBC baseline and from PRP done by doctors and nurses.

Discussion

The previous vitro studies recommended PRP that

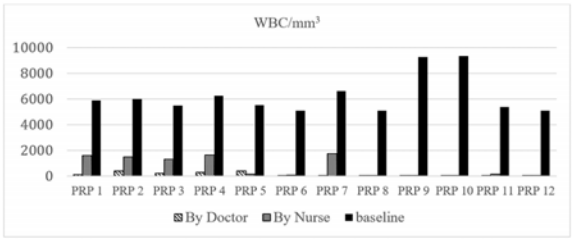

Figure 5. Comparison of WBCs from CBC baseline and from PRP done by doctors and nurses.

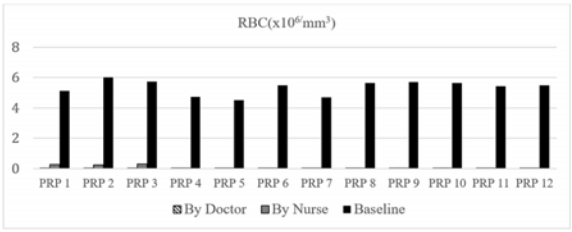

Figure 6. Comparison of RBCs from CBC baseline and from PRP done by doctors and nurses.

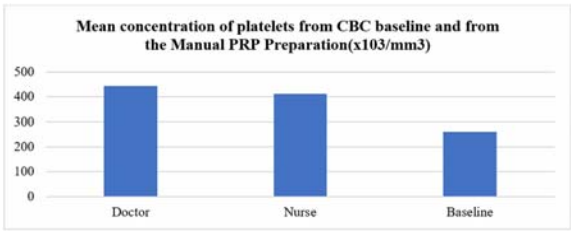

Figure 7. Comparison of the mean concentration of platelets from CBC baseline and from the Manual PRP Preparation done by doctor and nurse in the 12 volunteers.

would be used for treatment should have a platelet concentration of 2 to 4 times higher than in CBC baseline for a treatment[12]. However, there is no standard definition of how much platelet concentration needed comparing to the platelet in CBC baseline to consider as a PRP[13]. The variation of methods in preparing and the amount of PRP could possibly give different results on the cellular composition and biomolecular characteristics[14-16]. Another previous study developed a manual PRP preparation with the goal of producing platelet concentration of approximately two times higher than the CBC baseline. The manual PRP preparation uses the centrifuge of 1,000 RPM for 3 minutes with the result of 1.7 times higher of platelet concentration when compared with that from CBC baseline[11]. However, the steps in performing the manual PRP preparation can affect the quality of PRP due to human errors. Nevertheless, there had been no study of its clinical outcome as well as its cost and effectiveness from using the PRP from manual preparation.

From the result, both the doctors and nurses, which were the representatives of medical staffs, produced the mean concentration of platelets of nearly 2 times higher in PRP from the Manual PRP Preparation than in CBC baseline. The mean concentration of platelets obtained by the doctors was 441.667x10^3/mm^3 which was 1.7 times higher than in CBC baseline. The mean concentration of platelets obtained by the nurses was 410.75$\pm$118.1156x10^3/mm^3 which was 1.6 times higher than in CBC baseline. The results in this study show there was a significant difference in quality of PRP from the Manual PRP Preparation done by the doctors and nurses. The PRP performed by doctors had a higher concentration of platelet and lower concentration of WBCs when compared with PRP performed by the nurses.

If the manual PRP preparation is approved and

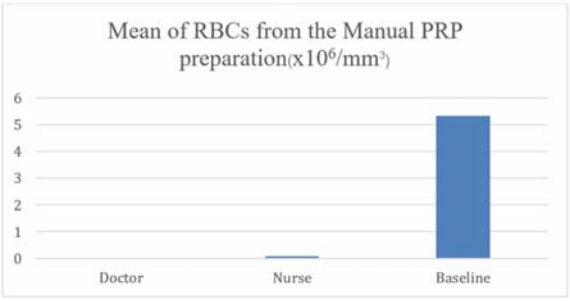

Figure 8. Comparison of the mean RBCs from the PRP from the Manual PRP Preparation done by doctor with that done by nurse in 12 volunteers.

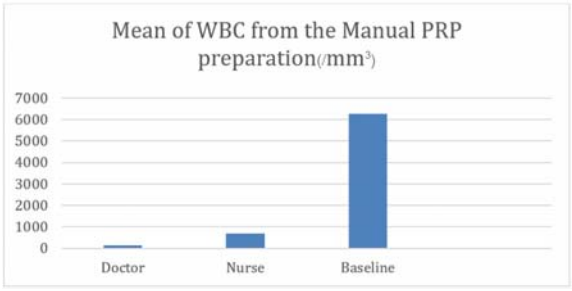

Figure 9. Comparison of the mean WBCs from the PRP from the Manual PRP Preparation done by doctor with that done by nurse in 12 volunteers.

Table 1. Comparison of the mean concentration of platelets from the Manual PRP Preparation with that from the CBC baseline

Platelets of PRP from manual PRP preparation	Folds from baseline	n	SD
Doctor	1.697	12	0.1396
Nurse	1.578	12	0.0535

Table 2. Paired sample test of PRP from Doctor and Nurse

Different of Doctor and Nurse	Mean	SD	95% confidence interval of the difference		p-value (≤0.05)
			Lower	Upper	
Platelets	30.9167 x10^3/mm^3 (7%)	39.1186	6.0619	55.7714	0.019
RBC	0.5917x10^6/mm^3 (75%)	0.10466	-0.12566	0.00733	0.076
WBC	560.833/mm^3 (80%)	721.809	-1019.449	-102.218	0.021
The fold of platelets concentration	0.118719	0.15589	0.019667	0.2177723	0.023

used in out-patient department, the performers, orthopaedic doctors and nurses, could follow the instruction of the protocol sheet. However, the basic knowledge and experienced skills in each career can contribute to human error in producing the PRP. The main key point that influences the outcome of the PRP is the step of drawing the plasma layer without drawing the RBC layer. This study uses doctors and nurses as the representatives of different professions for studying the human error in producing PRP using the manual PRP preparation. In clinical setting, nurses usually are the people who prepare the PRP. The present study would help point out that in this preparation, the step in pipetting the PRP should be emphasized.

Regardless of the exact same procedure performed to extract the PRP, there are factors influencing the outcome of the quality of the PRP, which includes the basic knowledge of platelet concentration. After the centrifuge of a blood sample, three layers are formed which are RBCs in the bottom, platelets and WBCs in the middle layer, and plasma in the top layer. From this study, the doctors could extract more platelet concentration using the Manual PRP Preparation than the nurses did. Different concentration of platelet can affect the quality outcome of PRP. Thus, if the Manual PRP Preparation is approved in clinical setting in the future, factors of performers that can affect the outcome of PRP should be considered and studied. Limitation of the present study is small number of volunteers. Therefore, the differences in results might be underestimated. Furthermore, this study showed PRP from the manual PRP preparation had a lower cost than the commercial kit, which is 300 baht and 3,000 baht, respectively. However, there is no clinical study comparing the quality of PRP between the manual PRP preparation and other methods. Therefore, it is necessary in the future studies.

Conclusion

The different medical profession of the performers, which doctors and nurses were the representatives in this study, gave a statistically significant difference in platelet concentration and the amount of WBCs and RBC. Using the manual PRP preparation, the PRP performed by doctors had a higher concentration of platelet when compared with PRP performed by the nurses.

What is already known on this topic?

The preparation of PRP by spinning method can produce PRP that platelets concentration are higher than baseline.

What this study adds?

The manual PRP preparation with 1,000 rpm/3 mins can present the platelets rich plasma about 1.6 to 1.7-fold concentration above baseline and human error factor was important for different of platelets concentration.

Acknowledgements

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Potential conflicts of interests

The authors declare no conflicts of interests.

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