Cost Effectiveness of a Platelet-rich Plasma Preparation Technique for Clinical Use

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ABSTRACT

Introduction. Despite limited clinical evidence, platelet-rich plasma (PRP) is currently used for the treatment of various soft tissue injuries, but optimal use of PRP has yet to be determined. In many instances, PRP is prepared using commercial devices that lack standardized preparation techniques and consistent quality of the PRP produced. **Objective.** The aim of this study is to explore a simple, easy, economical method of PRP preparation that is practical for clinical use. **Materials and Methods.** This cross-sectional study was conducted at the Sports Medicine Clinic at the University of Malaya Medical Centre, Malaysia. Participants were healthy postgraduate students and staff at the Sports Medicine Department. The PRP was prepared using a single centrifugation technique. Leukocyte and platelet levels were compared with that of a whole blood baseline and a commercial preparation kit. **Results.** The PRP produced using this technique contained significantly higher mean platelet (1725.0 vs. 273.9 x 10^9/L) and leukocyte (33.6 vs. 7.7 x 10^9/L) levels compared with whole blood. There was no significant difference in the mean platelet and leukocyte levels between the PRP produced in this study and by a commercial PRP system. **Conclusions.** A single-centrifugation protocol using readily available materials in a typical clinical setting could produce PRP of comparable quality to those of a commercial PRP production system.

KEY WORDS

platelet-rich plasma, cost effectiveness, tissue repair, growth factors, sports medicine, biologics, stem cells

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Administration of autologous biological substances has gained considerable attention for the management of soft tissue injuries/conditions.[1-3] Substances such as autologous blood and blood products, including autologous condition serum, platelet rich in growth factors, and platelet-rich plasma (PRP), are currently being used in clinical settings despite limited evidence.[4]

Over the last 2 decades, there has been growing evidence to support the use of PRP for soft tissue healing. Several studies[5-7] reported significantly faster healing of chronic ulcers among patients treated with PRP gel. Recent meta-analyses[8,9] concluded PRP could shorten acute wound healing time and length of hospital stay as well as have positive effects in controlling wound infection. In addition, PRP is used for musculoskeletal conditions such as lateral epicondylitis (tennis elbow), muscle injury, and knee osteoarthritis.[1-4,10,11] The rationale behind PRP use in treatment is the notion that growth factors and cytokines liberated from platelet granules would augment the natural healing process.[12-14] Despite such belief, PRP use for soft tissue conditions still remains controversial because research has demonstrated inconsistency in clinical effects following PRP administration.[10,15-19] These inconsistencies could be attributed to lack of standardization in PRP treatment protocol, including platelet concentration, dosages, timing of treatment, frequency of administration, mode of delivery (blind vs. ultrasound guidance), post administration care, and rehabilitation programs.

The majority of PRP used in previous studies[10,11,15,16,20] were prepared using commercially available systems from various pharmaceutical companies. The method of PRP preparation varies between different systems; accordingly, the PRP yield differs in its qualities. Moreover, these kits are costly and may influence the frequency of PRP administration for each participant as well as the total number of participants recruited. In Malaysia, a commercial PRP system costs between RM500 to RM2000 (USD: $116.95–$467.80; Euro: €97.85–€391.40; based on currency rates as of August 28, 2017), excluding other hospital/clinic charges, and currently is not covered by any medical insurance. Hence, developing a PRP preparation method that is simple, safe, and cost effective could encourage more research in this area. Also, such a technique could potentially make PRP treatment accessible to the less economically privileged.

The objective of this study is to explore a simple, easy, cost-effective method of PRP preparation that is practical for clinical use. The quality of PRP produced using this method was

Figure 1. Tubes centrifuged at 3200 RPM for 10 minutes.

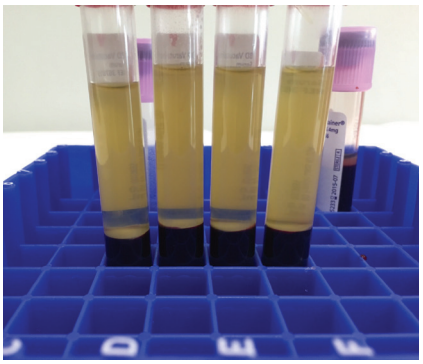

Figure 2. Tubes allowed to rest for 5 minutes.

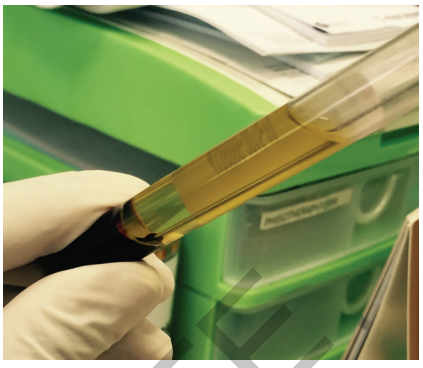

Figure 3. Gentle aspiration of buffy coat.

compared with PRP prepared using a commercial kit from a previous study conducted by the present author.[10]

MATERIALS AND METHODS

The University of Malaya Medical Centre (UMMC) Ethics Committee (Kuala Lumpur, Federal Territory of Kuala Lumpur, Malaysia) approved this study (MEC Ref no. 20149-534).

A convenience sampling of postgraduate students and staffs who were working at the Sports Medicine Clinic, UMMC, were invited to participate in this study. Participants consisted of healthy individuals who were free from any chronic illnesses and not taking any regular medication, including aspirin and nonsteroidal anti-inflammatory drugs. Prior to recruitment, the study objectives and procedures involved were explained to participants, and each signed an informed consent form. A total of 27 individuals who volunteered were screened prior to participation. All individuals fulfilled the inclusion criteria and consented to be included as participants in this study.

PRP preparation

The PRP preparation technique was adapted from previously published methods.[21–23] Whole blood was drawn using a 21 G x 1.9 cm butterfly needle (Becton Dickinson & Co, Franklin Lakes, NJ) from the participant's antecubital vein into 4 plain 6.0 mL BD Vacutainer tubes (Becton Dickinson & Co). Each tube was prefilled with 0.6 mL of the anticoagulant citrate dextrose solution-A to prevent collected blood from clotting. An additional 2 mL of whole blood was collected into an ethylenediaminetetraacetic acid (EDTA) tube to acquire baseline whole blood platelets and leukocytes (WBC) value. All 4 plain tubes were centrifuged at 3200 RPM for 10 minutes using a table top Horizon Model 755VES centrifuge (The Drucker Co, Port Matilda, PA; **Figure 1**). The speed and duration of the centrifugation process used in this study were based on work by previous researchers.[21,23,24]

After centrifugation, the tubes were placed in a test tube rack and allowed to rest for 5 minutes to facilitate the settling of platelets onto the buffy coat (**Figure 2**). The PRP was collected using a needle attached to a 5-mL syringe measuring 18 G x 4.5 cm under naked eye visualization. With the tube stoppers removed, the needle tip was positioned so as to just touch the buffy coat. The syringe plunger was gently raised to vacuum up platelets on the buffy coat, and the needle tip was slowly moved along the buffy layer (**Figure 3**).

A total volume of 0.5 mL to 0.75 mL buffy coat was extracted from each tube into the collection syringe. A total PRP of 2.0 mL to 3.0 mL per participant was collected and transferred into an EDTA tube for analysis. The amount of platelets and WBCs present in the venous blood and the PRP were determined using the Sysmex XN-10 and XN-20 (Sysmex Corporation, Kobe, Japan) high-performance automated hematology analyzer in the UMMC outpatient laboratory. The ratio of platelet levels in venous blood to PRP was calculated to determine the ability of the current method to concentrate platelets.

Statistical analysis

Data obtained were analyzed using SPSS software for Mac (Version 22; IBM Corp, Armonk, NY). Descriptive analysis of participants' characteristics was performed. Continuous variables were reported using mean and standard deviation (SD) or median and interquartile range (IQR), depending on data distribution based on Shapiro-Wilk test of normality. Categorical data were presented as frequencies and percentages. Paired sample *t* test or its nonparametric equivalence test were performed to determine the differences in platelet and WBC content between the whole blood and PRP produced by the technique used herein.

In addition, platelet and WBC content produced using the current method were compared with those produced using GPS III Platelet Separation System (Biomet Inc, Warsaw, IN) from a previous study.[10] For all analyses, a value of $P < .05$ was considered statistically significant.

RESULTS

Twenty-seven participants (15 men; 12 women) with a median age of 33.0 ± 12 (IQR) years volunteered in this study. The mean baseline platelet and WBC counts present in the whole blood were 273.9 ± 55.4 (SD) x 10^9/L and 7.7 ± 2.4

Table 1. Platelets and leukocytes (WBC) levels among participants

	MALE (n=15)	FEMALE (n=12)	T/Z[a] SCORE	*P* VALUE
Age (Median ± IQR)	30.5±9.8	34.0±13.0	-1.62[a]	.106
Platelets (x10⁹/L)				
Whole blood (Mean ± SD)	260±53.4	291.6±55.1	-1.37	.183
PRP (Mean ± SD)	1638.9±783.4	1834.6±784.7	-0.78	.444
WBC (x10⁹/L)				
Whole blood (Mean ± SD)	7.7±1.9	7.3±2.0	0.01	.991
PRP (Median ± IQR)	38.3±31.2	27.2±16.0	-0.87[a]	.381

WBC: white blood cells (leukocytes); IQR: interquartile range; SD: standard deviation; PRP: platelet-rich plasma

[a] Mann-Whitney *U* test

Table 2. PRP contents comparison with commercial preparation kit

	CURRENT METHOD	COMMERCIAL METHOD[a]	t/Z[b] SCORE	*P* VALUE
Whole blood (x10⁹/L)				
Platelet (Mean ± SD)	286.0±73.0	234.0±57.5	-1.73	.083
WBC (Median ± IQR)	7.5±1.8	7.3±1.3	-0.33[b]	.745
PRP (x10⁹/L)				
Platelet (Mean ± SD)	1725.0±773.8	1324.0±340.7	1.98	.055
WBC (Median ± IQR)	33.6±30.1	37.2±19.8	-0.90[b]	.368

PRP: platelet-rich plasma; IQR: interquartile range; SD: standard deviation; WBC: white blood cells (leukocytes)

[a] Biomet GPS III (Zimmer, Warsaw, IN)

[b] Mann-Whitney *U* test

(IQR) x 10⁹/L, respectively. There was no significant difference between the mean age, platelet, and WBC values between gender (**Table 1**). The mean number of platelets in the PRPs prepared with the current technique was 1725.0 ± 773.8 (SD) x 10⁹/L, and the mean number of WBCs was 33.6 ± 15.1 (SD) x 10⁹/L. The current PRP preparation technique contained a significantly higher (6x) number of platelets compared with whole blood (t[26] = -10.46; $P < .001$). Further, Wilcoxon signed-rank test also demonstrated a significantly higher (4x) number of WBCs present in the PRP (Z = -4.4; $P < .001$).

No significant difference in the mean number of platelet and WBC contents between the current method of PRP preparation and commercially produced PRP (**Table 2**) was found. The PRP produced in this study was classified as P4-x-A according to the Platelets, Activation, White cells classification system.[25]

All preparation processes were performed in the Sports Medicine Clinic, UMMC, and took approximately 25 to 30 minutes from blood drawing to final PRP production. The total cost of consumables for the entire process was < MYR 30.00 (USD: $7.02; **Table 3**) and inexpensive compared with commercial PRP systems available in Malaysia (**Table 4**).

DISCUSSION

The use of autologous PRP for soft tissue injuries/diseases remains controversial with contradictory clinical responses. These inconsistencies could be partially explained by differences in the method used to prepare PRP by previous researchers.[10,16,17,20,26,27] In many instances, researchers used commercially available PRP systems that differ in preparation protocol as well as quality (platelet and WBC content) of the PRP produced.[25] Also, these kits are expensive, with costs reported to be between USD $175.00 to USD $1550.00.[28] It is possible that such high prices may limit the number of participants recruited, thus affecting study outcomes.[3,10,15] Developing a standardized, simple, more cost-effective technique of PRP production might encourage more clinical studies on PRP effects for soft tissue injuries/diseases.

Several methods of PRP protocol have been described in the literature, including centrifugation and apheresis techniques.[25] Although an apheresis technique has high repeatability and yields consistently higher platelet concentration with a lower risk of contamination, it is costly and impractical for outpatient clinical settings as it requires specialized equipment.[29]

Despite the ability to produce significantly higher amounts of platelets, the double centrifugation technique was questioned because of potential alterations in platelet morphology, which might affect functions. In addition, the double centrifugation technique is more sensitive to processing errors and carries a higher risk of contamination from the repeated handling of blood products.[30,31]

The present study demonstrated that a single centrifugation technique using convenient, inexpensive, readily available consumables (vacutainers,

needles, and syringes) could produce PRP of comparable quality to that produced using a commercial system. A mean platelet count 6 times higher than baseline (mean platelet count of 1725.04 x 10^9/L) was achieved using the technique herein.

The PRP content (platelets and WBCs) in this study was comparable to that observed by previous authors[10,15,17,26] and well within the therapeutic level previously reported.[25,31] Also, a mean platelet count of 874.2 x 10^9/L to 1369.0 x 10^9/L in PRP was reported in studies using similar single-spin centrifugation techniques.[21,22]

LIMITATIONS

Several limitations of this study need to be considered. The sample size (27 subjects), though consistent with another study,[22] may have impacted study results. Lack of growth factor concentration analyses may have limited the investigator's ability to demonstrate the magnitude of increase in growth factor (GF) concentration in the PRP. However, it is reasonable to assume there was a higher GF level present in the PRP because the majority of the GFs are stored within the alpha and dense granules in the platelet cytoplasm.

CONCLUSIONS

This study demonstrated it is possible to produce PRP in the clinical practice setting. Platelet-rich plasma with quality comparable to that of commercially available kits can be prepared using components (consumables) readily available in most clinics, hospitals, and pharmacies.

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Table 3. Total costs of consumables used for PRP preparation

ITEM	PRICE PER UNIT/ ML SOLUTION	NO. OF ITEMS USED	COST (USD; $)
Butterfly needle	7.10	1 unit	7.10
Plain vacutainer bottle	1.44	4 bottles	5.76
EDTA vacutainer bottle	1.76	1 bottle	1.76
ACDA solution	1.73	2.4mL	4.14
10mL syringe	1.22	2 units	2.44
18G x 4.5cm needle	7.82	1 unit	7.82
	Total cost		**29.02**

PRP: platelet-rich plasma; EDTA: ethylenediaminetetraacetic acid; ACDA: anticoagulant citrate dextrose solution-A

Table 4. Price, time of preparation, and platelet concentration factors of several PRP kits in Malaysia

PRP KIT	PRICE (RM)	PREPARATION TIME (MIN)	PLATELET CONCENTRATION FACTOR
Product 1 (60mL)[a]	1600.00	15	3.2
Product 2 (60mL)[b]	1512.00	15	4.9
Product 3 (30mL)[c]	480.00	15	5.4 – 6.0
Product 4 (20mL)[d]	400.00	20	N/A

PRP: platelet-rich plasma; RM: ringgit (Malaysia)

[a] Biomet System GPS III (Zimmer Biomet, Warsaw, IN; www.biomet.com)

[b] SmartPrep 2 System (Harvest Technologies, Lakewood, CO; www.harvesttech.com)

[c] TriCell Documentation (REV-MED International, Munich, Germany; www.revmedinc.com)

[d] Dr.PRP Separation Kit (Rmedica, Geumcheon-Gu, Korea; www.rmedica.com)

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