Standardization of Platelet-Rich Plasma Preparation protocol: for desired and consistent composition of platelet.

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ABSTRACT: Introduction: *Platelet-rich plasma is widely used in different fields of medical science, because platelets are rich in growth factors and cytokines which play important role in wounds healing and tissue regenerative processes. The wide variation in reported PRP preparation protocol leads to variable composition and biological response. Detailed, precise and stepwise description of PRP preparation protocol for consistent composition is needed.*

Aim: *Obtain desired composition of platelets in PRP.*

Materials and method: *For PRP preparation 5ml of whole blood (4.38ml blood+0.614ml CPDA-1 anticoagulant) from 60 participants (30 study group-A and 30-B for data validation) in two subgroup test tube. Test tubes are centrifuged for 1st spin: subgroup B-I at 200g for 12min, B-II at 700g for 4min and 2nd spin: B-I at 1500g for 6min, B-II at 700g for 15min. By leaving calculated PRP-2 volume required for desired platelet concentration according to formula, remaining upper platelet poor plasma (PPP) was separated from PRP-1.*

Results: *In B-I, PRP-1 volume was 2.166±0.287 ml with platelet concentration 393.8±117.7x10^3/µL and in B-II; PRP-1 volume was 2.148±0.273 ml with platelet concentration 402.0±105.77x10^3/µL. In B-I, PRP-2 volume was 0.7996±0.234 ml with platelet concentration 1017.37±11.62x10^3/µL (5.532±1.484-fold) and residual WBC 0.8392±0.5441%. In B-II, PRP-2 volume was 0.7766±0.201 ml with platelet concentration 1019.57±18.07/µL (5.529±1.418-fold) and residual WBC 1.6705±0.9846%.*

Conclusion*: Desired composition of platelet can be prepared by using appropriate centrifugal force, time and adjusting final PRP volume according to their individual platelet concentration.*

Key Words: *Platelet-rich plasma; Growth factors; Cytokine; Regeneration.*

1. Introduction:

Platelet-rich plasma (PRP) is autologous preparation, containing platelets count above peripheral blood level, in small volume of plasma.[1, 2] PRP have been used since the 1970s and becomes popular since the 1990s.[3] PRP preparation methodology continues to broaden from conventional blood centrifugation to commercial systems.[4-6] PRP treatment has Additional advantages of easy methodology, low or non-invasive procedures, cost-effective, safe (autologous product), shorten the recovery period, more lasting effect than conventional therapy, therefore improving life quality of patients.[7-10] Clinical application of PRP in numerous studies have demonstrated appreciable and notable results for wound healing and regenerative processes, in different areas of medical science.[11-35]

Platelets are formed in the marrow from megakaryocytes and present in peripheral blood like cytoplasmic fragments, approximately 2µm in diameter.[36-37] Platelets are rich in growth factors like- platelet-derived growth factor, transforming growth factor-ß, platelet-derived epidermal growth factor, vascular endothelial growth factor, insulin-like growth factor-1, fibroblastic growth factor and epidermal growth factor [38] which play an important role in soft and hard tissue healing.[39] Their bioactive proteins have a fundamental role in haemostasis or tissue healing.[40] Platelets also has anti-inflammatory, analgesic effects.[41-46] antibiotic effect.[47] After endothelial injury platelets are activated by contact with collagen, they secrete stored intercellular mediators and cytokines from their cytoplasmic pool and release their α-granule content (growth factors) after aggregation. Their secretion is intense in first hour and at least one week they continue synthesizing more cytokines and growth factors from their mRNA reserves.[48] Many proteins are secreted into surroundings [49] having a paracrine effect on different cell types: endothelial

cells.[50] tendon cells,[36,51-53] myocytes,[51] mesenchymal stem cells from different origins,[54-55] chondrocytes,[56] osteoblasts,[57] fibroblasts.[58] These signaling cytokines and growth factors regulate inflammation, collagen synthesis, stimulating cell proliferation, cell migration, angiogenesis and synthesis together, results in tissue regeneration with remodeling of newly formed tissue.[59-61] Some authors considered, a platelet count of ≥1 million/μL (approximately 4-7 fold of baseline platelet count) as therapeutically effective concentration of platelets in PRP.[62-64]

Although application of PRP in chronic injuries of bone and cartilage has given promising result, reduces pain and improves knee function and is superior than hyaluronic acid.[18] After *in vitro study* of PRP and mesenchymal stem cells combination some authors concluded, PRP favoured the osteogenic differentiation[54] and others demonstrating a chondrogenic compromise.[55,65] Some studies reported improvement in androgenetic alopecia with PRP treatment [21,66-67] while some reported no improvement.[68-69] These variations could be due to different RPP preparation protocol, their platelet and leukocyte concentration, which can modify its growth factor content.[2] Studies have reported growth factor decreases after use of higher centrifugal force [70] and cooling may retard platelet activation during PRP preparation.[71] Some authors believe presence of WBC provides natural protection against infections and allergic responses.[72] Other authors correlated presence of high WBC concentration with their catabolic cytokine concentration (matrix metalloproteinase-9 and IL-1β),[73] catabolic gene expression,[73-74] and release of toxic reactive oxygen species,[75] destroying surrounding tissue, even if the tissue is not injured [76-77] and thus increasing inflammation and reducing tissue regeneration.[75] At the moment, nomenclature and definition of PRP has significant controversy.[3,78-83] There is no universally established clear gold standard protocol for collection, PRP preparation, quality control, administration of the obtained products, threshold dose required for therapeutic benefit and lack of regulation.[8, 84 -86].The detailed, precise, and stepwise description of the PRP preparation protocol (centrifugal conditions, consistent platelet and leukocyte concentration, type of anticoagulant used) is required.[8, 12, 83 -88]

Therefore, the purpose of this study is to develop a standard PRP preparation protocol, for obtaining desired concentration of platelet and WBCs in PRP consistently, at standard temperature 22 ^{o}C (20°C-24°C) by using appropriate centrifugal force, time and adjusting PRP volume.

2. Materials and method:

Blood collection- After the approval of Hospital Local Ethics Committee, 60 healthy adult volunteer participants between 18-60 years of age was enrolled to the study. Out of total 60 subjects, 30 were processed in the study group (group-A) and rest 30 (group-B) were used for validation of results. All participants gave informed consent. Blood collection was performed following aseptic procedure. For blood collection from each participant, 1.4 mL of anticoagulant CPDA-1was collected in a 12 mL syringe, needle was replaced with 18-guage needle and 10 mL of blood was collected in the syringe (total volume, anticoagulant + blood=11.4 mL) from antecubital region within 2-4 min. Blood was mixed properly with the anticoagulant to prevent clotting and 5 mL of whole blood was transferred in each two separate test tubes namely subgroup-A-I and A-II for PRP preparation. For baseline blood cell analysis, remaining 1.4 mL of whole blood was transferred in a separate test tube for each participant. Blood cell count was performed with cell counting equipment (Sysmex KX-21, Sysmex Corporation Japan).

2.1 PRP Preparation:

All centrifugal process was done at 22°C, in a Refrigerated centrifuge model (ROTO SILENTA 630 RS, Hettich, Instruments LP. Germany). We use two centrifugal steps for PRP preparation, Brief protocol described in figure-1.

2.1.1 First Spin:

For 1st spin, subgroup A-I, whole blood was centrifuged at RCF-200g for 12 min and subgroup A-II, at RCF-700g for 4 min. The upper layer of platelet rich plasma was collected into other empty test tube, by using micropipette with care to avoid mixing of RBC and labeled as PRP-1. Collected volume ranged between 1.5 to 2.5 mL. PRP-1 was adequately mixed to homogenize and Blood cell count was performed with cell Hematology analyzer (Sysmex KX-21). Platelets and WBCs recovery in PRP-1 was calculated using the following formula/equations:

1. Total cell count = total volume in μL X cell concentration /μL

2. Percentage Platelet recovery in PRP1 $= \frac{\text{Total Platelet in PRP 1}}{\text{Total Platelet in Baseline}} X100$

3. Percentage residual WBC in PRP1 $= \frac{\text{Total WBC in PRP1}}{\text{Total WBC in Baseline}} X100$

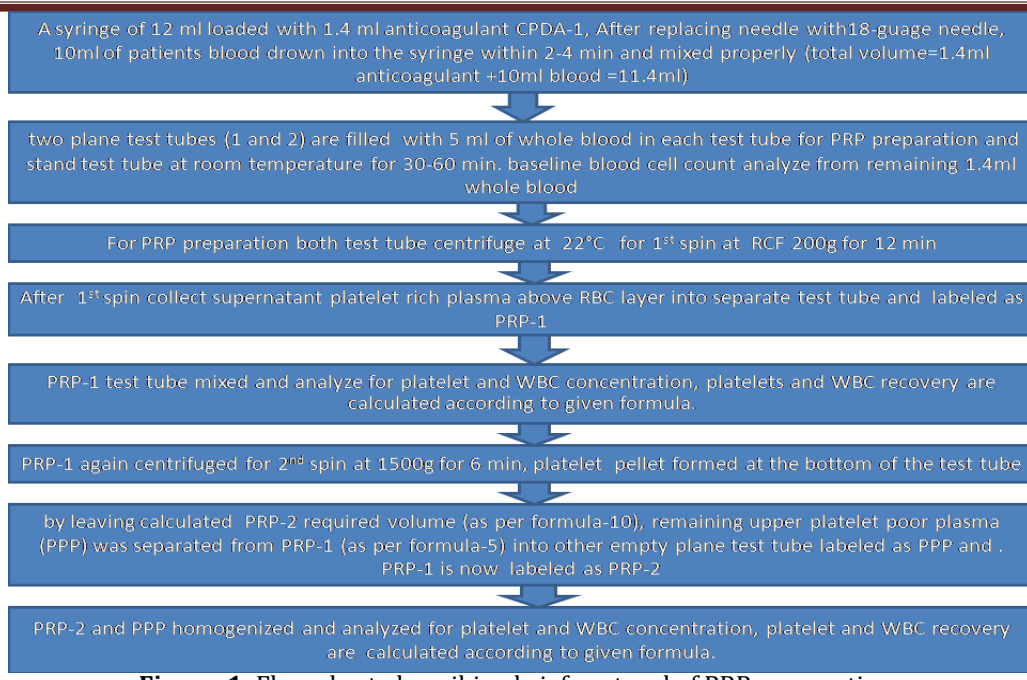

Figure-1: Flow chart, describing brief protocol of PRP preparation.

2.1.1 Second spin:

For 2nd spin, PRP-1 of subgroup A-I was centrifuged at RCF-700g for 15 min and subgroup A-II at RCF-1500g for 6 min. Platelets pellet was formed at the bottom of test tube. For obtaining platelet concentration consistently ≥1 million/µL, by leaving calculated plasma volume required in PRP-2 (as per equation-4) remaining upper platelet poor plasma (PPP) was separated into other plane test tube by the help of micropipette. PRP-1 is now named as PRP-2 (platelet concentrate). PRP-2 and PPP were homogenized and analyzed for platelets and WBC concentration. Platelets and WBCs recovery in PRP-2 were calculated using the following formula/equations:

4. PRP 2 volume required for desired platelets count/µL= $\frac{\text{Total platelts count PRP 1}}{\text{desired concentration of platelets /µL}}$

5. PPP volume separated = PRP1 volume – PRP 2 volume required for desired platelet count/µL

6. Percentage platelet recovery PRP 2 from baseline= $\frac{\text{Total platelets PRP 2}}{\text{Total platelets baseline}} X100$

7. Percentage platelet recovery PRP 2 from PRP 1 = $\frac{\text{Total platelets PRP 2}}{\text{Total platelets PRP 1}} X100$

8. Percentage residual WBCs in PRP 2 from baseline= $\frac{\text{Total WBCs in PRP 2}}{\text{Total WBCs in Baseline}} X100$

9. Percentage residual WBCs in PRP 2 from PRP1= $\frac{\text{Total WBCs in PRP 2}}{\text{Total WBCs in PRP 1}} X100$

2.2. Statistical analysis:

The results are presented as mean ± standard deviation. For statistical analysis data were entered into software Microsoft excel (sheet) and analysis were performed with the software Statistical Package for Social Sciences (SPSS 13.0) for Windows (SPSS Inc., Chicago, IL, USA). Inter subgroup comparison was assessed using students t-test. A p. value of <0.05 was considered statistically significant.

3. Results and observations:

3.1.1. Composition after First spin:

After 1st spin, group-A platelets and WBCs recovery in PRP-1 were given in table-1. The difference between subgroup A-I and A-II (table-1) of PRP-1 volume, platelet concentrationPRP-1, total platelet count PRP-1, fold increase platelet concentration PRP-1 from baseline and percentage platelet recovery was not statistically significant. But WBC concentration PRP-1, total WBC count PRP-1, percentage residual WBC in PRP-1 from baseline was statistically significant (All $P < 0.05$, Pair wise *t*-test).

After 1st spin (PRP-1, table-1) in PRP-1 of subgroup A-I, PRP-1 volume was 2.165±0.342mL with total

platelet count 812.57±247.53x10^6, percentage platelet recovery 85.30±6.24% and percentage residual WBC from baseline 0.92±0.57%. In subgroup A-II, PRP-I volume was 2.146±0.347 mL with total platelet count 830.41±252.21x10^6, percentage platelet recovery 87.19±5.58% and percentage residual WBC from baseline 1.86±1.41%.

Table 1: Result of group-A after 1st spin (platelets and WBCs recovery in PRP-1)

	Subgroup A-I (n=30)	**Subgroup A-II (n=30)**
PRP-1 volume in mL	2.165±0.342	2.146±0.347
Platelet concentration PRP-1x10^3/µL	383.17±122.39	395.60±128.62
Total platelet count PRP-1x10^6	812.57±247.53	830.41±252.21
Fold increase platelet concentrationPRP-1from baseline	2.00±0.28	2.07±0.31
Percentage platelet recovery PRP-1	85.30±6.24	87.19±5.58
WBC concentration PRP-1/µL	130.7±82.5	261.3±188.82
Total WBC count PRP-1x10^3	276.94±163.77	535.5±372.45
Percentage residual WBC in PRP-1 from baseline	0.92±0.57	1.86±1.41

Participant's (group-A, n=30) platelet concentration was 190.07±53.83x10^3/µL with WBC concentration 6.09±1.27x10^3/µL and total platelet count 950.33±269.15x10^6 with total WBC count 3.04±0.63x10^7 in 5 mL.

3.1.2 Composition after the Second Spin:

The difference between subgroup A-I and A-II of platelet concentration (PRP-2), percentage platelet yield (PRP-2 from PRP-1), percentage platelet yield (PRP-2 from baseline), platelet count in PPP, total platelet count in PPP, platelet yield of PPP from PRP-I,). WBC concentration, total WBC count and percentage residual WBC in PRP-2 from baseline, was statistically significant (All $P < 0.05$, Pair wise *t*-test).

After 2nd spin in subgroup A-I the calculated PRP-2 required volume 0.813±0.247 mL according to formula- 4, to obtain platelet concentration 1million/µL. The platelet concentration was 932.77±31.59x10^3/µL with percentage platelet recovery (PRP-2 from PRP-1) 93.27±3.15%, percentage loss platelet PPP (from PRP-1) 5.64±2.86% and percentage residual WBC from baseline 0.927±0.56%. In subgroup A-II, PRP-2 volume was 0.830±0.252mL with platelet concentration 952.1±30.72x10^3/µL, percentage platelet recovery (PRP-2 from PRP-1) 95.21±3.07%, percentage loss platelet PPP (from PRP-1) 3.03±1.90% and percentage residual WBC from baseline 1.823±1.32%.

Table 2: Result of group-A samples after 2nd spin (platelet and WBC yield and recovery in PRP-2)

	Subgroup A-I (n=30)	**Subgroup A-II (n=30)**
PRP-2 volume in mL	0.813 ±0.247	0.830 ±0.252
PPP volume discarded in mL	1.353 ±0.39	1.316 ±0.40
Platelet concentration in PRP-2x10^3/µL	932.77±31.59	952.1±30.72
Fold increase in pl. count in PRP-2 from PRP-1	2.72±1.02	2.68±0.97
Fold increase in pl. count in PRP-2 baseline	5.34±1.69	5.44±1.69
Total pl. count in PRP-2x10^6/µL	758.24±230.84	792.95±250.69
Percentage pl. yield in PRP-2 from PRP-1	93.27±3.15	95.21±3.07
Percentage pl. yield in PRP-2 from baseline	79.57±6.46	83.05±6.51
WBC concentration in PRP-2/µL	371.7±257.9	705.3±543.65
Total WBC count in PRP-2x10^3	279.49±165.61	525.00±347.89
Percentage residual WBC in PRP-2 from baseline	0.927±0.56	1.823±1.32
Percentage residual WBC in PRP-2 from PRP-1	100.6±8.48	99.56±5.31
Platelet count in PPPx10^3/µL	38.93± 30.74	21.63±18.77
Total pl. count in PPPx10^6/µL	44.97±27.68	24.91±17.30
Percentage pl. yield in PPP from PRP-1	5.64±2.86	3.03±1.90
Percentage loss platelet PPP from baseline	4.78±2.39	2.66±1.68

3.2 Optimization of platelet concentration in PRP-2:

After 2nd spin platelet concentration of PRP-2 was <1 million/µL in both subgroup. This difference is due to loss of percentage residual platelet in PPP. So for obtaining PRP-2 with consistent concentration of platelet concentration ≥1million/µL, we modify equation no- 4 as follows-

$$10.\ \text{PRP2 vol. required for desired platelet concentration}/\mu\text{L} = \frac{\{\text{Total plateletcount PRP 1} - (\%\ \text{loss platelet PPP} + 2\text{SD})\}}{\text{Desired platelet concentration}/\mu\text{L}}$$

For data validation and optimization of the platelet-rich plasma, we run another 30 sample named group-B (subgroup -B-I and B-II), samples are centrifuged for 1St spin, B-I at RCF 200g for 12 min and B-II at 700g for 4 min, for 2nd spin, B-I at 1500g for 6 min and B-II at 700g for 15 min. Results are given in table-3 and 4. In subgroup B-I, PRP-2 volume was 0.7996±0.234 mL with platelet concentration 1017.37±11.62x10^3/µL,

Fold increase platelet concentration PRP-2 from baseline 5.532±1.484-fold and Percentage residual WBC PRP-2 from baseline 0.8392±0.5441%. In subgroup B-II, PRP-2 volume was 0.7766±0.201 mL with platelet concentration 1019.57±18.07/µL, Fold increase platelet concentration PRP-2 from baseline 5.529±1.418-fold and Percentage residual WBC PRP-2 from baseline1.6705±0.9846%.

Table 3: Result of group-B samples after 1st spin (platelet and WBC yield and recovery in PRP-1)

	Subgroup B-I (n=30)	**Subgroup B-II (n=30)**
PRP-1 volume in mL	2.166± 0.287	2.148±0.273
Platelet concentration PRP-1x10^3/µL	393.8±117.7	402.0±105.77
Total platelet count PRP-1x10^6	841.715±246.863	853.428±221.274
Fold increase platelet concentration PRP-1from baseline	1.92±0.265	2.055±0.272
Percentage platelet recovery PRP-1	85.00±6.073	87.095±5.712
WBC count PRP-1/µL	133±80.7	264.±148
Total WBC count PRP-1x10^3	287.63±176.54	563.30±310.97
Percentage residual WBC count PRP-1 from baseline	0.839±0.549	1.654±0.99

Participant's (group-B, n=30) whole blood platelet concentration was 195.87±49.03x10^3/µL with WBC concentration 7.023±1.1685x10^3/µL and total platelet count 979.333±245.173x10^6/µL with total WBC count 3.5116±0.5842x10^7 in 5mL.

Table 4: Result of group-B samples after 2nd spin (platelet and WBC yield and recovery in PRP-2)

	Subgroup B-I (n=30)	**Subgroup B-II (n=30)**
PRP-2 volume in mL	0.7996±0.234	0.7766±0.201
PPP volume discarded in mL	1.367±0.359	1.371±0.322
Platelet concentration PRP-2x10^3/µL	1017.37±11.62	1019.57±18.07
Fold increase platelet concentration PRP-2 from PRP-1	2.87±1.078	2.739±0.846
Fold increase platelet concentration PRP-2 from baseline	5.532±1.484	5.529±1.418
Total platelet count PRP-2x10^6/µL	813.978±240.0	794.191±214.5
Percentage platelet recovery PRP-2 from PRP-1	96.64±1.10	92.78±1.64
Percentage platelet recovery PRP-2 from baseline	82.17±6.225	80.82±5.76
WBC count PRP-2/µL	385±251	771±437
Total WBC count PRP-2x10^3	287.49±174.25	569.71±311.69
Percentage residual WBC in PRP-2 from baseline	0.8392±0.5441	1.6705±0.9846
Percentage residual WBC in PRP-2 from PRP-1	100.18±3.761	100.4±3.4234
Platelet concentration PPPx10^3/µL	7.50±11.646	4.73±12.988
Total platelet count PPPx10^6/µL	21.714±13.202	44.994±12.519
Percentage loss platelet PPP from PRP-1	2.52±1.215	5.431±1.47
Percentage pl. yield in PPP in from baseline	2.147±1.078	4.717±1.263

4. Discussion:

Present study was designed to develop a PRP preparation protocol for obtaining desired concentration of platelet in PRP consistently by using appropriate centrifugal force, time and adjusting PRP volume at standard temperature 22°C (20 °C-24°C). We obtained from 5 mL of whole blood (4.38 mL whole blood + 0.614 mL CPDA-1 anticoagulant), PRP-2 volume 0.7996±0.234 mL with platelet concentration 1017.37±11.62x10^3/µL (5.53±1.48-fold and 82.17±6.22% recovery from baseline) and WBC concentration 385±251/µL (0.83±0.54% from baseline) after centrifugation, 1st spin at RCF 200g for 12 min and 2nd spin at RCF 1500g for 6 min. Similar platelet concentration and recovery with slightly higher residual WBC concentration 771±437/µL (1.6705±0.9846% from baseline) after 1st spin at RCF 700g for 4 min and 2nd spin at RCF 700g for 15 min.

These findings explain selection of appropriate centrifugal force with time and required volume of PRP play an important role in PRP preparation. They affect overall quality and quantity of platelets in PRP. Numerous protocols have attempted to optimize PRP preparation by using various parameters of centrifugal force and time. Araki *et al.* [89] reported similar platelet recovery 70-80% with different WBC recovery higher (10-35%) and lower (4.1%-5.8%) respectively, after 1st spin, at 70g for10 min and at 230g– 270g for 10 min. They also reported that, WBCs precipitated with increase of centrifugal force of ≥840g. Amanda *et al.*[90] reported, 70%-80% platelet recovery and concentration 5-fold, after centrifugation of 3.5 mL of whole blood for 1st spin at 100g for 10 min and 2nd spin at 400g for 10 min and removal of 2/3 plasma. They also reported longer centrifugal time of 1st spin, slightly increase platelet recovery and decrease WBC in PRP. In our study, the percentage platelet yield was similar at RCF 200 g for 12 min and at RCF 700g for 4 min with statistically significant difference in percentage residual WBC lower 0.8392±0.5441% and higher

1.6705±0.9846% respectively. Our result shows WBC precipitate with higher centrifugal time as well as with higher centrifugal force during 1st spin. So considering their potential pro-inflammatory effect [42, 78, 91] WBC contamination can be minimized in PRP by centrifugation of whole blood by adjusting centrifugal force and time.

Some authors studied on low temperatures and higher RCF for PRP preparation. Rachita Dhurat and MS Sukesh [92] reported consistently platelet count >1 million/mL after centrifugation on temperature 16°C, 1st spin at RCF 900g for 5 min and 2nd spin at 1000g for 10 min, in the lower 1/3rd of the plasma. Amable *et al.* [93] reported on temperature 12°C highest 87% platelet recovery after the 1st spin at 300g for 5 min and highest 97.4% platelet yield from PRP-1 after the 2nd spin at 700g for 17 min. They obtained 3.6-fold platelet concentration in 300µL PRP-2. But Macey *et al.* [71] reported that cooling may retard platelet activation so temperature is also an important factor for viable platelets in PRP. Transfusion medicine technical manual DGHS, [94] Standard for Blood Bank and Blood Transfusion Services NACO Govt. of India [95] and AABB manual [96] recommends temperature 22±2°C for PRP preparation. Considering these we maintained temperature 22°C during PRP Preparation. Jo *et al.* [97] studied on centrifugal time and relative centrifugal force (RCF) and reported highest (92%) platelet recovery with platelet concentration 310.7±78.5×103/mm3 from 9 mL whole blood, after centrifugation 1st spin at 900g for 5 min. After 2nd spin at 1500g for 15 min they obtain highest 84% Platelet recovery with 633 .2±91.6×103/mm3 platelets concentration (4.2-times from baseline). Dugrillon *et al*. [70] reported when centrifugal force is less than 800g, Growth factors TGF-β1 increases with increase of platelet concentration and decreases when forces are above 800g. They stated quality is more important than platelet concentration in PRP. Considering these, we selected centrifugal force 200g and 700g for the 1st spin.

Anitua E *et al* [11] in 1999 proposed one spin method for PRP preparation. They centrifuge 4.5 mL of whole blood, at 460g for 8 min and collect 0.5 mL of plasma as PRP, located just above the Buffy coat. The upper fraction was discarded as PPP. The platelet concentration was 2.67-fold from the baseline value. Landesberg *et al.* [98] reported, PRP with 3.2-fold baseline platelet concentration after centrifugation of 5 mL of whole blood two spin at 200g for 10 min per spin. Bausset *et al*. [99] obtained 2.0 mL of PRP with 3.47-fold platelet concentrations from baseline, after centrifugation of 8.5 mL of whole blood and reported centrifugation of 130g or 250g for 15 min for two spin was optimal for the two spins procedure. Kececi *et al.*[100] obtained 1.5 mL PRP from 9 mL whole blood. After 1st spin at 250g (250-270g) for 10 min and the 2nd spin in increasing order at- 300g, 500g, 750g, 1000g, 1500g and 2000g for 10 min. Platelet concentration increases 1.92-fold, 2.16-fold, 2.80-fold, 3.48-fold, 3.67-fold, and 3.76-fold respectively. They concluded to select centrifugal force according to their baseline value, for obtaining a standard platelet concentration. Our study shows desired platelet concentration can be obtained by adjusting their PRP volume simply.

Some authors compare, commercially available PRP kits: Kevy SV *et al.* [101] reported, platelet recovery from PRP-kits- SmartPReP-72% (4-fold), 3iPCCS-58%, Sequestra-54%, Secquire-31%, CATS-31%, Interpore Cross-27% and Biomet GPS-42.6%. Sundman *et al*. [78] reported, increase of platelet concentration 1.99-fold with decrease of WBC count 0.13-fold by using Arthrex ACP kit, they also reported increase of platelet concentration 4.69-fold and WBC 4.26-fold, by Biomet GPS III kit. Mazzucco *et al.* [65] compared three commercial kits (Fibrinet, RegenPRP-Kit and Plateltex) and a homemade procedure. They reported 1.6-fold to 4.4-fold increase in platelet concentration. Le *et al.*[72] compared four commercial kits (Curasan PRP kit, Plateltex, GPS II, RegenLab) and a homemade protocol. They reported 2.75 -fold, 3.43-fold, 1.89-fold, 1.55-fold and 1.77-fold increase in platelet count and 32.0%, 20.0%, 22.6%, 79.0% and 45.6% platelet recovery respectively. Castillo *et al*. [102] compared three commercial kits- Biomet GPS III, MTF Cascade and Arteriocyte Magellan systems. They reported 2.07-fold, 1.62-fold and 2.80-fold increase in platelet count and 22.6%, 67.6% and 65.5%, platelet recovery respectively. Tamini *et al*. [103] reported comparison of two PRP preparation systems: Double centrifugation (ACE system; Surgical Supply and Surgical Science Systems, Brockton, MA, USA) and single centrifugation (Nahita System; Nahita, Navarra, Spain). For ACE system, 8.5 mL whole blood was centrifuged for 1st spin at 160g (1300rpm) for 10 min and 2nd spin at 400g (2000rpm) for 10 min, For Nahita System, 3.5 mL whole blood was centrifuged at 280g (1500rpm) for 7 min, Platelet concentration was 3.36-fold in ACE system and 2.27-fold in Nahita system. Rachita Dhurat and MS Sukesh [92] stated commercially marketed PRP kits are widely variable; they produce different composition of platelets and WBCs depending on their centrifugal force and time, which influence variability of growth factors concentration.

Our PRP preparation is highly purified as WBCs are minimized (0.83±0.54% from baseline) and platelet count (1017.37±11.62x10^3/µL with 5.53±1.48-fold) with recovery (82.17±6.22%) in PRP optimized. These parameters are better than those reported in current literatures including commercial PRP kits.

This study protocol may be helpful for personnel of blood transfusion medicine, researchers and clinicians of regenerative medicine for obtaining desired concentration of platelets with standard quality and quantity in PRP consistently, which can be used reliably in regenerative medicine and study, evaluating regenerative ability of PRP in different regenerative processes.

5. Conclusion:
Desired and consistent composition of platelet can be prepared by using appropriate centrifugal force with time and adjusting final PRP volume according to their individual platelet count.

6. Conflict of interest: Nil

7. Disclosures: None to disclose.

8. Acknowledgment: Nil

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