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RESEARCH ARTICLE

COMPARISON OF DIFFERENT METHODS OF CENTRIFUGATION FOR OPTIMUM PREPARATION OF PLATELET RICH PLASMA (PRP)& FRAMING A CUSTOMIZED PRP PROTOCOL-AN INNOVATIVE APPROACH

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Abstract

Introduction: The rationale behind the therapeutic potential of the high concentration of platelets in PRP (Platelet Rich Plasma) for intervention procedures of soft tissue injuries is often challenged by the ability of the centrifugation methods followed in extracting the high quality PRP with prompt contented quantity too.This study aims to unravel the basic science of PRP preparation involved in centrifugation protocols, spin methods, speed and time duration of centrifugation to obtain consistent therapeutic platelets yield.

Materials and Methods:30 participants were subjected to intervention procedures using PRP. For therapeutic purpose 3 centrifugation protocol types are followed.

- Protocol 1-Conventional Old protocol;
- Protocol 2-Customised New protocol;
- Protocol 3-Spin Reversal of the routine -hard spin followed by soft spin;

The Platelets and WBC concentration and composition in the final autologous PRP sample harvested as a result of double centrifugation method are compared for quantity and efficiency.

Results: The maximum PCF-3.21 times above baseline and PRR -64.21% are through new customized spin proving its efficiency. As determined by one-way ANOVA, there is statistically significant difference between the PROTOCOL groups (1,2,3) for the variables PRP platelet count, PRP Leucocytes, PCF, PRR.

A Tukey post hoc test revealed that there is statistically significant value for Protocol 2 and Protocol 3 compared to the Protocol 1 for PRP platelet count, PCF and PRR whereas for PRP Leucocytes, there was a statistically significant difference between the Protocol 2 and Protocol 3 which again implicates the necessity of spin reversal in yielding better quantity of leucocytes.

Conclusion: To produce PRP samples with consistent and reproducible compositionsof platelets and leucocytes with better quality control standard through a detailed, precise and stepwise description of the manufacturing protocol has been explained in our study. Our perspective is in standardizing a safe, simple protocol that can be

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followed to obtain an optimal consistent platelet yield without the use of commercial kits, which has been proved statistically too.

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Introduction:-

Platelet Rich Plasma is a biological solution enriched with supra-physiologic amounts of various bioactive proteins and essential growth factors that trigger the regenerative efficacy of the injured soft tissues by promoting healing and repair.[10] There exists lack of standardisation and clarity regarding the biological complexity and optimal preparatory protocols which may have an impact on the treatment efficacy. The quantity of growth factors released could be related to the system of preparation employed. The methods of PRP manual preparation are elaborated in this article, highlighting their haematological yield and vice versa.

Literature Review:-

Variable protocols have been tried to initiate the centrifugation procedure optimally using performance-based standards and parameters of centrifugation

Table 1:- Summary of the available literature evidences.

S.No	Author	Centrifugation speed	No.of spin steps	Platelet concentration factor (from baseline)	WBC recovery efficiency	Platelet recovery efficiency	Volume of whole blood used	Remarks
1	Anitua et al. [1]		1	2.67				additional spins are needed to achieve higher platelet concentration factors (>3×)
2	Kahn et al. [4]	centrifugal acceleration of 3730 ×g for a period of 4 min	1				478 ml	
3	Slichter and Harker [5]	1000 ×g for a period of 9 min	2			80%	250–450 mL	subsequent centrifugation step of 3000 ×g for a period of 20 min decreased the platelet viability.
4	Landesberg et al [3].	two spins at 200 ×g for 10 min per spin	2	3.2			5ml	
5	Amanda G. M. Perez et al [9]	I-100 ×g for 10 min; II-400*g for 10 min	2	Aft first step-2; Aft second step-3 to 5		70–80%	3.5 ml	
6	Jo et al [2]	I-900 ×g for	2	4.2		I-92%; II-		

S.No	Author	Centrifugation speed	No.of spin steps	Platelet concentration factor (from baseline)	WBC recovery efficiency	Platelet recovery efficiency	Volume of whole blood used	Remarks
		5 min ;1500 ×g for 15 min				84%		
7	Bausset et al. [6]	130 or 250 ×g for a period of 15 min	2	3.47			8.5ml	
8	Araki et al. [7]	I-70 ×g for 10 min; I-230–270 ×g for 10 min; II-2300 ×g for 10 min	2	7.4	I-10 to 35%; I-4 to 6%	70-80% (with EDTA as anticoagulant); 35% (with ACD as anticoagulant)		
9	Mazzocca et al. [9]	I-1500 rpm for 5 min					10	low platelet (382 × 103 /mm3) and low WBC (0.6 × 103 /mm3)

Aim

The aim of the present study is to compare the biological characteristics of PRP focusing on the platelet & leukocyte concentration and composition, derived out of different centrifugation methods from a physiatry view.

Objectives:-

1. To make steps to yield PRP with optimum mean therapeutic dose of platelets as described in literature
2. To find out a unique, custom made, cost effective protocol with common laboratory centrifuge and readily available materials in a GovernmentTertiary Care Centre setup.

Study design-Prospective comparative cross-sectional study
Study area-Government Kilpauk Medical College-PMR Outpatient Department
Study period-3 months from October 2019 to December2019
Study Population-Patients visiting KMC-PMR OPD with musculoskeletal complaints

Inclusion Criteria: -

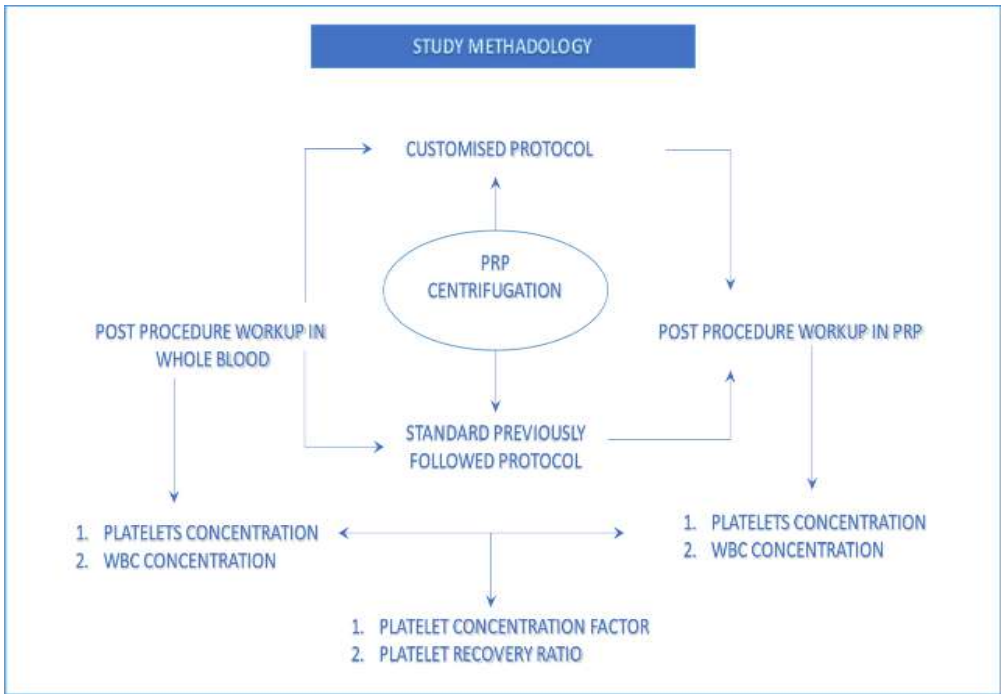
The Participants are the patients with various musculoskeletal conditions like Degenerative-Articular/Tendinous pathologies like Osteoarthritis of knee, Rotator cuff tendinitis, Supraspinatus Tendinitis, Medial and Lateral Epicondylitis, De Quervain'stenosynovitis, Retrocalcaneal bursitis, Plantar Fascitis, Pressure ulcers with symptoms >3months and no response to conservative management.

Exclusion Criteria-

Patients who had steroid intervention in last 3months. Cellulitis/acute infections, History of trauma, Use of anticoagulants and NSAIDS for past 7days, non-cooperative patients

Sample Size-

Total-30 Inclusive of-Osteoarthritis of knee-10; Rotator cuff tendinitis-9; Medial and Lateral Epicondylitis-3; DeQuervain'stenosynovitis-1; Retrocalcaneal bursitis-3; Plantar Fascitis-2; Pressure ulcers-2



Flow Chart 1:- Description of Methodology

Materials And Methods:-

Figure 1:- Image of the commercially available table top centrifuge used.

Operational procedure

Whole blood is initially collected in tubes that contain anticoagulant Acid Citrate Dextrose (preserves platelet viability) in the ratio of 6:1.[11]

The first spin step is performed at constant acceleration to separate RBCs from the remaining WB volume. After the first spin step, the WB separates into three layers: an upper layer that contains platelets poor plasma, an intermediate layer that is known as the buffy coat and that is rich in WBCs, and a bottom layer that consists mostly of RBCs. Only the upper layer or the upper layer plus buffy coat is transferred to an empty sterile vacutainer.

Figure 2:- Sample derived at end of spin 1.

The second spin step is then performed. The upper portion of the volume that is composed mostly of PPP (platelet-poor plasma) is removed to create the PRP (Platelet-Rich Plasma).

The concentrations of platelets and WBC in the final sample obtained are measured to characterize the quality of PRP**Centrifugation protocol design-**Initially we followed the **CONVENTIONAL** centrifugation protocol which was followed at The Department of Transfusion Medicine, Government Kilpauk Medical college- which was

- FIRST SPIN-1500 RPM/min-for 10 mins (soft spin)
- SECOND SPIN-3700 RPM/min-for 10 mins (hardspin)
- Centrifuge used: -8 holed bucket type table top REMI centrifuge
- Procedure DONE-Manual double centrifugation -tube method following all strict aseptic precautions in fumigated room by using sterile, pyrogen free 4 ml vacutainers
- The yielded final PRP samples are testedfor Platelet Concentration by Automated Hematology Cell Analyzer in The Department of Clinical Pathology and the results are tabulated. Then the idea of framing a new **CUSTOMISED** protocol based on the technical parameters of the centrifuge available in the department is formulated as follows:

- **How the New Protocol Arrived…. /?!**

Figure 3:- Measurement of radius of rotor.

Formula RCF=1.12*R(S/1000)^2*gWhere ‘g’ is the effect of earth’s gravitation field; RCF (Relative Centrifugal Force), R is the radius of the rotor (from center of rotor to sample) in millimeters and S is the speed of the centrifuge in revolutions per minute.[4]
Radius of Rotor-125 mm

Ispin-100g=850 rpm (FOR PLATELET SEPARATION) [9]

II spin-400g =1700 rpm (FOR PLATELET CONCENTRATION) [9]
Note: -100 g and 400 g respectively for first and second spins are already proposed nominal values that have yielded better results in previous studies.[9]

As per the centrifugal acceleration force generated is represented in terms of g force (effect of earth's gravitation field) than RPM,[9] the new spin methods (**PROTOCOL 2)** calculated according to the formula and the protocol are used to harvest autologous PRP and the concentration factor and recovery efficiency of platelets yielded are compared with the old conventional centrifugationmethod (**PROTOCOL1**). Likewise, the WBC concentrations are also compared between the two spin methods. Since literature recommends [13] spin reversal (hard spin followed by soft spin for better WBC yield in PRP), that is also tried. (**PROTOCOL 3)**

Variables Assessed-

a) Mean therapeutic platelet concentration of platelets /micro liter of PRP
b) Platelet concentration factor=platelet concentration in PRP yielded/platelet concentration in whole blood
c) Platelet recovery ratio= (platelet concentration in PRP*volume of PRP yielded)/ (platelet concentration in whole blood*volume of whole blood) *100
d) Mean therapeutic WBC concentration /micro liter of PRP.

Statistical Analysis-

a) The output values of the centrifugation processes are analysed through SPSS descriptive analysis by the Statistician, Tamil Nadu Dr. M. G. R. Medical University and the following parameters are collected: -
b) Mean and standard deviation of the platelet's concentration in whole blood and the PRP samples derived and comparing the values.
c) Mean and standard deviation of WBC concentration in whole blood and the PRP samples derived and comparing the values.
d) Mean and standard deviation of the platelet concentration factor (PCF) derived from PRP
e) Mean and standard deviation of the platelet recovery ratio (PRR) derived from PRP

Results:-

PROTOCOL	WB LEUCOCYTES				PRP LEUCOCYTES			
	Mean	SD	95% Confidence Interval for Mean		Mean	SD	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
1	6186.25	1985.1	4526.63	7845.872	2175	2917.2	-263.84	4613.84
2	6175	1455.8	4957.94	7392.06	5562.5	4012.458	2208.001	8916.999
REVERSAL	6292.86	2511.65	4842.67	7743.043	7292.86	3125.69	5488.137	9097.577

PROTOCOL	WB PLATELET				PRP PLATELET			
	Mean	SD	95% Confidence Interval for Mean		Mean	SD	95% Confidence Interval for Mean	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
1	258.5	44.954	220.918	296.082	118	85.298	46.689	189.311
2	234	35.24	204.54	263.46	397.13	193.875	235.041	559.209
REVERSAL	231.6	55.36	199.605	263.538	411.14	112.31	346.298	475.988

Of the 30 participants,19 participants are female and 11 are male with mean age of 52.23 years.8 followed the old spin protocol,8 followed new spin protocol and 14 followed the spin reversal technique.10 underwent intra articular knee joint injections.16 underwent intra tendinous injections.2 had intra bursal PRP and 2 had topical PRP application to chronic non healing pressure ulcers.

Table 2:- Comparative table depicting the Mean and Standard deviation (SD) of the outcome variables.

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
WB platelet count	Between Groups	4009.771	2	2004.886	0.864	0.433
	Within Groups	62685.43	27	2321.683		
	Total	66695.2	29			
PRP platelet count	Between Groups	487758.8	2	243879.389	13.775	0
	Within Groups	478014.6	27	17704.244		
	Total	965773.4	29			
WB leucocytes	Between Groups	94556.79	2	47278.393	0.01	0.99
	Within Groups	1.24E+08	27	4608513.823		
	Total	1.25E+08	29			
PRP leucocytes	Between Groups	1.33E+08	2	66721815.48	6.019	0.007
	Within Groups	2.99E+08	27	11084386.51		
	Total	4.32E+08	29			
Platelet concentration factor	Between Groups	9.75	2	4.875	9.943	0.001
	Within Groups	13.238	27	0.49		
	Total	22.988	29			
Platelet recovery ratio	Between Groups	5132.466	2	2566.233	11.714	0
	Within Groups	5914.856	27	219.069		
	Total	11047.32	29			

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) spin1_cat	(J) spin1_cat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PRP platelet count	1	2	279.1250*	66.53	0.001	114.2	444.08
		3	-14	58.97	0.969	-160	132.2
	2	1	-279.1250*	66.53	0.001	-444	-114.2
		3	-293.1429*	58.97	0	-439	-146.9
	3	1	14.02	58.97	0.969	-132	160.23
		2	293.1429*	58.97	0	146.9	439.36

Table 3:- Minimum and maximum values of the outcome variables.

PROTOCOL	PCF				**PRR**			
	Mean	**SD**	**95% Confidence Interval for Mean**		**Mean**	**SD**	**95% Confidence Interval for Mean**	
			Lower Bound	**Upper Bound**			**Lower Bound**	**Upper Bound**
1	0.465	**0.4**	**0.1313**	**0.7987**	**10.81**	**9.26**	**3.069**	**18.5585**
2	1.93	**0.85**	**1.2204**	**2.6421**	**42.2413**	**18.52**	**26.7552**	**57.7273**
REVERSAL	1.59	**0.74**	**1.1651**	**2.0163**	**39.11**	**14.97**	**30.466**	**47.7497**

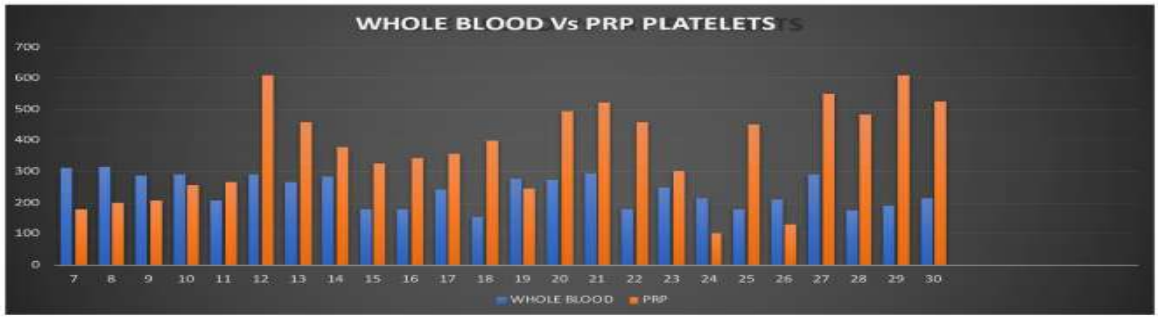
PRP Leucocytes	1	2	3388	1665	0.123	-740	7514.9
		3	-1730	1476	0.479	-5389	1928.2
	2	1	-3388	1665	0.123	-7515	739.89
		3	-5117.8571*	1476	0.005	-8776	-1459
	3	1	1730	1476	0.479	-1928	5388.9
		2	5117.8571*	1476	0.005	1459	8776.4
Platelet concentration factor	1	2	1.46625*	0.35	0.001	0.598	2.3343
		3	0.341	0.31	0.524	-0.43	1.11
	2	1	-1.46625*	0.35	0.001	-2.33	-0.598
		3	-1.12571*	0.31	0.003	-1.9	-0.356
	3	1	-0.34	0.31	0.524	-1.11	0.4289
		2	1.12571*	0.31	0.003	0.356	1.8952
Platelet recovery ratio	1	2	31.42750*	7.4	0.001	13.08	49.776
		3	3.133	6.56	0.882	-13.1	19.398
	2	1	-31.42750*	7.4	0.001	-49.8	-13.08
		3	-28.29411*	6.56	0.001	-44.6	-12.03
	3	1	-3.13	6.56	0.882	-19.4	13.131
		2	28.29411*	6.56	0.001	12.03	44.559

Table 4:- One-way Anova results.

As determined by one-way ANOVA, there was a statistically significant difference between the **PROTOCOL** groups (1,2,3) for the variables PRP platelet count, PRP LEUCOCYTES, PLATELET CONCENTRATION FACTOR and PLATELET RECOVERY RATIO whereas, there was no statistically significant difference for WB platelet count and WB LEUCOCYTES variables.

Table 5:- Post Hoc (Tukey HSD).

RESULTS-PLATELET CONCENTRATION RANGING FROM 33*10^3 TO 611*10^3/micro lit



Whole Blood Vs PRP Platelets

Graph 1:- Platelet concentration Range.

Inference:-

A Tukey post hoc test revealed that there was statistically significantly lower for **PROTOCOL** 2 and **PROTOCOL** 3 compared to the PROTOCOL 2 forPRPplateletcount, PLATELETCONCENTRATIONFACTOR and PLATELETRECOVERYRATIO whereas for PRPLEUCOCYTES, there was a statistically significant difference between the **PROTOCOL** 2 and **PROTOCOL** 3 which again implicates the necessity of spin reversal in yielding better quantity of leucocytes.

A Tukey post hoc test also revealed that there was no statistically significant difference between the **PROTOCOL** 1 and **PROTOCOL** 3 for PRP platelet count, PLATELET CONCENTRATION FACTOR and PLATELET

RECOVERY RATIO whereas for PRP LEUCOCYTES, there was not statistically significantly lower for category 2 and **PROTOCOL** 3 compared to **PROTOCOL** 1.

Detailed inference for each significant dependent variable:
Dependent Variable: PRP platelet count
A Tukey post hoc test revealed that the PRP platelet count is statistically significantly lower for **PROTOCOL** 2(397.1 ± 193.9 min, p = .001) and **PROTOCOL** 3 (411.1 ± 112.3 min, p = .000) compared to the **PROTOCOL** 1 (118 ± 85.3 min). There was no statistically significant difference between the **PROTOCOL** 2 and **PROTOCOL** 3 groups (p = .969).

Dependent variable: PRP leucocytes
A Tukey post hoc test revealed that the PRP LEUCOCYTES has statistically significant difference between the **PROTOCOL** 1 and **PROTOCOL** 3 groups (p = .005). There was no statistically significant difference between **PROTOCOL** 2 (2175.0 ± 2917.2 min, p = .123) and **PROTOCOL** 3 (7292.9 ± 3125.7 min, p = .479) compared to **PROTOCOL** 1(5562.5 ± 4012.5 min)

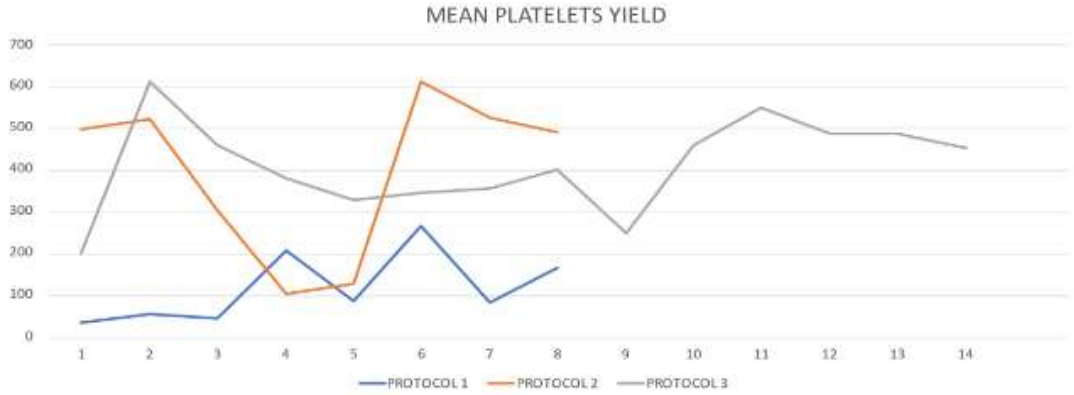
Dependent Variable: platelet concentration factor
Tukey post hoc test revealed that the PLATELET CONCENTRATION FACTOR is statistically significantly lower for **PROTOCOL** 1(1.93 ± .85 min, p = .001) and **PROTOCOL** 3 (1.59 ± .74 min, p = .003) compared to the **PROTOCOL** 2 (.47 ± .40 min). There was no statistically significant difference between the **PROTOCOL**2 and **PROTOCOL** 3 groups (p = .524).

Dependent Variable: platelet recovery ratio
A Tukey post hoc test revealed that the PLATELET RECOVERY RATIO is statistically significantly lower for **PROTOCOL** 1 (42.2 ± 18.5 min, p = .001) and **PROTOCOL** 3 (39.1 ± 14.97 min, p = .001) compared to the **PROTOCOL** 2 (10.8 ± 9.3 min). There was no statistically significant difference between the **PROTOCOL**2 and **PROTOCOL** 3 groups (p = .882).

At least more than half of the outcome variables assessed have showed significant statistical difference by ANOVA and there is statistically significant difference between protocol 1*3 and protocol 1*2.but not between 2&3 by post hoc Tukey test. Hence the alternate hypothesis of customisation of new centrifugation protocol based on rationalisation of parameters shall be accepted and expected to set a standard to enhance the quality of PRP optimally.

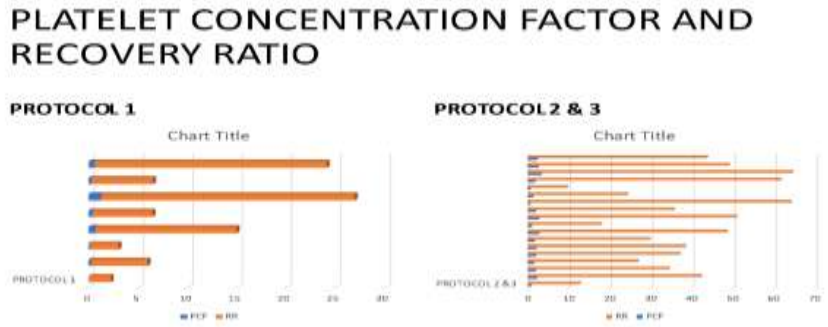
CONSISTENCY OF PRP YIELD AS PER LITERATURE
Figure 6:- PRP yield as per literature

Figure7:- LPRP yield as per our study.

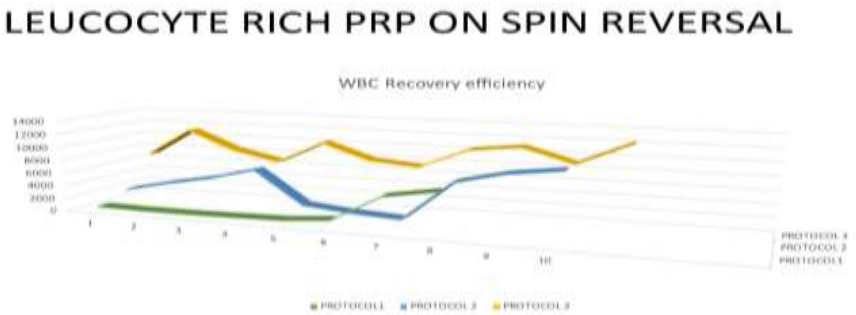


Graph-2:- Mean platelets yield-protocol wise.

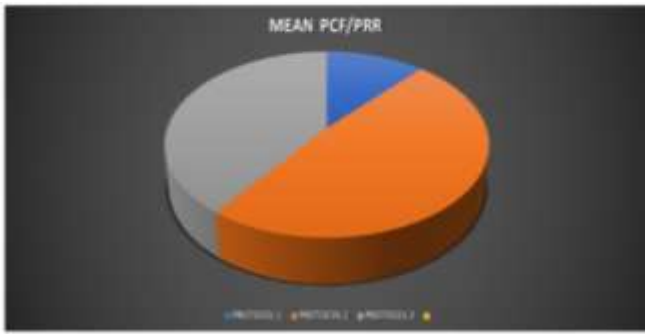
Graph-3:- Mean Leucocyte yield – whole blood vs PRP.



Graph 4:- Platelet concentration factor and Recovery Ratio-protocol wise.



Graph5:- Leucocyte PRP yield.



Graph 6:- Mean Platelet concentration factor and recovery ratio-protocol wise

Leucocyte rich PRP is meant for articular cartilages /structures with type 2 collagen Leucocyte poor PRP gives better result in places of tendons/structures with type 1 collagen

What is already available in literature
Not all PRP preparations are created equal

PRP can be affected by-1. Platelet recovery efficiency; 2. Final volume of PRP derived; 3. Presence and/or absence of anticoagulant in the sample; 4. Presence and/or absence of WBC 's in the sample; 5. The addition/absence of calcium chloride or thrombin

The temperature in which centrifugation is carried out

Leucocyte rich PRP is meant for articular cartilages /structures with type2 collagen Leucocyte poor PRP gives better result in places of tendons/structures with type 1 collagen [20]

What we learnt from the study
Double spin is better than single spin of centrifugation (single spin would not produce a true PRP. Instead, a mixture of PRP and PPP with disappointingly low platelet counts. will be produced).[3]

1. I Spin-platelet separation
2. II spin-platelet concentration
3. Variations in the volume of blood processed (5 to 120 ml)
4. Centrifugation shall be customized according to the centrifuge used
5. RPM (rotations per minute)-the term is now being replaced by g force
6. Customized preparation of PRP`~-Commercial PRP kits
7. Processing Qualitative Standards (PQLSs)-Centrifugation speed, duration, anticoagulants, temperature and the type of centrifuge and technical precision play a major role in determining the fruitful clinical outcome [15]
8. Platelets may get distorted at higher speed affecting extrapolation of clinical and experimental results [9]

Discussion:-

According to the physics of the centrifugation process, time and acceleration are the fundamental parameters that define the composition of the PRP sample after the first spin step.[16] According to the physical behavior, the extrapolation of the operating parameters is not straight forward, since it involves an exponential relationship with the distance traveled by the particles in the centrifugation. The method for quality assurance of individual PRP preparations should be given weightage equally and assessed. Still there are few unknowns/least considered facts regarding the processes that may have an impact on treatment efficacy for musculoskeletal conditions being dealt by the Physical Medicine and Rehabilitation team [17]

The high concentrations of cells are important, as the white blood cell count in PRP samples has frequently been ignored, being considered insignificant. These findings demonstrate that leukocytes strongly influence the quality of PRPs.[19] Therefore, modifying the PRP preparation method according to the pathology is essential to achieve better clinical results with PRP therapy. There is currently disagreement in the literature over whether the presence of WBC in PRP provides any benefit. Proponents of PRP containing high WBC concentrations (Leukocyte and

Platelet-Rich Plasma (L-PRP) according to Ehrenfest classification believe that the presence of WBC provides natural protection against infections and allergic responses [13]

1. The minimum PRP platelet count is from old spin method (PROTOCOL 1)
2. The minimum PRP WBC is from both old and new spin methods which in turn depicts that reversal of spins favors increased WBC yield.
3. The minimum PCF and PRR is from new customized spin. (PROTOCOL 2)
4. The maximum PRP platelet and WBC count is from spin reversal method and the same has been reflected in the mean values too.
5. The maximum PCF and PRR is from PROTOCOL 1but the mean values are maximum through PROTOCOL 2 Which recommends that customization yields better quantity platelets.
6. The lowest mean of PRP Platelets and WBC, PCF, PRR are from old spin method.
7. The maximum PCF-3.21 times above baseline and PRR -64.21% are through new customized spin proving its efficiency
8. The results are variable in PRP method and buffy coat method and the platelets and WBC concentration also varies according to the centrifugation protocol followed which definitely has a huge impact in the expected clinical responses and the functional outcome measures.[20]
9. The mean therapeutic dose of PRP is 1 million/cu mm of PRP in 5 ml of PRP as per literature [9], yet we are able to achieve $411*1^{03}$/cu mm of PRP
10. The mean PCF of 3 to 4 and PRR of 70-80% as per literature [7] are near normally reached by manual customized techniques also
11. The injection being carried out in the tendon, the volume of PRP should thus be minimal (to decrease the intratendinous pressure and to minimize pain). Hence it is necessary that PRP should also have raised platelet count invariably for producing desired output. The quantity of released growth factors could be related to the system of preparation employed [6]
12. The notification of these aspects ensures the overall quality of PRP by allowing the diversity of results to become narrowed only to the autologous nature of the product. This is the starting point for comparison of biological results as well as for the standardization of the PRP in cost-effective wayfor specific clinical applications than the commercial PRP kits.

Conclusion:-

Customization of PRP preparation protocol definitely helps in standardization and hence better yield of WBC and Platelets in the final PRP Sample. The quality of the PRP contents in terms of WBC as well as Platelets yielded, improves the quality of the intervention performed and better functional outcome.

The Platelet concentration factor and Recovery Ratio of the platelets sequentially increased on refining and customizing the centrifugal acceleration forces.

Low speed centrifugation concept (LSCC) selectively enriches leukocytes, platelets and growth factors within fluid PRP.[13]

A novel, custom made, economical protocol enabling us to get the procedure done in a simple and efficient manner with readily available resources, could produce PRP of comparable quality to those of a commercial PRP processing system making us to be more resilient, inclusive, cost effective and productive for a substantial better clinical outcome [8]

Future Scope-

Confirmatory studies will no doubt need to be completed in future.[15] Performance based standards shall be improved. Additionally, the high cost of commercially available PRP kits, hinders the liberaluse of PRP over a larger population which can be addressed in future with customised protocols of rationalisation.

Limitations

The Sample after first spin is not analyzed in my study which shall be considered for comparison in future studies. However, a manual method is susceptible to operator error, preluding to chances of bias.[12]

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