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Optimising Platelet Rich Plasma Preparation: Investigating the Effects of Temperature on platelet viability and growth factor release

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ABSTRACT

Background: Platelet rich plasma is an autologous plasma suspension of platelets & growth factors for treating multiple non-haemostatic condition. The usage and interest in platelets' regenerative qualities has grown dramatically over the last 50 years in a wide range of medical specialties worldwide.

Objective: The purpose of the study is to compare the levels of growth factors and platelet elevation in PRP samples that have been processed using the single spin & the double spin method, as well as the impact of temperature.

Method: we enrolled 28 subjects aged between 20-45 yrs. PRP prepared by single and double spin protocols. Single Spin Protocol: 10 ml blood was collected in heparinised falcon tubes and blood into 2 tubes (5ml each), processed at 2 different temperatures – 22°C and 37°C at 2700 rpm for 10 mins. In Double Spin, Sample collection & division was similar to single spin protocol, but 1st spin was performed at 1800 rpm for 12 minutes, 2nd spin at 3400 rpm for 6 minutes. After PRP preparation, the amount of growth factors and thrombocyte elevation was assessed.

Results: The mean concentration of VEGF was elevated (6.42±1.66ng/ml) when PRP was prepared at 22°C using double spin protocol, significant difference was observed at 37°C (5.49± 1.66 ng/ml). the amount of PDGF was not much affected by temperature but in double spin preparation the levels were higher (10.49± 3.88 ng/ml & 10.23± 3.92). Considering thrombocyte elevation, it was more in double spin preparation (22°C) i.e. 605.12 ± 93.03 * 103/µl.

Conclusion: The Double spin centrifugation protocol isolated increased thrombocytes and growth factors compared to single spin protocol. We observed that at 22°C the amount of growth factors and platelets were increased than at 37°C.

KEYWORDS: Alopecia; Platelet Rich Plasma; Growth factors; Thrombocytes.

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INTRODUCTION

Article Highlights:

Alopecia is a condition that causes baldness on head and other regions of body where hair is usually present. It is characterised by abrupt beginning of non-scarring hair loss in often clearly defined areas that ranges in size from small patches to large. There could be multiple reasons associated to the condition including genetics, hormones, drugs and lack of nutrition.

- **PRP Therapy**: there are multiple therapies currently available for managing this condition but each of them are associated with certain side effects. PRP therapy is autologous preparation of plasma rich with platelets having nearly no side effects and potential efficacy.
- **Study Objective**: The primary aim of the study is to standardise and Optimise a protocol for preparing PRP considering single and double spin protocol and temperature as the primary parameters.

Key Results:

SingleandDoubleSpinProtocol: In this study we observed that by using double spin method of PRP preparation, good yield of growth factors and platelets was observed compared to single spin protocol**.**

Temperature: We considered two different temperatures i.e., 22 & 37 for preparing PRP using both methods. The amount of VEGF and thrombocyte counts displayed a significantly elevated value at 22. The concentration of PDGF was elevated at 22 but there was no significant difference observed.

- **Conclusion:** Our Study suggests that method of PRP preparation and temperature highly impacts the proportion of growth factors and dose of platelets concentrated on which the potency of the therapy is dependent.

INTRODUCTION

Plateletsare specialised blood cells derived from megakaryocytes with In-Vivo half-life of nearly 7 days. Platelet rich plasma is one in which the baseline platelet concentration is more than twice that of plasma. According to definition it contains platelet count above the physiological levels i.e., minimum of 1,000,000 platelets /µl in 5ml of plasma, linked with 3 to 5- fold increase in amount of growth factors. It contains bioactive compounds that support systematic tissue healing response to damage, which includes phases of wound healing that includes inflammatory, reparative & remodelling of tissue. Platelets consist of glycogen, lysosomes, alpha granules and dense granules. Alpha granules contain growth factors such as Vascular Endothelial Growth Factor (VEGF), Platelet Derived Growth Factor (PDGF), Epidermal growth factor (EGF), Insulin growth factor-1& 2 (IGF 1&2) that have a critical role to play in chemotaxis, proliferation, cell differentiation & angiogenesis. The dense granules carry bioactive factors required for tissue recovery such as serotonin, dopamine, histamine, adenosine & calcium. But these granules are released only after the process of platelet activation. Critical growth factors & other signalling molecules including fibrinogen and leucocyte derived catabolic cytokines which control tissue healing but they are available only after the process of platelet activation. In dermatology PRP is being used as promising option for the treatment of several dermatological conditions including tissue regeneration, scar revision, wound healing & some types of alopecia. Alopecia is a prevalent aesthetic concern affecting millions of individuals globally impacting both men & women. It's a disorder that results in baldness on the head or other areas of body where hair normally grows, with a negative impact on the quality of life as it affects psychologically & has social repercussions. For the purpose of hair restoration, PRP is applied by intradermal injections to affected areas of skin. Alopecia comes in various forms, including Androgenic, alopecia areata, chemotherapy-induced, anagen effluvium, telogen effluvium traction, and trichotillomania, depending on the underlying reasons of the disorder [6]. Androgenic alopecia (AGA) commonly discussed as female or male pattern hair loss or balding & is the utmost typical kind of hair loss. It is thought to be caused by a sophisticated shrinkage of the hair follicle, which causes the hair to gradually diminish and bald spots to appear on the scalp [7]. The most prevalent form of hair loss, AGA, is influenced by heredity and hormones, particularly dihydrotestosterone (DHT). DHT causes hair follicles to shrink, shortening the anagen phase and leading to thinner, shorter hair growth [10]. Additional factors such as stress, nutritional deficiencies, medications, and certain medical conditions can also add on to hair loss [11]. There are numerous treatment possibilities currently available for managing this condition such as using medications-Minoxidil, it is a topical medicine It is a topical medicine that extends the hair growth during anagen phase for male and female pattern baldness. The major challenges with this drug is daily basis applications, also having limited effectiveness in advance hair loss and creates potential scalp irritation [12]. Another medication is available. Finasteride is an oral medication that prevents testosterone from being converted to DHT, a hormone that causes men's hair follicles to shrink. Its major uses for Androgenetic alopecia in men and ineffective for women due to hormonal differences. Potential side effects reported with the use of this drug is decrease in libido and erectile dysfunction [13]. Currently Hair Transplantation procedures such as Follicular unite Extraction (FUE) and Follicular unit transplantation (FUT) are also being used for managing the condition but each of them have their own advantages and disadvantages. Newer techniques like robotic hair transplantation are being explored, but their long-term efficacy requires further investigation. While hair transplantation offers a viable solution for hair loss, it's important to be aware of the challenges involved. PRP use has been expanding gradually because to its biocompatibility, ease of preparation, affordability, and patient and clinician acceptance of its minimally invasive administration method and immunogenic safety resulting from autologous blood derivation.[14]

MATERIAL & METHODS

After signing an informed consent form, we recruited 28 participants in this study, ranging in age from 20 to 45 years. To rule out acute or chronic illnesses, the entire medical history was taken into consideration.

Single Spin- Centrifugation protocol

Ten ml of whole blood was withdrawn in a vacutainer holding heparin of concentration 1: 5000 IU as anticoagulant. The samples were divided into two tubes (5ml each), one to be centrifuged at 22°C and other to be centrifuged at room temperature (37°C) and single spin was performed at 2700 rpm for 10 minutes.

Double Spin- Centrifugation protocol

Another 10ml whole blood was taken into heparinised vacutainer- the sample was divided into two tubes (5ml each) one to be centrifuged at 22°C and other at temperature of 37°C.

For first spin, the sample centrifugation was performed at 1800 rpm for a duration of 12 minutes, the sample got separated into 3 layers: top layer contains platelet poor plasma, layer in the middle has buffy coat- rich in platelets and leucocytes and at the bottom erythrocytes are present. The upper and middle layer is taken into a sterile tube and second centrifugation step is performed at 3400 rpm for 6 minutes- A platelet pellet is obtained at the bottom of the tube & now the plasma is rich with platelets and leucocytes, the platelet pellet is homogenized with the remaining plasma.

Quantification and Analysis

After PRP preparation, the quantity of growth factors VEGF & PDGF was analysed in all the above-mentioned scenarios (controlled temperature and room temperature for both single and double spin methods) using commercially available ELISA kits. The platelet count of PRP samples for all the groups was analysed on Sysmex XE5000 haematology counter.

RESULTS VEGF concentration Initially PRP was prepared using single spin method at both the temperatures i.e. at 22°C and at 37°C. we observed that in single spin at 22°C the concentration of VEGF was 5.49±1.66 and at 37°C the concentration was 5.27±1.57. since the concentration of growth factors was higher at 22°C but the difference in the variables was insignificant (p value= 0.29); followed by this, PRP was prepared using double spin method both at 22°C and at 37°C, we observed the mean concentration of VEGF was 6.42±1.664 & at 37°C the value was 5.49±1.66. it was observed that there was significant difference in the levels of growth factors when PRP was produced using double spin method. Lately, we observed the effect of temperature which influenced the release of growth factors i.e., we observed that at 22°C the concentration of growth factors was comparatively better than at 37°C as shown below.

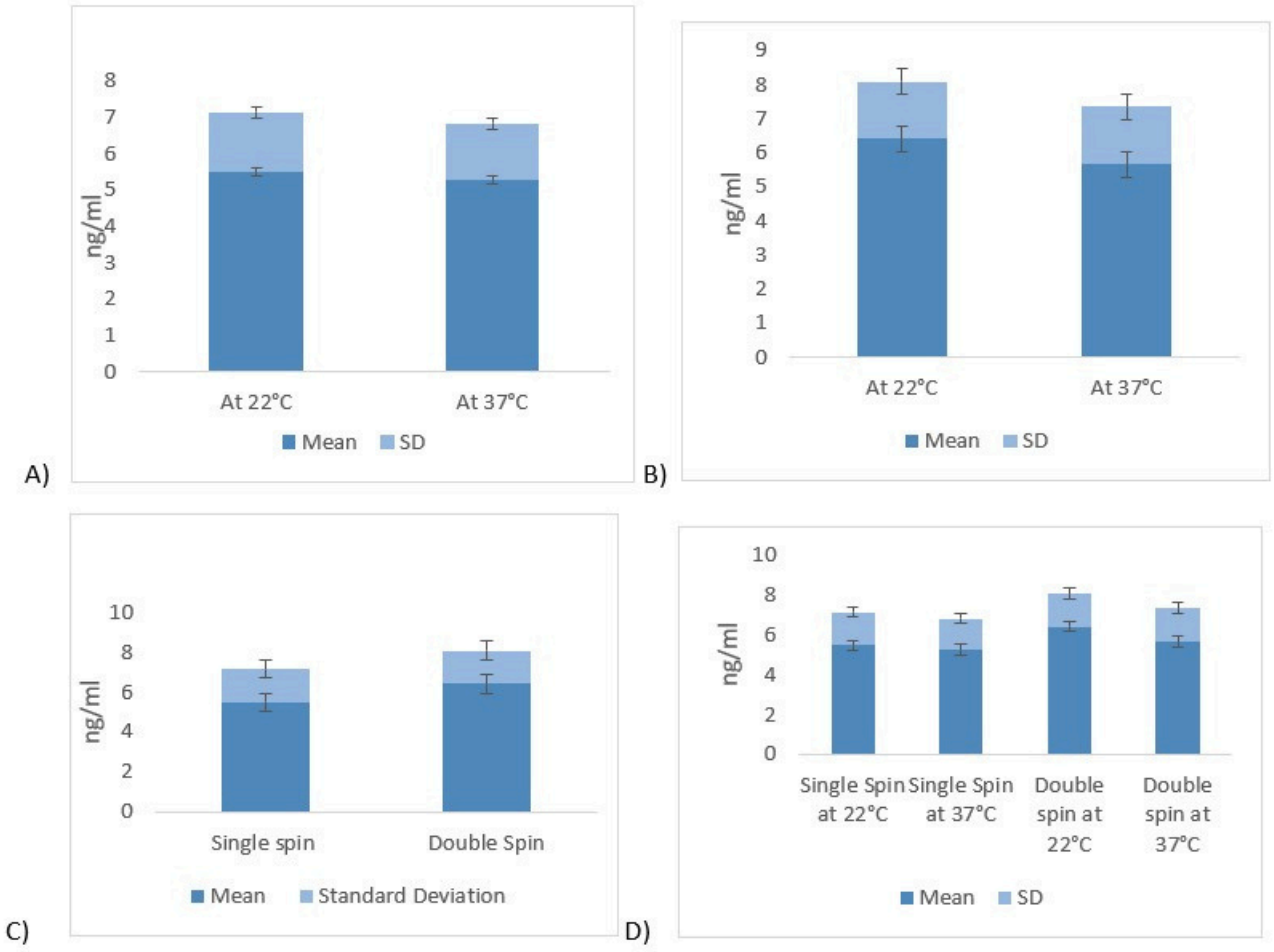

Figure 1. A) Concentration of growth factor VEGF when PRP Prepared using single spin method at two temperatures (at 22°C & 37°C): we observed that the conc. of VEGF was higher at 22°C but there was insignificant difference (p> 0.05) observed at both the temperatures. Similarly On Double spin method there was increased release of growth factors at 22°C (B). On comparing the method of preparation as in figure C, it was observed that there was significantly higher (p< 0.05) release of growth factors in double spin method. It was further observed that on comparing both the methods at both temperatures, the release of VEGF was maximum in double spin method performed at 22°C.

PDGF Concentration PDGF is a potentmitogen and has a significant role to play in developmentof blood vessels, growth of blood vessels from already existing blood vessel tissue etc. In the current study we observed the impact of temperature on the release of PDGF from alpha- granules of platelets. It was observed that when PRP was prepared by single spin method at 22°C, the concentration of PDGF was 10.37± 3.88 and at 37°C it was 9.94± 3.87. similarly, when PRP was prepared using double spin method at 22°C & 37°C the concentration was 10.49± 3.88 & 10.23± 3.92 respectively which was nearly similar. Lately, we compared the concentration at 22°C, we observed that the conc. was maximum in double spin method of preparation but the in both the methods at two different temperatures were nearly similar and the maximum yield of growth factors and platelets were maximum in double spin method, considering the above we observed that the concentration of PDGF is not much affected by the temperature but the no. of Spins does affect the isolation efficiency of PDGF growth factor.

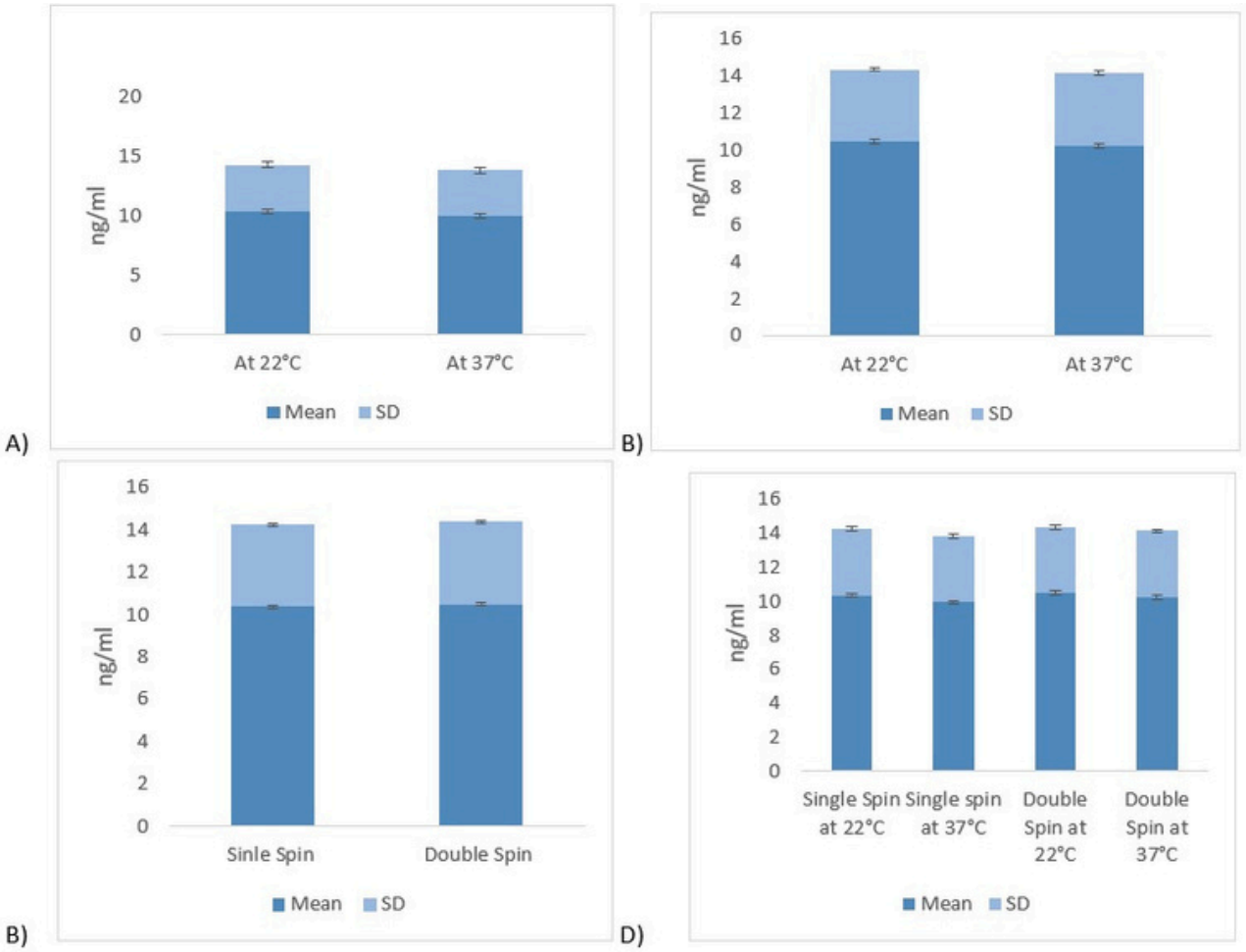

Figure 2. The concentration of PDGF was maximum at 22°C in single spin method and also in double spin method than at 37°C as seen in A & B. But on comparing both the methods at 22°C, it was observed that concentration was comparatively higher in double spin method (mean= 10.49 ±3.88) but the difference was insignificant (p>0.05) (C). On Comparing both the method at two different temperatures, it was observed that double spin method performed at 22°C released maximum amount of growth factors.

Platelet Concentration Indouble spin methodthe initial centrifugation is done to separate RBCs followed by second spin in which the platelets are suspended in final volume as pure PRP. When we performed double spin method at 22°C the mean concentration of platelets was 605.12 ± 93.03 whereas, when the temperature was elevated to 37°C the platelet count decreased to 536.27± 86.39. whereas, when we isolated PRP using single spin at 22°C & 37°C, platelet count was nearly similar i.e. 499.70 ± 78.34 & 499.76 ± 75.15 respectively, but the levels were comparatively low at 37°C as observed in double spin. Lately when we compared the platelet count at 22°C in single & double spin method, a statistically significant difference in the levels of platelets was observed. Here we observed that method of PRP preparation and temperature both have significant effect on number of platelets isolated in PRP as shown above.

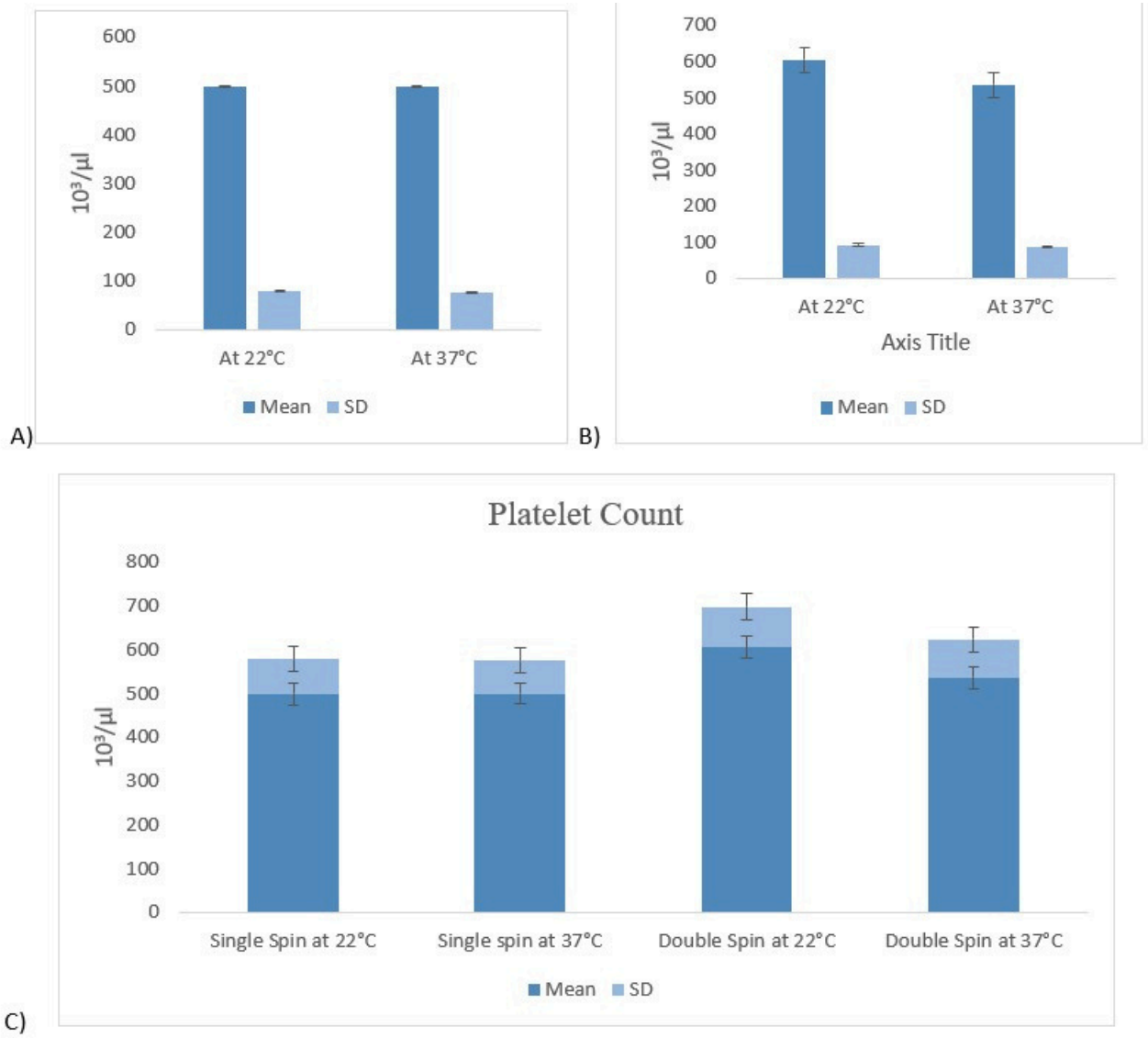

Figure 3. (A) The concentration of platelets was maximum at 22°C than at 37°C but insignificant difference was observed in single spin method whereas, in double spin method there was significantly higher($p<0.05$) number of platelets in double spin method performed at 22°C than at 37°C as shown in figure B, on comparing both method at both temperatures, there was significantly higher concentration of platelets at 22°C in double spin method.

DISCUSSION

Inthe last 20 years or more, PRP-based regenerative therapy has become more and more popular. Because it has been hypothesized that the concentration of growth markers in each PRP preparation contributes to some of its effectiveness, as this therapy concentrates on the availability of growth factors [15]. The centrifugal force acting in the radial direction, the gravitational force acting downward, and the drag force acting in the opposite direction of the particle motion all influence how the particle moves during centrifugation. The perceived mass of the particle determines the centrifugal force's magnitude. Temperature has a great role to play as the enzymatic activity as well as protein stability are highly heat sensitive parameters. Heat production during centrifugation might cause protein or enzymatic denaturation as well as premature, untimely activation of platelets and subsequent release of growth factors. Platelet activation can be delayed by cooling.

Kurita et al observed that double spin centrifugation produced considerably higher platelet concentration than single spin[16]. In a similar study, Saqlain et al conducted a cross-sectional study compared platelet count and platelet amount/ yield in PRP samples using single and double spin centrifugation protocols Platelet yield or platelet concentration (%) was calculated, the mean platelet count was maximum in the 2nd group when PRP was produced using double spin technique, the mean platelet count in single spin was 594.6 ±157.4 * 103/µl however, in double spin it was 923.06 ± 127.58 * 103/µl, compared to a single centrifugation procedure, it produced a greater platelet amount and yield with less red blood cell contamination[17]. Contradictory to the above results, Dubey et al studied the efficacy of PRP produced using single and double spin method for treating chronic ulcers, they observed a significant difference in the platelet count between whole blood and PRP prepared using both methods but in their study the one spin PRP system exhibited substantial improvements in healing parameters showcasing their future for managing chronic ulcers [18].

In our study we observed similar results that concentration of Platelets, Vascular endothelial & platelet derived growth factor was maximum in double spin isolation. In addition, we observed that temperature also affects the levels of VEGF and platelets count. The level of VEGF & platelet count was maximum & statistically significant difference was observed when the double spin was performed at 22°C and minimum when the single spin was performed at 37°C. whereas, the concentration of PDGF was not affected by the temperature, nearly equal levels of PDGF was observed at 22°C in both single and double spins, similarly at 37°C the levels were similar in both single and double spin but the maximum release of PDGF was seen in double spin method performed at 22°C. The first spin should be carried out at constant acceleration to separate RBCs from the remaining WBC volume when making pure PRP, and the second spin should be just sufficient to help create soft pellets. Currently, we might observe multiple protocols that describe the ideal conditions for centrifugation, number of spins, time duration for spins & which anticoagulant is ideal for sample collection, but have not considered the effect of temperature on growth factors and platelet count. Our study has primarily focussed on whether the concentration of GFs & platelets gets affected in single and double spin procedures and the impact of temperature that has a vital role in release of growth factors when isolating platelet rich plasma from the whole blood.

CONCLUSION

Although PRP preparation technology is comparatively advanced, each preparation method and approach have its own set of inherent drawbacks. Standardizing individual preparation protocols that are affordable and simple to modify for use in a clinical environment is advised, PRP preparation is significantly impacted by centrifugation parameters, including centrifugal force, blood volume, time, and the quantity of serum separating gel needed. Considering the above analysis, we have observed that while preparing PRP, method of PRP preparation and temperature does affects the concentration of growth factors and dose of platelets isolates which are seldom factors taken into account and computed to determine the most effective and efficient PRP. **Conflict of Interest**: None **Acknowledgement:** We want to express our earnest gratitude to the enthusiastic team for their precious patronage, which was essential to this study's success**.** Their work was essential for getting the samples we needed for our study. The subject's cooperation and desire to participate was essential to the overall success of our research, thus we would also want to thank them for their agreement to engage in this study.

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