Comparison of Platelet Concentration Levels Obtained from Ethylenediaminetetraacetic Acid Tube (EDTA) versus Commercially Available Platelet Rich Plasma (PRP) Kit in Filipino Adult Males with Androgenetic Alopecia: A Cross-Sectional Quantitative Study*

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ABSTRACT

Background: Platelet rich plasma (PRP) is increasingly used as an adjunct treatment for androgenetic alopecia (AGA). PRP preparation involves anticoagulant tubes, such as PRP kits or regular tubes such as EDTA. Studies have shown that platelet concentration in PRP correlates with the growth factors present. However, there is limited data regarding the platelet yield across different anticoagulant tubes. This study will aid in the use of EDTA tube as an alternative to the commercially available PRP kits.

Objective: This study aims to determine the platelet concentration level obtained in EDTA tubes versus the commercially available PRP kit in patients with AGA.

Methodology: This was a cross-sectional, quantitative study in 27 adult males with AGA. The venous blood extracted were placed in EDTA and PRP kit tubes and were centrifuged. A hemoanalyzer was used to obtain the platelet concentration levels. Statistical analyses using paired t-test was performed using the STATA MP Software.

Results: Comparative analyses indicated that the mean difference in platelet concentration between the PRP kit and EDTA was -472.67 (95% CI = -575.40 to - 369.93). Thus, the mean platelet concentration (t=-9.46, p=0.001) was statistically different between the two groups with a significantly higher mean platelet concentration with EDTA as compared to the PRP kit.

Conclusion: The platelet concentration levels obtained from the EDTA tubes were higher than the PRP kit. Thus, the EDTA tube may be a good alternative to the costly commercially available PRP kit.

Keywords: Platelet Rich plasma, EDTA, PRP Kit, Androgenetic alopecia

Commercial funding: N/A

Conflict of interest: N/A

INTRODUCTION

The prevalence of androgenetic alopecia (AGA) has substantially increased worldwide affecting 80% of men and 50% of women in the course of their life. Platelet rich plasma (PRP) preparations are increasingly being used in regenerative medicine, as well as for hair growth.[1] Platelet levels at 1,000,000 platelets/μL or 2-7 times its native concentration in whole blood is considered as the therapeutically effective in inducing tissue regeneration. Activated PRP was proved to contain the therapeutic platelet levels capable of promoting hair growth due to its antiapoptotic effects by activating antiapoptotic regulators like Bcl-2 and Akt; thus, extending dermal papilla cell lifespan.[2] The rationale for the use of high platelet concentration is based on their capacity to release large amounts of growth factors and cytokines from alpha granules, which augment healing and repair in tissues with low regenerative potential.[3]

All systems of PRP preparation follow a similar method which involves collecting blood in anticoagulant tubes such as sodium citrate or ethylenediaminetetraacetic acid (EDTA), centrifuging to separate RBCs and platelets, and activating the platelets to release growth factors (GF). However, PRP composition may vary due to differences in platelet and GF concentrations, collection volume, anticoagulant, centrifuge parameters, and activators. Clinical outcomes may differ based on injection methods, frequency, and intervals.[4]

There are a variety PRP kits and anticoagulant tubes for which PRP preparation may be performed; varying in price, size, anticoagulant used and PRP yield. These contain different anticoagulants and separator gels, which may influence platelet yield and its therapeutic effect.

Commercially available PRP kits are designed to produce autologous PRP by centrifuging peripheral blood

Disclosures: *The author has formally acknowledged and signed a disclosure affirming the absence of any financial or other relationships (including personal connections), intellectual biases, political or religious affiliations, and institutional ties that could potentially result in a conflict of interest.*

***1st Place, 31st Philippine Medical Association Residents' Research Presentation Original Paper, May 17, 2025**

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in a vacuum tube. Some of these kits use a thixotropic gel for cell separation and sodium citrate as an anticoagulant. They are simpler systems with a single centrifugation step in a closed system with a high platelet recovery.

Currently, there is only a single Food and Drug Administration (FDA) approved PRP kit available in the country. These kits offer the simplicity and ease of preparation of PRP at the expense of high costs. On the other hand, an economical variety of anticoagulant tubes are available, such as the blood collection tubes with EDTA, sodium citrate and acid citrate dextrose solution A (ACD-A). However, data regarding the platelet concentrate yield of EDTA tubes, in comparison with commercially available PRP kits and their effectiveness for PRP injection in patients with AGA remains to be limited, especially in the Philippines.

The outcome of the study included the platelet concentration level and mean platelet concentration level obtained from the EDTA and PRP tubes, in order to determine their significant difference, and evaluated the demographics and clinical profile of male patients with androgenetic alopecia.

REVIEW OF RELATED LITERATURE

Platelet Rich Plasma

Platelet-rich plasma (PRP) is defined as the processed liquid fraction of autologous peripheral blood that exhibits a platelet concentration exceeding baseline levels. PRP is prepared by centrifugation of autologous, anticoagulated whole blood in either 1 or 2 steps to separate it into 3 components composed of a top plasma layer, middle leukocyte layer, and bottom red blood cell (RBC) layer in order to collect a concentrate of platelets in plasma.[3] Within the platelets are the alpha granules which contain various growth factors, including platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epithelial growth factor (EGF), transforming growth factor-beta (TGF-3), and insulin-like growth factor (IGF) which facilitate the mitogenesis and differentiation of monocytes, fibroblasts, stem cells, keratinocytes, and endothelial cells. [5]

Platelet Rich Plasma for Androgenetic Alopecia

PRP has been used for AGA treatment, although its precise mechanism in promoting hair growth remains inadequately defined. Activated PRP is thought to enhance hair cycling by prolonging the anagen phase, inhibiting apoptosis and the catagen phase, and facilitating a more rapid transition from telogen to anagen phase. The growth factors in PRP stimulate the stem cells located in the bulge area of hair follicles, promoting neovascularization and folliculogenesis.[6]

Anticoagulant Tubes

A number of anticoagulant tubes are available for the collection of blood and in the preparation of PRP. Commonly used tubes are EDTA, ACD-A and sodium citrate tubes.

In a study by do Amaral, et al., (2016)[7], blood samples collected using EDTA demonstrated a higher platelet count compared to those collected with sodium citrate and ACD. On average, the platelet count in SC was 16.28% lower than that in EDTA, while the count in ACD was 23.01% lower than in EDTA and 7.94% lower than in SC.

Comparison Between Commercially Available PRP Kits and Anticoagulant Tubes

In a study conducted by Trevisson-Redondo et al., (2021)[8], the efficacy of three PRP concentration systems, namely the Easy PRP Kit, GloPRP, and Wego, was compared, alongside two types of anticoagulant tubes, EDTA and sodium citrate. The results indicated no significant differences in the average concentrations of PRP platelets and white blood cells between GloPRP when using either EDTA or sodium citrate; however, the Easy PRP Kit demonstrated significantly superior results compared to the other methods highest PRP: whole blood ratio (2.41 times versus 0.00- 1.13 times baseline for Easy PRP Kit) .

Remarkably, both tube-based methods achieved efficiencies of 56% (EDTA) and 60% (sodium citrate) of the total platelets concentrated by the Easy PRP Kit, surpassing the commercial GloPRP systems, which recovered only 46%.

In a narrative study conducted by Collin, Alexander, and Barkatali (2021)[9], results have shown that numerous commercial PRP preparation kits are available, each exhibiting varying concentrations of components. Currently, there is no consensus regarding the optimal concentrations of these components. Several classifications of platelet PRP have been proposed; however, none have been rigorously validated. The exact implications of the varying components of these systems are still unknown. It is evident that there is significant heterogeneity among the various commercial PRP systems. Therefore, this limits conclusions drawn from pooled PRP studies.

A small number of studies have been done comparing commercially available tubes with EDTA, sodium citrate and ACD-A as anticoagulant tube for PRP but results have been inconsistent and varied. A relatively few number of commercially available PRP tubes have been used in the country with only a single FDA approved

PRP tube. Currently, there are only limited studies comparing the efficacy of these tubes in achieving a high mean platelet concentration in comparison to the anticoagulants tubes (e.g. EDTA, sodium citrate, ACD-A).

Significance of the Study

This study will aid in the use of EDTA tubes as an alternative to the commercially available PRP kits. Thus, increasing the availability of PRP injection as a therapeutic option for AGA and other disease for which PRP may be of use, e.g. wound healing in trauma, joint injury and, aesthetics. This will aid in providing a greater number of patients with PRP as treatment option for AGA, not only in the Philippines but also in the greater parts of the world.

Research Question

Among patients with AGA, is the platelet concentration obtained from EDTA tubes comparable to the commercially available PRP kit?

OBJECTIVES OF THE STUDY

General Objective

- To determine the platelet concentration level obtained in EDTA tubes versus the commercially available PRP kit in patients with AGA

Specific Objectives

- To determine the demographic profile (age and civil status) and clinical characteristics (age at diagnosis, stage of AGA, smoking history, alcohol use, treatment history, family history of AGA) of the participants
- To determine the platelet concentration levels of PRP obtained with the use of EDTA tubes
- To determine the platelet concentration levels of PRP obtained with the use of the commercially available PRP kit
- To determine if there is a significant difference in the platelet concentration levels among the two

METHODS

Study Design

This is a cross-sectional, quantitative study that determined and compared the platelet concentration levels obtained from the EDTA tubes and commercially available PRP kit via a platelet determination test among adult male patients with AGA. The protocol was reviewed and approved by the Technical Review Board and the Institutional Ethics Review Board of the institution.

Study Setting

This was conducted in the outpatient department of a tertiary government hospital fully equipped study center for this study from November 2023 to July 2024.

Study Population

This study included all adult male androgenetic alopecia patients, aged 18-60 seen at the outpatient department of a tertiary government hospital.

Inclusion Criteria

All clinically diagnosed adult male patients, aged 18-60 years old with AGA seen at the outpatient department of a tertiary government hospital were included in this study.

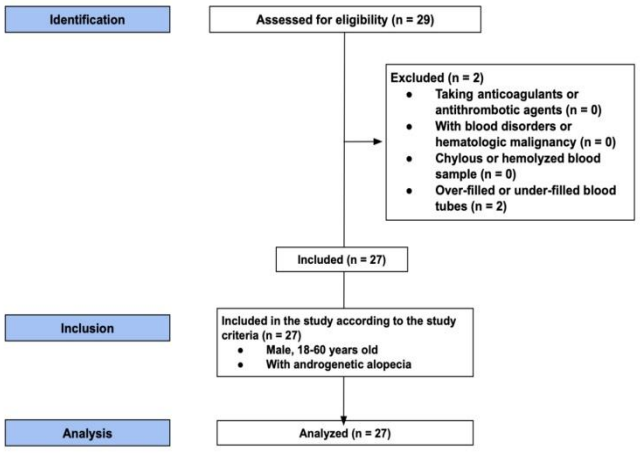
Exclusion Criteria

Participants taking anticoagulants or antithrombotic agents, with blood disorders or hematologic malignancy and those who declined to give their consent to undergo the procedure were excluded in this study. Blood samples from participants which are chylous, hemolyzed, with over-filled, and under-filled tubes were excluded from the study.

Withdrawal Criteria

The patients may withdraw from the study anytime that they decide not to participate in the study without any implications.

Figure 1. Study Framework



As seen in *figure 1,* during the period from November 2023 to July 2024, 29 male patients were assessed for eligibility. Of these 29, 27 patients were included according to the exclusion criteria wherein two participants had under-filled blood sample tubes.

Data Gathering Procedures

- Participants were recruited via a convenient non-probability sampling.
- An informed consent was given to each participant prior to the procedure.
- A total of 18 ml of venous blood were collected from the vein in the antecubital fossa of each participant using 10 ml syringes. These were distributed in two separate tube types, 8 ml for the EDTA tube and the other 10 ml were used for the commercially available PRP kit tube
- Centrifugation protocol used for the EDTA tubes [10]:
    - First spin: 100 g for 10 min
    - Recovery of the upper layer
    - Second spin: 400 g for 10 min
    - Upper ½ of the volume (platelet poor plasma) was discarded
    - Collection of PRP in a Red Top Tube
- Centrifugation protocol used for the commercially available PRP kit:
    - 1,500 RCF
    - Collection of PRP in a Red Top Tube
- *Extraction and Centrifugation process were done in the outpatient department of a tertiary government hospital
- PRP samples were ran under the hemoanalyzer (Mindray CAL 6000) at the clinical laboratory of a tertiary government hospital for platelet count determination. Prior to processing, the PRP were mixed by inversion, manually for 30 seconds for minimization of the gradient.

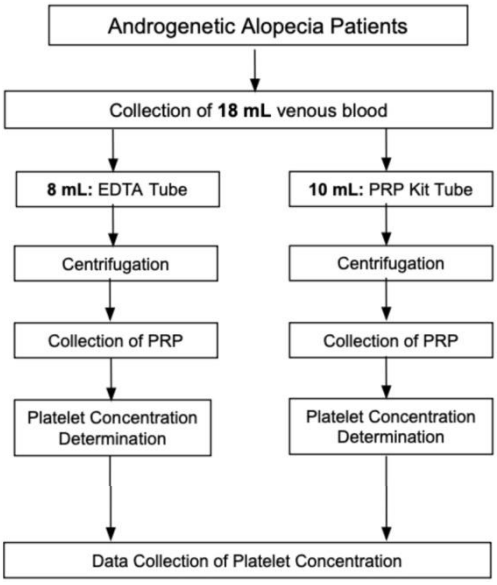


Figure 2. Study Methodology

t tests - Means: Difference between two dependent means (matched pairs)

Analysis: A priori: Compute required sample size

Input:	Tail(s)	=	One
	Effect size dz	=	0.5
	α err prob	=	0.05
	Power (1–β err prob)	=	0.8
Output:	Noncentrality parameter δ	=	2.5980762
	Critical t	=	1.7056179
	Df	=	26
	Total sample size	=	27
	Actual power	=	0.8118316

Figure 3. Sample size calculation using G*Power software

Sample Size

Figure 3 shows that the minimum sample size for this study was computed using G*Power software based on platelet mean difference between two tubes with effect size = 0.5 (medium), level of significance = 0.05 and power = 0.80 as shown below. Thus, the researcher included at least 27 patients for this study whose blood samples were used for both the EDTA tube and commercially available PRP tube. [11]

Sampling Method

Convenient, non-probability sampling was done to recruit 27 adult male patients aged 18-60 years old, diagnosed with AGA at the outpatient department of a tertiary government hospital.

Statistical Analysis

Statistical analyses were performed using STATA MP – Parallel Edition Statistical Software, Version 18, College Station, TX: StataCorp LP. A *p*-value <0.05 was considered statistically significant. Descriptive statistics included mean and standard deviation for normally-distributed, continuous data; median and interquartile range for ordinal data and non-normal, continuous data; and, frequency and proportion for nominal data. Data normality was analyzed using skewness, kurtosis, and Shapiro- Wilks test. Comparative analyses of the platelet concentration was conducted using paired *t*-test. In addition, the mean difference and their respective 95% confidence intervals (CI) were reported.[12]

RESULTS

Demographic Characteristics

Table 1. ***Demographic Characteristics of the Participants (N = 27)***

Characteristics	Frequency (f)	Percentage (%)	Mean (SD) or Median (IQR)
Age (Years; x, SD)			37.67
Age of Onset of Androgenic Alopecia (*f*, %)			
18 to 30 Years Old	12	44.44%	
31 to 40 Years Old	5	18.52%	
41 to 60 Years Old	10	37.04%	
Marital Status (*f*, %)			
Single	9	33.33%	
Married	18	66.67%	
Smoking Status (*f*, %)			
Non-Smoker	21	77.78%	
Smoker	6	22.22%	
Alcohol Consumption (*f*, %)			
Non-Drinker	9	33.33%	
Drinker	18	66.67%	
Psychoactive Substances (*f*, %)	0	0.00%	
Metabolic Diseases (*f*, %)			
Thyroid Disease	0	0.00%	
Diabetes Mellitus	3	11.11%	
Hypertension	10	37.04%	
Dyslipidemia	2	7.41%	
Treatment History (*f*, %)	6	22.22%	
Minoxidil	4	66.67%	
Hair Growth Shampoo	2	33.33%	

Table 1 presents the demographic characteristics of the participants. It can be noted that the mean age of the participants was 37.67 years old (SD = 12.12), and the majority had the onset of androgenic alopecia at the age of 18 to 30 years old (44.44%). Results also indicated that most participants were married (66.67%), were non-smokers (77.78%), were alcoholic beverage drinkers (66.67%), and had not used psychoactive substances (0.00%). The most prevalent metabolic disease among the participants were hypertension (37.04%) and diabetes mellitus (11.11%). Results also showed that 22.22% of the participants previously received treatment for hair loss, and among them, the most common was the use of minoxidil (66.67%).

Descriptive Statistics of Male Pattern Baldness or Hair Loss

Table 2. ***Descriptive Statistics of the Male Pattern Baldness or Hair Loss using the Norwood Hamilton Scale among the Participants (N = 27)***

Norwood Hamilton Scale	Frequency (*f*)	Percentage (%)	95% Confidence Intervals (CI)
Stage II	1	3.70%	0.09% to 18.97%
Stage IIA	6	22.22%	8.62% to 42.26%
Stage III	8	29.63%	13.75% to 50.18%
Stage IV	5	18.52%	6.30% to 38.08%
Stage IVA	2	7.41%	0.91% to 24.29%
Stage V	4	14.81%	4.19% to 33.73%
Stage VI	1	3.70%	0.09% to 18.97%

The descriptive statistics of the male pattern baldness or hair loss using the Norwood Hamilton Scale are shown in *Table 2*. It can be noted that the most prevalent male pattern baldness was Stage III (29.63%), followed by Stage II-A (22.22%) and Stage IV (18.52%). Only 3.70% had Stage II and Stage VI hair loss among the recruited participants.

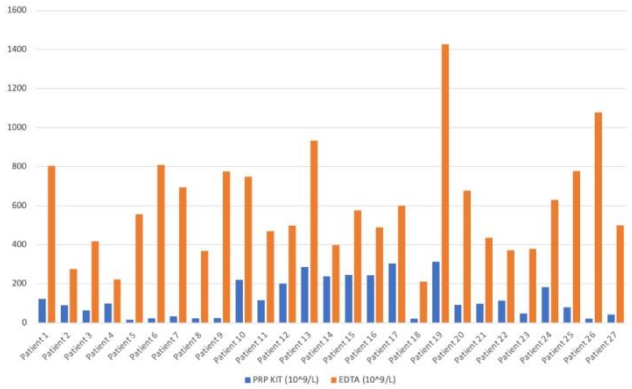


Figure 4. Comparative Graphs of the Platelet Concentration Obtained in PRP Kit and EDTA Tube

Comparative Analyses of Platelet Outcomes

Table 3. ***Comparative Analyses of the Outcomes (Platelet Concentration) among the Participants according to the Type of Platelet Rich Plasma (PRP) Tube Used (N = 27)***

Outcomes	Type of Platelet Rich Plasma Tube (PRP) (N = 27)				Mean Difference (95% CI)	Test Statistic	*p*-value (Two-Tailed)
	PRP Kit		EDTA				
	Mean (SD) [Range]	95% CI	Mean (SD) [Range]	95% CI			
Platelet Concentration (10^9/L)	124.52 (97.75) [15.00 To 313.00]	85.85 To 163.19	597.19 (271.53) [212.00 To 1,427.00]	489.77 To 704.60	−472.67 (−575.40 to −369.93)	−9.46*	0.001

*Significant at 0.05

Table 3 and *Figure 4* illustrates the comparative analyses of the platelet concentration according to the type of PRP tube used. Analyses indicated that the skewness and kurtosis of the data were 0.61 (p=0.145) and 1.91 (p=0.141), respectively, and Shapiro-Wilk's Test yielded a p-value of 0.056. These statistical tests indicate that the outcome was normally-distributed. It can be noted that the mean platelet concentration using the PRP kit tube was 124.52 (SD=97.75; Range=15.00 to 313.00), while the EDTA tube had a mean platelet concentration of 597.19 (SD=271.53; Range=212.00 to 1,427.00). Comparative analyses indicated that the mean difference in platelet concentration between the PRP kit tube and EDTA tube was −472.67 (95% CI = −575.40 to −369.93). This result indicated that the mean platelet concentration (t=−9.46, p=0.001) was statistically different between the

PRP kit tube and EDTA tube. In particular, it can be noted that the mean platelet concentration was significantly higher in the EDTA tube compared with the PRP kit tube.

DISCUSSION

Androgenic alopecia continues to be the most prevalent hair disorder for which no satisfactory treatment exists. Currently, there have been a variety of treatment options for AGA, with only two FDA approved medications, the topical minoxidil and oral finasteride. Given the limited effectiveness of current therapies for AGA, PRP has emerged as a promising alternative treatment. With the advent of PRP, currently, there is only a single FDA approved PRP kit in the country and it utilizes sodium citrate as an anticoagulant. In an effort to enhance the economic accessibility of PRP for all patients with androgenetic alopecia, blood collection anticoagulant tubes, such as those containing EDTA, may serve as alternative to proprietary PRP kit tubes.

Platelet rich plasma is defined as a volume of plasma derived from a patient's own blood with a platelet concentration higher than the baseline level. For clinical effectiveness, the platelet concentration should be at least 1,000 x 10^3 cells/µL in the plasma.[13] The regenerative potential of PRP relies on the concentration of released growth factors. Growth factors in platelet granules of PRP attach to the bulge area of hair follicles, thus, stimulating hair growth with a crucial role in starting and sustaining the healing process.[14] In a study by Eppley, et al., (2004)[15], the quantification of platelet concentration combined with growth factor analysis and its implications in wound healing was determined which revealed that the concentration of growth factors also increased with increasing platelet number. Therefore, a higher platelet concentration would indicate an elevated level of growth factors, thereby enhancing the regenerative potential of the obtained PRP.

Platelets are rich in growth factors that facilitate tissue repair mechanisms, including epidermal growth factor (EGF), platelet-derived growth factor (PDGF-AA and -AB), and transforming growth factor (TGF)-3. These growth factors function in accelerating angiogenesis and promoting collagen synthesis, ultimately facilitating wound healing and tissue regeneration. In a study conducted by Lee, et al., (2024)[16], they determined the correlation of growth factors with the platelet levels in PRP, Pearson correlation analysis indicated a strong positive correlation between PDGF, TGF, and EGF levels and the platelet concentration in the final PRP product, with correlation coefficients of $r = 0.697$ ($p < 0.0001$), $r = 0.488$ ($p < 0.0001$), and $r = 0.572$ ($p < 0.0001$), respectively.

Dermal papilla cells regulate hair morphogenesis and growth by producing growth factors which are essential for maintaining the hair follicle in the anagen phase. Thus, upregulating these growth factors within dermal papilla cells represents a potential strategy for prolonging the anagen phase and subsequently increasing hair density, most especially in patients with androgenetic alopecia.[17]

Table 3 shows that the mean platelet in the EDTA group was significantly higher than the PRP Kit group. Consequently, the maximum platelet concentration value obtained from the EDTA group was at 1,470,000 cells/ul as compared to the maximum platelet concentration of the PRP kit group at 313,000 cells/ul. Thus, the EDTA group achieved the level at which platelet concentration was deemed to be effective for PRP use in 2 of the participants.

In a systematic review by Fadadu et al., (2018)[18] the platelet concentration factor varied significantly across 33 systems studied, ranging from 0.52 to 9.7 times the baseline platelet concentration from the whole blood. Only 11 of the 33 systems and protocols met the ideal concentration of PRP at 1,000 ,000 cells/µl. These systems varied in their PRP tubes, spin settings, anticoagulants, activators and separator gels. Although 1,000 x 10^3 cells/µl was the platelet concentration level for which the regenerative capacity of PRP is therapeutically effective, an optimal platelet concentration for PRP has not yet been established.

A potential key difference in methodology between the two groups appears to be the use of tubes containing thixotropic polyester separator gel, the anticoagulant and the centrifugation methods used. The centrifuged blood in the PRP kit tube resulted into three distinct layers: a clear, yellowish plasma layer on top, which included a platelet poor plasma (PPP) layer above and a PRP layer below, a middle layer of cell separator gel containing leukocytes, and a red blood cell layer at the bottom. The tube was then resuspended to mix the plasma, thrombocyte, and leucocyte, forming the final PRP. Conversely, the EDTA tubes utilized lacked a separator gel. Thus, manual separation of the plasma from the centrifuged whole blood was done and PRP was obtained after two centrifugation steps as compared to the single centrifugation step done in the PRP kit group. The initial centrifugation (or soft spin), which applies a low centrifugal force, separates whole blood into three distinct layers: red blood cells, the buffy coat, and plasma. The subsequent centrifugation (or hard spin), which involves a higher centrifugal force, concentrates the platelets at the bottom of the plasma phase in order to obtain the PRP. [19]

In a study conducted by Reksodiputro, M. H. (2021)[20], wherein the same PRP kit was used in the determination of platelet count in PRP, they have concluded that the low platelet count obtained using the PRP kit may probably be caused by the trapping of platelets in the separator gel tube. This may explain the results described in table 3 and figure 4 wherein the PRP Kit group had a significantly lower platelet concentration levels as compared to the EDTA group (t=−9.46, p=0.001).

The PRP kit employed in this study used sodium citrate as an anticoagulant, in contrast to the ethylenediaminetetraacetic acid used in EDTA tubes. According to Carvalho, et al. (2024)[21], the platelet concentration levels obtained from EDTA tubes were significantly higher than the sodium citrate tubes with a mean platelet concentration in PRP for SC-collected samples at 1,858,410 cells/mm^3 (range, 102,000 to 4,260,000), and 3,458,222 cells/mm^3 (range, 1,476,000 to 5,322,000) for EDTA-collected samples. The platelet recovery rate was higher with EDTA in comparison to SC (51.04% vs. 29.85%, p = 0.005). Similarly, the results described in table 3 show that the EDTA tube group was superior to the PRP kit which uses sodium citrate as an anticoagulant.

PRP kits offer the advantage of ease and simplicity of use within a closed system. On the other hand, the use of EDTA tubes entail a double spin and separation process in order to obtain the PRP. Despite the ease of use of PRP kits, certain factors such as the presence of separator gels, which may decrease the platelet levels, and the anticoagulant utilized may affect the platelet concentration levels in the PRP produced, potentially decreasing its therapeutic efficacy.

CONCLUSION

The platelet concentration levels obtained from the EDTA tubes were significantly higher than the commercially available PRP kit levels. Indirectly, an increase in platelet concentrate results in an increased level of growth factors. Therefore, we can conclude that the EDTA tube may be a good alternative to the costly commercially available PRP kit.

RECOMMENDATIONS

With the results of the study, it is recommended to have further clinical studies with the use of EDTA tubes in hair restoration therapies. Additional studies may be done with a larger sample size to encompass a larger representative of the population wherein EDTA may be compared to other variants of PRP kits in combination with the anticoagulant tubes available in the country. Further research on a randomized controlled trial comparing the therapeutic effect of PRP using EDTA tubes versus PRP kits in relation to the platelet concentration levels in patients with androgenetic alopecia (AGA) may be conducted.

LIMITATION

This study is limited to the collection of data from the laboratory results of the platelet concentration levels of the participants and did not include the administration of PRP injection in the participants. Furthermore, it was limited to the use of a single type of commercially available PRP kit and did not completely represent all variants. This study only included adult male patients with AGA and did not include females and other age groups.

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