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ORIGINAL ARTICLE

Different platelet-rich plasma preparation protocols in Female pattern hair loss: Does it affect the outcome? A pilot study

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Abstract

Background: Platelet-rich plasma (PRP) injection is a promising modality for hair regeneration in female pattern hair loss (FPHL). A standard protocol on best methods for PRP preparation has not been established.

Objectives: To optimize standard PRP preparation protocols and evaluate its clinical efficacy in FPHL.

Methods: Comparative study enrolled 40 female patients with FPHL divided randomly into 4 equal groups. Each group received 3 sessions of monthly intradermal injection of PRP prepared by different methods regarding number of spins, centrifugation speeds, type of the centrifuge, and the size of PRP tube. Patients were evaluated by trichoscan before and 1 month after the 3rd session for number of terminal, vellus hair, and average hair width.

Results: A statistically significant increase in platelet count in PRP prepared by combination of digital centrifuge, large-sized sodium citrate tube, and low centrifugation speed (900 rpm). All patients showed statistically significant increase in percentage of terminal hair and average width of hair after treatment as assessed by trichoscan, without statistically significant difference between studied groups.

Conclusions: Digital centrifuge, large-sized sodium citrate tubes, and a single spin with low centrifugation speed (900 rpm) were ideal for PRP preparation. PRP is an effective and safe modality in FPHL therapy.

KEYWORDS
digital centrifuge, FPHL, PRP, trichoscan

1 | INTRODUCTION

Female pattern hair loss (FPHL) or Androgenic alopecia (AGA) is the commonest non-scarring alopecia that affects nearly 50% of females.[1] Female pattern hair loss (FPHL) is characterized by frontal hairline retention with diffuse decrease in hair density of the crown and frontal scalp. FPHL is presented by frontal accentuation taking Christmas tree pattern, diffuse central thinning, frontotemporal recession but uncommon, and bitemporal thinning.[2] Hair growth can be stimulated by platelet-rich plasma through the promotion of angiogenesis and vascularization, in addition, it motivates hair follicles to enter the growth cycle with extension of the anagen phase duration. This process is mediated by growth factors that stimulate activation of wingless (Wnt)/b-catenin, extracellular signaling regulated kinase (ERK), and protein kinase B (Akt) signaling pathways, thus, ending by cellular proliferation and differentiation.[3]

Platelets within 10 min from their activation, secrete growth factors including platelet-derived growth factor (PDGF) a and b, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), transforming growth factor (TGF) α and β, connective tissue growth

factor (CTGF), fibroblast growth factor (FGF) and insulin-like growth factor-1 (IGF-1). So, platelets have anti-inflammatory action through release of many anti-inflammatory cytokines, including interleukin-1 receptor antagonist (IL-1ra), interleukin (IL)-4, IL-10, IL-13, soluble tumor necrosis factor (TNF) receptor (sTNF-R) I, and interferon γ.[4]

Potential efficacy of PRP is dependent on many factors, including the preparation method associated with the patient variables and the method of application, affecting the final therapeutic response.[5] Still lacking a standardized PRP preparation protocols, so we aimed to optimize standard PRP preparation protocols and evaluate its clinical efficacy in FPHL.

2 | MATERIALS AND METHODS

This comparative study was conducted on a total 40 female patients with FPHL, recruited from Dermatology Outpatient Clinic of Al-Zahraa University Hospital, Cairo, Egypt at the period from December 2018 to November 2019.

The study was done according to Declaration of Helsinki and approved by the Research Ethics Committee of Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. Before participation in the study informed written consent was signed by all patients after clarifying all steps of the study and taking their approval.

2.1 | Patients' selection

By convenience sample from all female patients attending the Dermatology Outpatient Clinic of Al Zahraa University Hospital and presented with FPHL to select patients according to the inclusion and exclusion criteria.

2.2 | Sample size

Included 40 female patients, based on the formula.

2.3 | Inclusion criteria

Female patients aged between 20- and 35 years old, with grade I and II according to Ludwig classification.[6] With normal serum free testosterone, serum ferritin ≥70 μg/ml.

2.4 | Exclusion criteria

Pregnant and lactating females, smokers, patients beyond selected age, also patients with any manifestations of hyperandrogenemia such as hirsutism, menstrual irregularities or polycystic ovary, associated chronic systemic diseases such as thyroid disorders, associated other types of alopecia, and patients who received treatment for FPHL in the previous 3 months were all excluded. Immunosuppression or being under any kind of treatment causing absolute or relative immunosuppression, patients of any bleeding/clotting disorder or using anticoagulants, for example, warfarin, and patients with abnormal CBC findings were also included in the exclusion criteria.

Female pattern hair loss clinical diagnosis was based on the pattern of hair thinning of the crown and widening of the central part of the scalp, retention of frontal hairline and done by 2 certified dermatologists.

Trichoscopic assessment was also done by (trichoscope model M lite, S/W compare view, SRH company) with 2 different lenses with 50 and 200 magnifying power which revealed presence of trichoscopic signs of FPHL (hair diameter diversity more than 20%, single follicular unit in frontal areas, increased proportion of thin and vellus hairs, perifollicular hyperpigmentation (may be present), and presence of variable number of yellow dots). The 50× lens is used for measuring terminal and vellus hair counts and terminal-vellus ratio while the 200× lens is for measuring average hair width. Trichoscan combines standard trichoscopy with automatic digital image analysis for the measurement of human hair. The computer software calculates hair density, width, and the terminal-vellus ratio [7].

10 ml of blood was obtained by venipuncture with complete aseptic precautions from all patients.

Patients were assigned by simple randomization method using sealed envelopes into 4 equal groups, according to the method of PRP preparation.

Group I: PRP was prepared by only 1 spin using centrifugation force of (3000 rpm or 1000 g for 10 min).

Group II: PRP was prepared by 2 spins using centrifugation force of (1500 rpm or 250 g for 10 min) for the first and (2000 rpm or 450 g for 10 min) for the second one.

Group III: PRP was prepared by 2 spins using centrifugation force of (1500 rpm or 250 g for 10 min) for the first and (3000 rpm or 1000 g for 10 min) for the second one.

The centrifugation speed and time in these 3 groups were chosen according to the study of Kececi et al.[8] Where g is the relative centrifugal force.

Preparation in the 3 groups was done using small sodium citrate tubes (VACUTEST 2 ml tube, 9NC Buffered Citrate 3.2%, made in Italy) and non-digital centrifuge (80-1Electronic Centrifuge, maximum speed: 4000 rpm, timer range (0–60 min), capacity: 20 ml×6, made in China). A study flow diagram was illustrated (Figure 1).

Group IV: PRP was prepared by 1 spin with centrifugation force of (900 rpm or 220 g for 10 min) using large sodium citrate tube (VACUETTE 9 ml tube, 9NC Coagulation sodium citrate 3.2%, made in Austria) and digital centrifuge (HERMLE z 326 k centrifuge, maximum speed 18 000 rpm, maximum capacity 4 × 100 ml, timer (10 seconds to 99 min), temperature range (−20 to 40C), made in Germany).

Platelet count was done before and after centrifugation for all groups (for the first session only). It was performed using automated hematology analyzer Sysmex KX21 N.

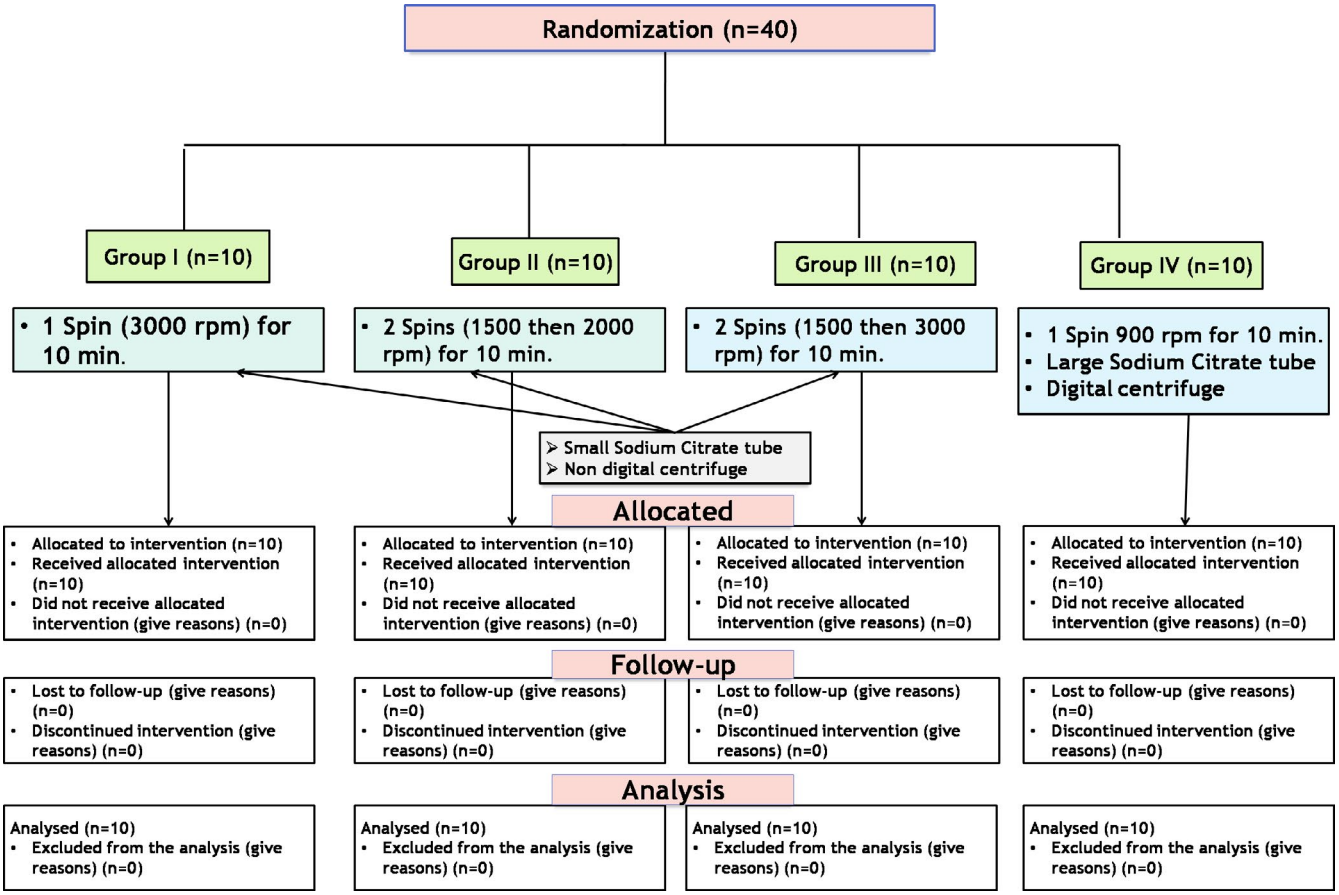

FIGURE 1 Flow chart of the study selected FPHL patients

Additional subgroups from the 10 patients of group (IV) was done by block randomization method just for platelets count of the obtained PRP. Illustrated by flow diagram (Figure 2).

Each patient received 3 sessions of monthly intradermal injection of PRP. The fronto-parietal area of the scalp was disinfected using alcohol and divided into 12 equal squares of 1 × 1 cm^2 diameter. The corners of every square were injected intradermally with 0.1 ml by insulin syringe making a total of 20 points injected on the fronto-parietal area/session in the 4 groups.

The area of evaluation was in the mid-line 2 cm behind the frontal hair line and it was fixed throughout the study. Patients were evaluated at baseline, each session, and 1 month after 3 monthly PRP sessions. Clinical photographs taken by (Sony Xperia Z2, 20.7 megapixel) and trichoscan were done before, and 1 month after 3 monthly PRP sessions. Photographs and trichoscan were randomly presented to two blinded dermatologists for evaluation. Degree of improvement was assessed according to grade of change in Ludwig classification types as mild and moderate improvement means there is improvement but still within the same grade and good improvement if there is changing in the grade.

The patients were asked to assess the degree of improvement in hair density, hair thickness, rate of hair fall by numeric values from −1 to 4 (−1 means worse, 0 means no change, 1 means mild improvement, 2 means moderate improvement, 3 means good improvement, and 4 means excellent improvement).

2.5 | Statistical analysis

Data were performed by version 23 of (IBM SPSS Statistics for Windows, Armonk, NY, IBM Corp.). Mean, standard deviations, and ranges were used to represent the quantitative variables with parametric distribution. The qualitative variables were formed as number and percentages. The relationship between qualitative data was created by chi-square test when the expected count in any cell <5. Independent *t* test was used for relationship between two independent groups with quantitative variables and parametric distribution. Non-parametric data were performed by Mann-Whitney test. The relation between two paired groups with quantitative data and parametric distribution was created by paired *t* test and Wilcoxon rank test for non-parametric data. One-way ANOVA used for comparison between more than two independent groups with quantitative data and parametric distribution while with non-parametric data were performed using Kruskal-Wallis test. Spearman correlation coefficients were used to assess the correlation between two quantitative parameters in the same group. *p* values <0.05 were statistically significant.

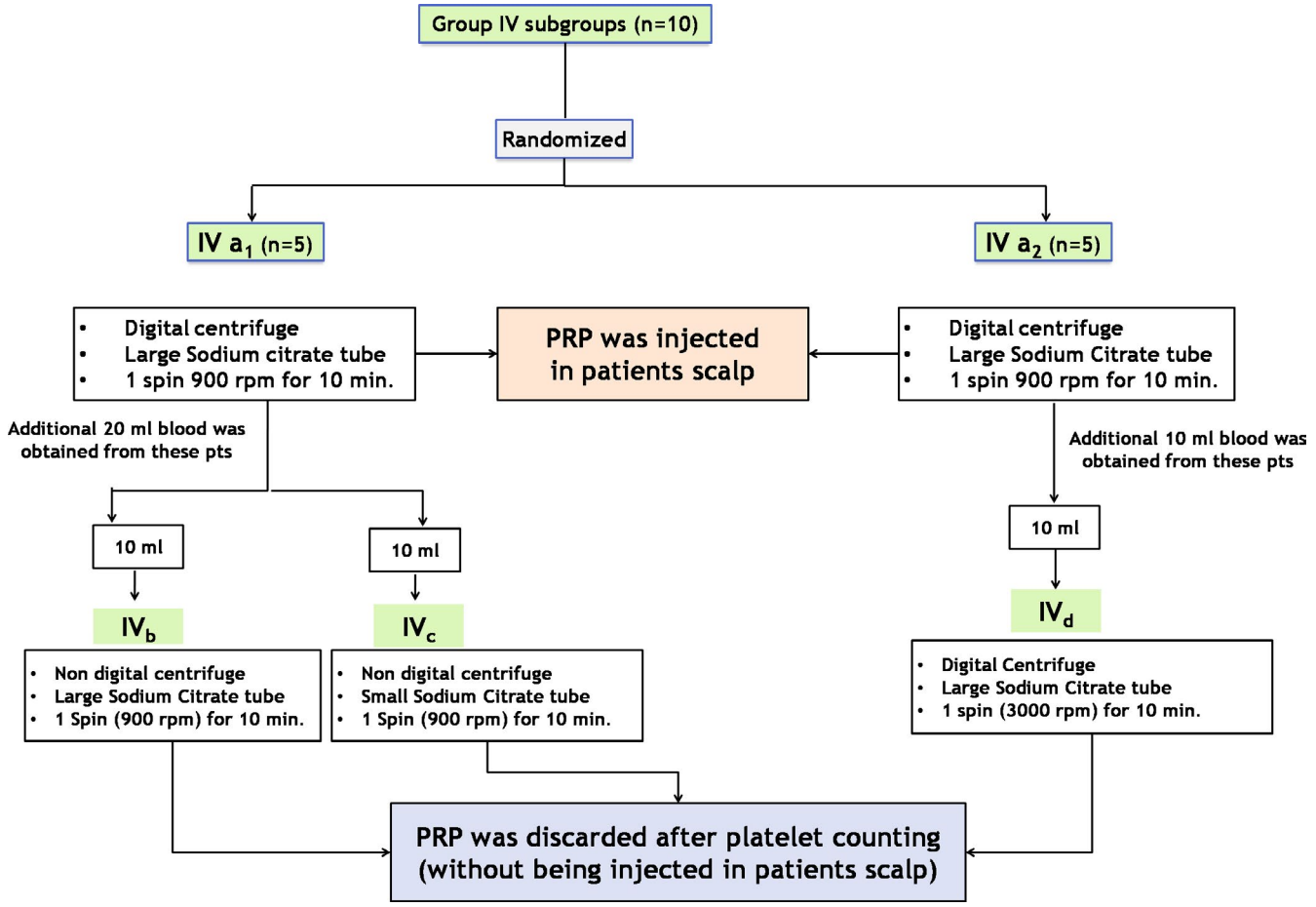

FIGURE 2 Flow chart of group IV subgroups

3 | RESULTS

The present study included 40 females with FPHL. Their age ranged between 20 and 35 years with a mean ± SD equal 25.75 ± 4.61 years. The duration of the disease ranged between 2 and 8 years with a mean ± SD equal 4.75 ± 1.64 years. Positive family history was found in all patients, and 21 patients were grade I (52.5%) and 19 patients were grade II (47.5%) according to Ludwig classification. No significant difference between the 4 studied groups as regards data before centrifugation and before treatment as shown in Table 1.

A statistically significant increase in % of change in platelet count after centrifugation in group IV than the other three groups with *p*-value <0.001. However, no statistically significant difference between the four studied groups regarding the other parameters (percentage of terminal hair, percentage of vellus hair, terminal: vellus ratio and average hair width in mm). Percentage of change of terminal hair and terminal: vellus were higher in group IV and also the percentage of change of vellus hair was lower in group IV than the other three studied groups, but they do not reach the statistically significant difference (Table 2) (Figure 3).

Clinical photographs of patients with FPHL before and 1 month after 3 sessions of monthly intradermal PRP injections showing moderate improvement (Figures 4 and 5).

Our results showed a statistically significant positive correlation between percentage of change of platelet count with terminal hair with *p*-value = 0.004 (Figure 6a) and also with terminal: vellus ratio with *p*-value <0.001 (Figure 6b). There was also significant negative correlation with % of change of vellus hair with *p*-value = 0.027 (Figure 6c) while there was no statistically significant correlation between % of change of platelet count and % change of average width of hair (Table 3).

Results of photographic assessment revealed that 10 patients (25%) showed no improvement, 13 patients (32.5%) showed mild improvement while 17 patients (42.5%) showed moderate improvement. None of our patients showed good improvement. No statistically significant difference in photographic assessment between the four studied groups (Table 4).

Also, patient self-assessment 1 month after last session showed no statistically significant difference between the four studied groups (Figure 7).

Non-statistically significant relation was found between age of the patients, duration and grading of FPHL and photographic improvement (Table 5).

Mild tolerable pain during the session that lasted for few hours was presented in all cases but pain that lasted more than 48 h was reported only in one patient (2.5%). Development of scalp dandruff was observed in 2 patients during the follow-up period (5%).

TABLE 1 Comparison between the four studied groups regarding demographic data, clinical characteristics, data before centrifugation and before treatment

	Group I	Group II	Group III	Group IV		
	N° = 10	*N°* = 10	*N°* = 10	*N°* = 10	Test value	*p*-value
Age (years)						
Mean ± SD	25.50 ± 3.92	27.50 ± 5.60	26.30 ± 4.85	23.70 ± 3.62	*F* = 1.219	0.317
Range	20–34	20–35	20–33	20–32		
Duration (years)						
Mean ± SD	4.80 ± 1.81	5.00 ± 1.83	4.80 ± 1.32	4.40 ± 1.78	*F* = 0.220	0.882
Range	2–7	3–8	3–7	2–6		
Grade						
Grade I	5 (50.0%)	5 (50.0%)	6(60.0%)	5 (50.0%)	$\chi^2 = 0.301$	0.960
Grade II	5 (50.0%)	5 (50.0%)	4 (40.0%)	5 (50.0%)		
F.H						
Positive	10 (100.0%)	10 (100.0%)	10 (100.0%)	10 (100.0%)	–	–
Platelet count (×10⁹/L)						
Mean ± SD	266.00 ± 28.26	274.80 ± 39.31	281.10 ± 41.38	315.50 ± 66.89	*F* = 2.202	0.105
Range	242–341	230–352	219–374	214–384		
Percentage of terminal hair						
Mean ± SD	75.03 ± 10.15	75.64 ± 7.21	74.94 ± 5.11	73.66 ± 6.99	*F* = 0.120	0.948
Range	61.5–88.6	66.7–86.7	66.7–82.8	65.2–84.8		
Percentage of vellus hair						
Mean ± SD	23.97 ± 9.33	24.31 ± 7.16	24.96 ± 5.08	26.38 ± 6.92	*F* = 0.214	0.886
Range	11.4–38.5	13.3–33.3	17.2–33.3	15.6–34.8		
Terminal: Vellus ratio						
Median (IQR)	3.10 (2–5.67)	2.95 (2.33–4.99)	3.03 (2.81–3.74)	2.28 (2.23–3.78)	*K* = 0.625	0.891
Range	1.6–7.77	2–6.52	2–4.81	1.87–5.44		
Average width of hair in mm						
Mean ± SD	0.026±0.006	0.025±0.006	0.025±0.008	0.024±0.005	*F* = 0.068	0.976
Range	0.016–0.034	0.018–0.038	0.018–0.041	0.013–0.031		

Abbreviations: F, One-way ANOVA test; F.H, Family history; K, Kruskal-Wallis test; *N°*, Number; χ^2, Chi-square test.

TABLE 2 Comparison between the four studied groups regarding % of change after centrifugation and after treatment

	Group I	Group II	Group III	Group IV		
% change	*N°* = 10	*N°* = 10	*N°* = 10	*N°* = 10	*K*	*p*-value
% change of platelets						
Median (IQR)	−85.84 (−88.93−−78.99)	−87.75 (−91.30−−86.06)	−87.84 (−90.68−−84.44)	57.21 (39.18–69.26)	22.864	<0.001*
Range	−90.51−−73.38	−92.42−−79.26	−91.98−−68.49	13.02–106.07		
% change of terminal						
Median (IQR)	3.76 (2.55–8.46)	5.22 (0.72–9.57)	4.18 (2.01–6.37)	11.54 (5.83–12.92)	5.893	0.117
Range	−0.45–10.43	−0.29–14.36	1.09–15.07	0.95–24.2		
% change of vellus						
Median (IQR)	−9.7 (−21.23−−2.9)	−13.35 (−25.23−−3.76)	−10.30 (−19.51−−7.60)	−28.24 (−48.48−−10.92)	5.461	0.141
Range	−29.13–13.86	−48.9–2.24	−38.46−−5.23	−61.54−−5.03		
% change of terminal: vellus						
Median (IQR)	17.02 (3.7–31.57)	20.67 (4.51–48.57)	16.15 (10.69–32.15)	56.89 (18.80–122.39)	6.367	0.095
Range	−3.83–53.38	−2.47–123.79	6.67–86.99	6.3–188.21		
% change of width						
Median (IQR)	17.86 (15.15–21.74)	14.91 (7.14–32.00)	19.31 (15.38–22.22)	10.00 (4.17–42.86)	2.606	0.456
Range	7.14–45	0–45.83	11.11–36.36	0–69.23		

Note: K: Kruskal-Wallis test.

Abbreviation: *N°*, Number.

*p- value <0.05 is considered statistically significant.

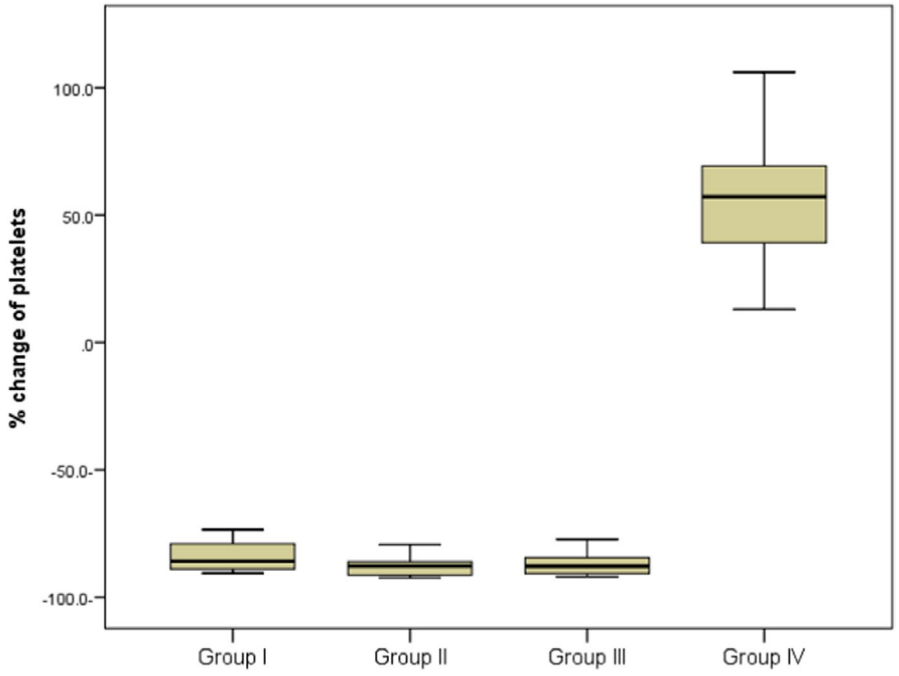

FIGURE 3 Percentage of change of platelet count in the four studied groups after centrifugation

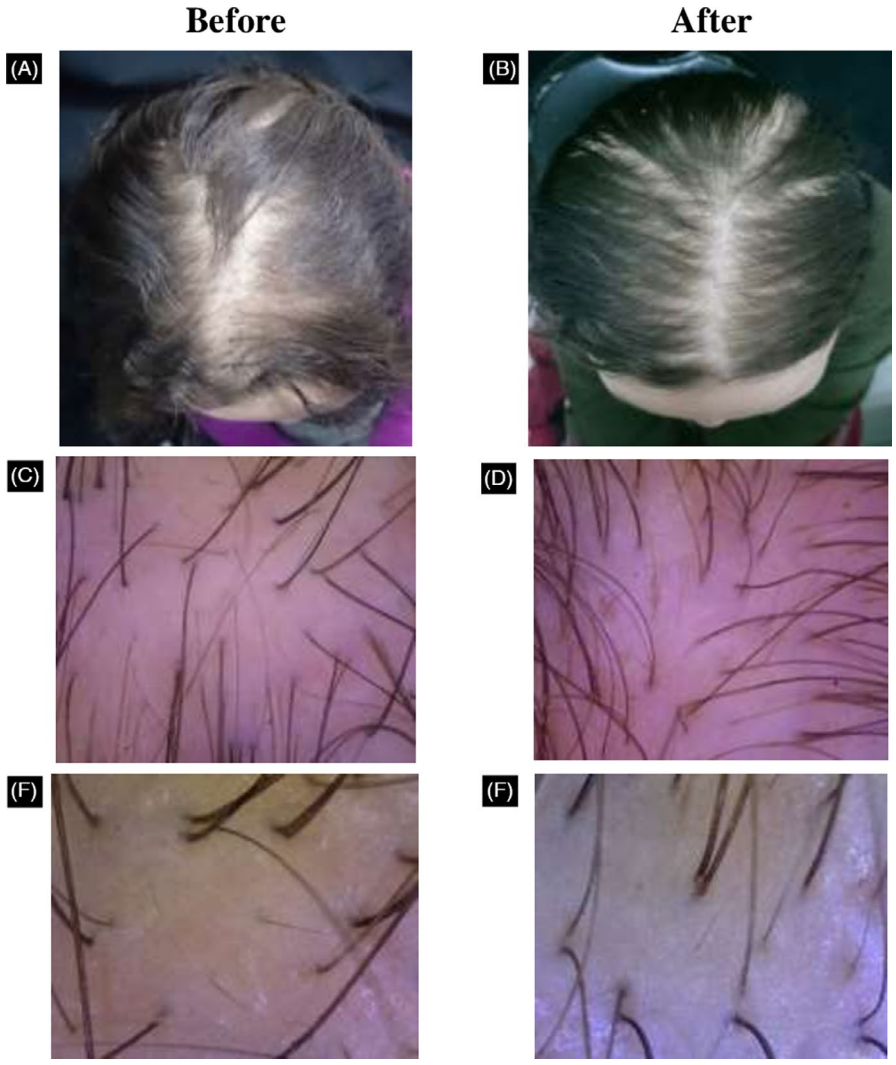

FIGURE 4 Female patient from group I, aged 25 years with FPHL grade II of 6 years duration, showing moderate improvement. (A) Before treatment. (B) After treatment. (C) Trichoscopic photograph before treatment with terminal hair count (61.5%) and vellus hair count (38.5%)/cm^2. (D) Trichoscopic photograph after treatment with terminal hair count (66.7%) and vellus hair count (33.3%)/cm^2 (with magnification power = 50× in C, D). (E) Trichoscopic photograph before treatment with average hair width (0.028 mm). (F) Trichoscopic photograph after treatment with average hair width (0.030 mm) (with magnification power = 200× in E, F)

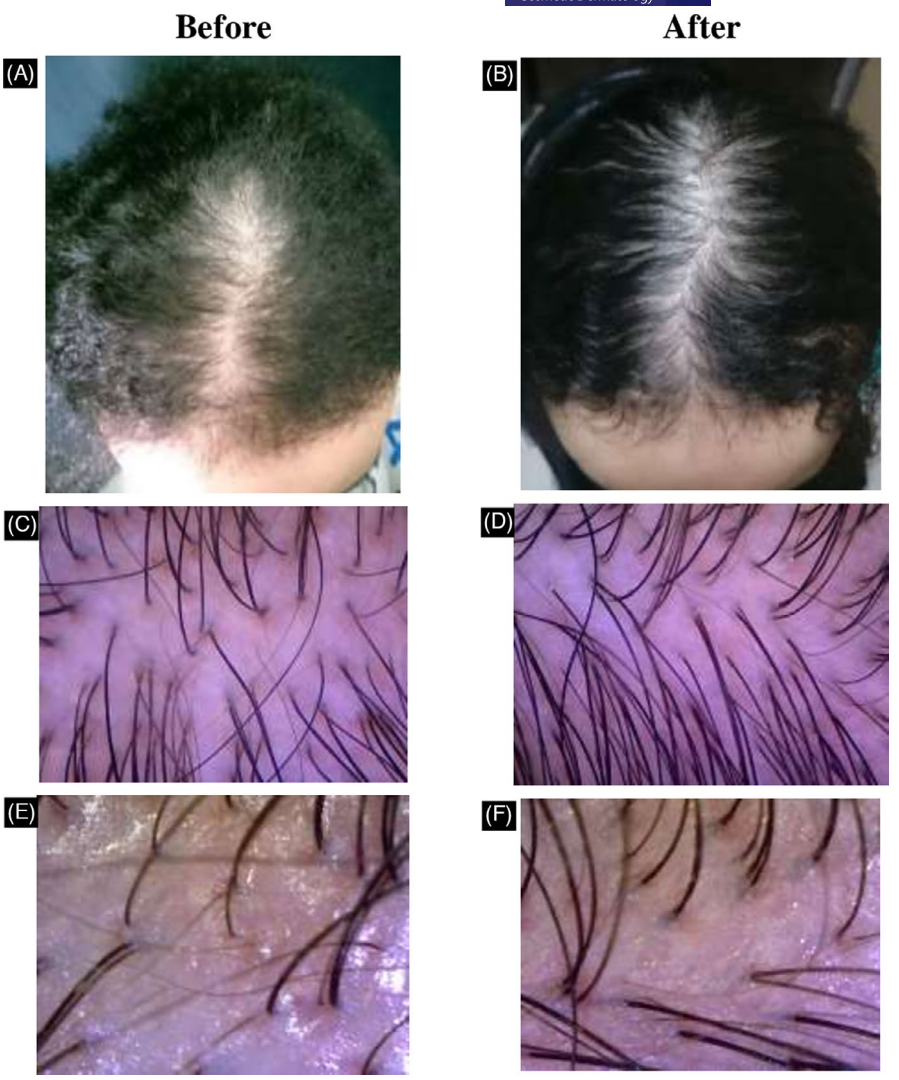

FIGURE 5 Female patient from group VI, aged 23 years with FPHL grade II of 6 years duration, with moderate improvement. (A) Before treatment. (B) After treatment. (C) Trichoscopic photograph before treatment with terminal hair count (69.5%) and vellus hair count (30.5%)/cm^2. (D) Trichoscopic photograph after treatment with terminal hair count (78%) and vellus hair count (22%)/cm^2 (with magnification power = 50× in C, D). (E) Trichoscopic photograph before treatment with average hair width (0.013 mm) (F) Trichoscopic photograph after treatment with average hair width (0.022 mm) (with magnification power = 200× in E, F)

Regarding group 1V subgroups, a statistically significant increase in platelet count after centrifugation in group (IV a1) than group (IV b) and (IV c) with *p*-value <0.001. The percentage of decrease in platelet count was higher in group (IV c) than group (IV b) with *p*-value <0.001 (Table 6) (Figure 8).

There was a statistically significant increase in platelet count in group IV a2, while a statistically significant decrease in group IV d was found with *p*-value <0.001 (Table 7) (Figure 9).

4 | DISCUSSION

In the current study 40 patients with FPHL, received intradermal injection of PRP prepared by different methods regarding number of spins, speed of centrifugation, type of centrifuge (non-digital versus digital), and size of the PRP tube.

The current study showed statistically significant decrease in platelet count after centrifugation, in PRP prepared by non-digital centrifuge and small sodium citrate tubes (2 ml) and high centrifugation speed as in groups I, II, and III. In spite of difference in number of spins and centrifugation speed in these 3 groups, there was no statistically significant difference in the decline in platelet count in these groups. Our findings agree with the study of Anbar et al.,[9] who found that platelet count was inversely proportional to the increasing centrifugation velocity. This can be explained by the possible damaging effect of the higher speed. In addition, Bausset et al.,[10] reported that the lower centrifugation speeds were better for preserving the resting platelet morphology than higher speeds.

Our findings disagreed with Kececi et al.,[8] who showed that platelet count was directly proportional to the increasing centrifugation velocity in PRP prepared by double-spin centrifugation (250 g for 10 min for the first spin while the second spin ranged from around 300–2000 g for 10 min). This higher increase in both centrifugation speed and platelet concentrations may be due to the use of different types of centrifuges and tubes compared with that used in our study.

The insignificant difference in platelet count in this study between the groups I, II, and III with different speed and number of spins, stands in contrast to the findings of Sabarish et al.,[11] who reported count difference according to centrifugation rate and time. This may be due to use of non-digital centrifuge and 2 ml sodium citrate tubes in these 3 groups.

The present results showed a statistically significant increase in platelet count in group IV compared with the other three studied

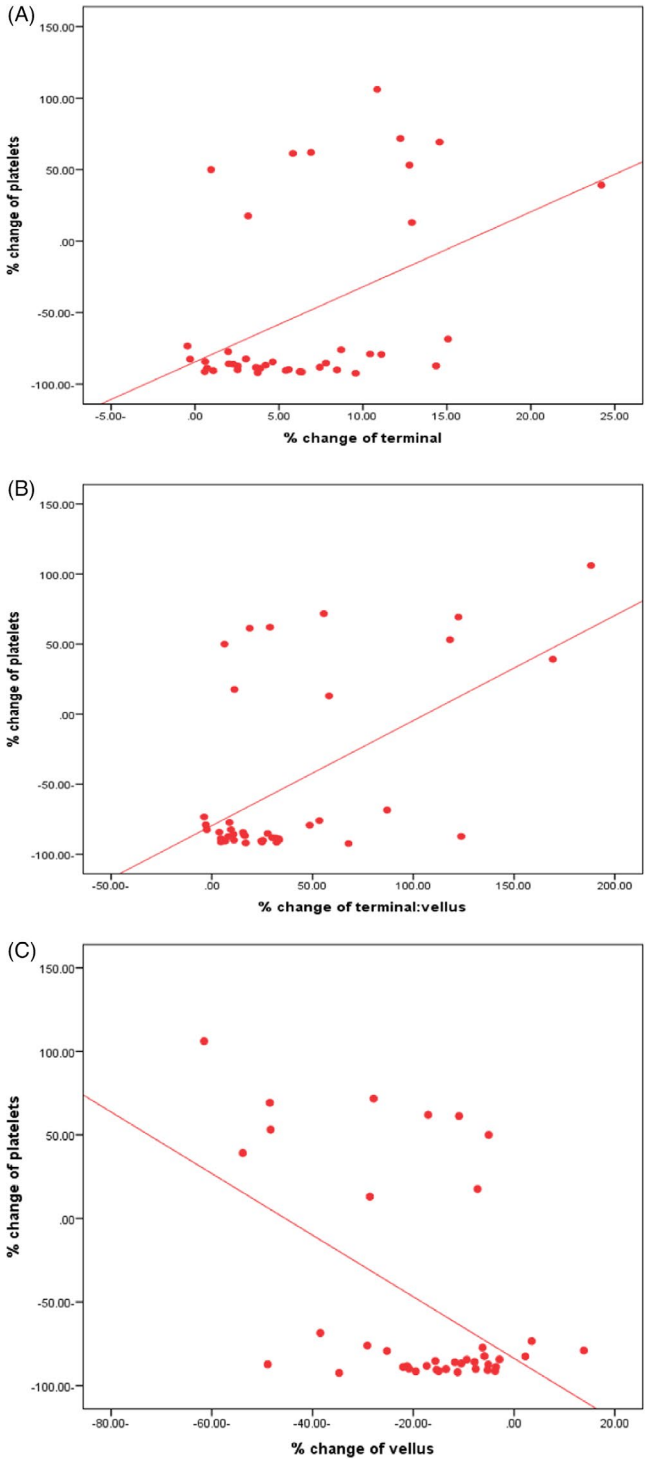

FIGURE 6 Correlation of % of change of platelet count with % of change in the other studied parameters in all patients. (A) Positive correlation between % of change of platelet count and % change of terminal hair in all patients. (B) Positive correlation between % of change of platelet count and % change of terminal: vellus hair in all patients. C, Negative correlation between % of change of platelet count and % change of vellus hair in all patients

groups. PRP in group IV was prepared by changing all the previous parameters using digital centrifuge, large sodium citrate tube (9 ml), and low centrifugation speed (900 rpm).

TABLE 3 Correlation of % of change of platelet count with percentage of change in the other studied parameters in all patients

% of change of hair parameters (All cases)	% of change of platelet count (All cases)	
	r	*p*- value
% change of terminal	0.444	0.004*
% change of Vellus	−0.493	0.001*
% change of terminal: Vellus	0.551	<0.001*
% change of width	0.051	0.752

Note: r: Spearman correlation coefficient.

**p*- value <0.05 is considered statistically significant.

Our findings agree with the study of Trink et al.,[12] who reported significant increase in platelet concentration of PRP prepared with low centrifugation speed (70 g or 800 rpm for 8 min) which is consistent with that obtained in our study.

However, the current study disagreed with Hsu et al.,[13] who found a 434% increase in platelet concentration of PRP prepared from 20 ml venous blood by means of double-spin centrifugation (2400 rpm for 10 min and 3500 rpm for 15 min). This higher increase in both centrifugation speed and platelet concentrations may be due to the use of different types of centrifuges and tubes in addition to a larger volume of blood compared with that used in our study.

But, the question which of the new parameters used in group IV (digital centrifuge, size of the sodium citrate tube, or low centrifugation speed) caused the significant increase in platelet count, was answered by IV subgroups. PRP in group IV b prepared by same parameters but with non-digital centrifuge showed significant decrease in platelet count when compared to groups IV a1 revealing, the advantage of using digital centrifuge for optimal PRP preparation.

Both groups IV b and IV c showed statistically significant decrease in platelet count, but the decrease in platelet count was more in group IV c than group IV b. These results clarify the advantage of the tube size for optimal PRP preparation.

Up to our knowledge, there have been no studies performed in this field comparing different methods of PRP preparation regarding type of centrifuge and size of the PRP tube as in our study.

Then, higher speed or lower one is suitable for standard PRP preparation; both groups IV a2 and IV d were prepared using digital centrifuge and 9 ml sodium citrate tube, but with 900 rpm in group IV a2 and 3000 rpm in group IV d. Group IV a2 showed statistically significant increase in platelet count while group IV d showed statistically significant decrease in platelet count. These results confirm the damaging effect of too rapid centrifugation on platelet integrity. The main limitation in our study is the small sample size.

The clinical efficacy of PRP prepared by different methods in the four groups was assessed by trichoscan using computer software analysis. Our results revealed statistically significant increase in percentage of terminal hair, terminal: vellus ratio and average hair width and a statistically significant decrease in percentage of vellus hair 1 month after 3 monthly intradermal PRP injection in the four

TABLE 4 Comparison between the four studied groups regarding photographic assessment

Photographic improvement	Group I N° = 10	Group II N° = 10	Group III N° = 10	Group IV N° = 10	χ^2	*p*-value
No	2 (20.0%)	3 (30.0%)	3 (30.0%)	2 (20.0%)	0.807	0.999
Mild	4 (40.0%)	3 (30.0%)	3 (30.0%)	3 (30.0%)		
Moderate	4 (40.0%)	4 (40.0%)	4 (40.0%)	5 (50.0%)		
Good	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		

Abbreviations: N°, Number; χ^2, Chi-square test.

FIGURE 7 Patient self-assessment in the four studied groups

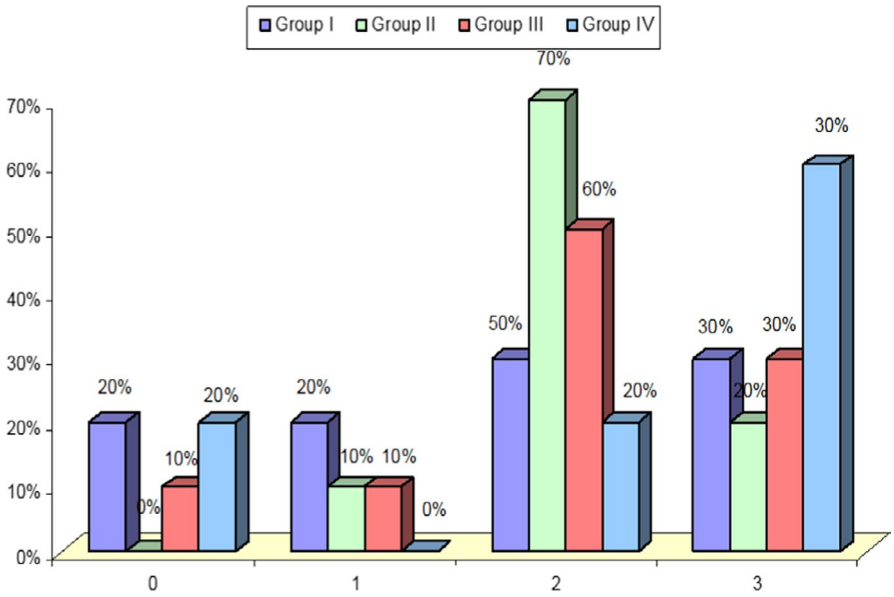

TABLE 5 Relation between age, duration, and grading of FPHL and photographic improvement

	Photographic improvement				
	No N° = 10	Mild N° = 14	Moderate N° = 16	Test value	*p*-value
Age (years)					
Mean ± SD	25.4 ± 5.68	27 ± 4.04	24.88 ± 4.4	$F = 0.826$	0.446
Range	20–34	22–33	20–35		
Duration (years)					
Mean ± SD	3.8 ± 1.62	5.29 ± 1.2	4.88 ± 1.82	$F = 2.667$	0.083
Range	2–8	3–7	2–7		
Grade					
Grade I	8 (80.0%)	5 (35.7%)	8 (50.0%)	$\chi^2 = 4.654$	0.098
Grade II	2 (20.0%)	9 (64.3%)	8 (50.0%)		

Abbreviations: F, One-way ANOVA test; N°, Number; χ^2, Chi-square test.

studied groups without statistically significant difference between all these groups.

Depending on these results it is logical to question what is the cause of the clinical improvement; the platelets, the growth factors, or the process of intradermal injection. The present study showed significant positive correlation between percentage of change of platelet count and percentage of change of terminal hair and terminal: vellus ratio and also significant negative correlation between percentage of change of platelet count and percentage of change of vellus hair. This favors that PRP has a role in clinical improvement. But what about the groups that showed statistically significant increase in percentage of terminal hair and average hair width in spite of statistically significant decrease in platelet count after centrifugation. We can explain it by supposing that the too rapid centrifugation speed may lead to morphological activation and disintegration of the platelets and subsequent release of the growth factors that caused the clinical improvement.

However, the possible role of the process of intradermal injection cannot be ruled out due to absence of a control group. The

TABLE 6 Comparison between subgroups (IV a1), (IV b), and (IV c) regarding percentage of change of platelet count

Platelet count	IV a1 N° = 5	IV b N° = 5	IV c N° = 5	F	p-value
Mean ± SD	59.68 ± 9.48	−50.15 ± 13.39	−92.60 ± 2.23	338.103	<0.001*
Range	47.66–71.74	−70.31–−33.43	−94.79–−90		
Post hoc analysis					
(IV a1) vs (IV b)		**(IV a1) vs (IV c)**			**(IV b) vs (IV c)**
<0.001*		<0.001*			<0.001*

Note: F: One Way ANOVA.

Abbreviation: N°, Number.

*p- value <0.05 is considered statistically significant.

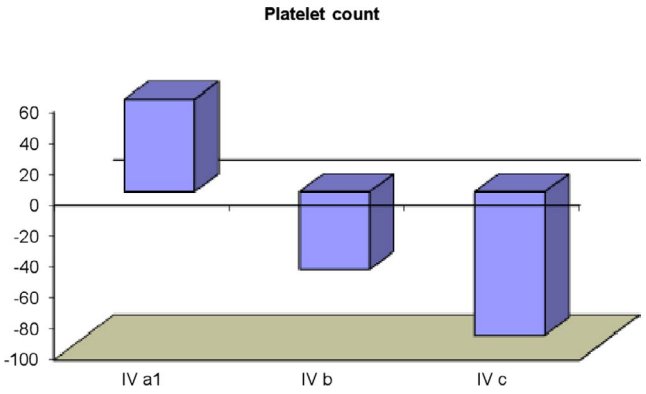

FIGURE 8 Comparison between subgroups (IV a1), (IV b), and (IV c) regarding percentage of change of platelet count

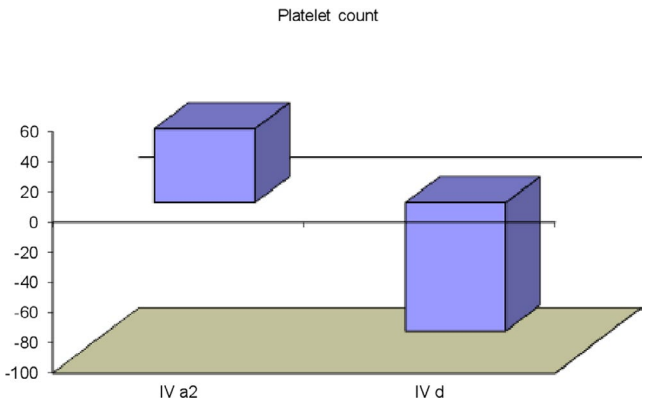

FIGURE 9 Comparison between subgroups (IV a2) and (IV d) regarding percentage of change of platelet count

TABLE 7 Comparison between subgroups (IV a2) and (IV d) regarding percentage of change of platelet count

Platelet count	IV a2 N° = 5	IV d N° = 5	t	p-value
Mean ± SD	49.03 ± 38.87	−85.00 ± 2.97	7.687	<0.001*
Range	13.02–106.07	−88.28–−80.84		

Note: t: Paired *t* test.

Abbreviation: N°, Number.

*p- value <0.05 is considered statistically significant.

limitations in our study were lack of assessment of growth factors and platelet function and morphology and absence of a control group. In addition, the short follow-up stands as a limitation in the present study. As it is difficult to separate true benefits of PRP from a possible short-term increase from needling/trauma to the scalp.

Compared with several studies, there is general agreement on the efficacy of PRP in treating AGA, but with great variations in the exact outcomes including hair density, growth rate hair, and shaft thickness depending on the protocol followed.[4,14–16] Similar to our results, Shapiro et al.[17] reported PRP with standardized preparation as an effective and safe therapeutic option in patients with AGA grade I and II, with improved hair diameter and density.

Finally, there are numerous protocols in the current literature that described the optimal conditions for PRP preparation. However, it is difficult to compare between them with respect to different variables of the process such as the volume and sampling of blood, number of spins, time of centrifugation, range of centrifugal acceleration, types of centrifuges and tubes.

PRP is not a simple a product, but biological properties of PRP are affected by many variables. Therefore, the PRP procedure requires optimization of the preparation protocols, composition, and application methods. PRP therapeutic effects are not only dependent on the platelet concentration. Other constituents, such as red blood cells, leukocytes, and growth factors, also contribute to the final result obtained from using PRP.[5]

5 | CONCLUSION

According to our study, using digital centrifuge, larger sodium citrate tubes (9 ml compared to 2 ml tube) and a single spin with low centrifugation speed (900 rpm) for 10 min is ideal for PRP preparation. PRP is an effective and safe procedure with a low complication rate in treatment of FPHL. Our results are encouraging as a start in field of standardization of PRP preparation protocols and call for further

large-scale, controlled, and randomized studies to confirm our initial encouraging results and recommend adding PRP maintenance sessions with more prolonged follow-up period. More investigations aiming to standardize other variables including centrifugation time, type of the anticoagulant, volume of blood sample, and assessment of other components including growth factors, red blood cells, and leukocytes are needed. Further studies evaluating PRP as monotherapy compared to combinations with approved drugs are also required.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Nayera Hassan Moftah contributed to conception, design and revision of the manuscript. Nour El-Eman Taha contributed in acquisition of data, analysis and interpretation of data. Mervat Hamdino performed the procedures, drafting the manuscript, and given final approval of the version to be published. Alshaymaa Mohamed Alhabibi contributed to the practical laboratory work and data analysis. All authors have read and approved the final manuscript.

ETHICAL APPROVAL

The study was approved by the Research Ethics Committee of Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. The study was following the Helsinki Declaration.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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