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ORIGINAL ARTICLE

Lyophilized growth factor intralesional injection in female pattern hair loss: A clinical and trichoscopic study

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Abstract

Various therapeutic modalities have been tried for female pattern hair loss (FPHL) treatment. To our knowledge, no previous studies had evaluated the therapeutic effect of lyophilized growth factor (L-GF) intralesional injection in FPHL. The current study aimed to evaluate the efficacy and safety of intralesional L-GF injection in FPHL by clinical and trichoscopic evaluation. This study included 20 patients with FPHL. All patients received three treatment sessions of intralesional injection of L-GF 4 weeks apart. Patients were followed-up for further 3 months. The outcome was evaluated by trichoscopy, photography score, patient's satisfaction score and side effects were reported. Trichoscopic evaluation showed significant posttreatment increase in all hair parameters associated with a significant decrease in vellus hair count. Ludwig's grade II showed posttreatment significant differences in all trichoscopic parameters from the baseline. No significant differences were detected regarding all trichoscopic parameters between the two Ludwig's grades posttreatment. 80% of patients showed photography score improvement that was significantly higher in Ludwig's grade II than in grade I. 100% of patients showed improvement in patient's satisfaction score with insignificant difference between Ludwig's grades. Intralesional injection of L-GF is safe and improved various trichoscopic hair parameters and clinical scores in FPHL.

KEYWORDS
female pattern hair loss (FPHL), lyophilized growth factor(s) (L-GF), patient's satisfaction score, photography score, trichoscopy

1 | INTRODUCTION

Androgenetic alopecia, commonly known as male pattern baldness and female pattern hair loss (FPHL), is the most common type of progressive hair loss.[1,2] An imbalance between growth factors and cytokines results in diffuse follicular keratinocyte apoptosis with premature termination of the anagen phase and subsequent follicular miniaturization.[3] This miniaturization process is reflected clinically as decrease and variation of hair density and thickness in a patterned distribution.[4] Besides, there is a decrease in terminal/vellus (T/V) hair ratio and anagen/telogen hair ratio.[5]

There is a rising attention to the valuable therapeutic effect of autologous platelet rich plasma (PRP) in androgenetic alopecia.[6-18] The regenerative power of PRP on follicular epithelium depends on the levels of various growth factors released upon activation.[7,8] PRP therapeutic effect lies in its ability to prolong hair follicle anagen phase and prevent the onset of apoptosis and premature catagen phase.[19] PRP treatment is not a standardized management and great variations have been reported regarding platelet concentrations, PRP volume, PRP preparation technique, the frequency and spacing of sessions and the evaluation procedures.[6,10,18] Alterations in PRP composition and treatment protocols have led to the variation in PRP treatment outcomes in the literature. [18]

In Contrast to PRP, lyophilized growth factors (L-GF) represent a standardized form of delivering platelet derived growth factors extracted from allogeneic platelet.[20,21] To the best of our knowledge,

L-GF has not been previously tried in FPHL. Accordingly, the current study aimed to evaluate the efficacy and safety of intralesional L-GF injection in FPHL based on subjective clinical and objective trichoscopic evaluation.

2 | METHODOLOGY

This experimental noncontrolled study included 20 randomly selected patients with FPHL attending the Dermatology Department Outpatient Clinic, Ain-Shams University Hospitals. Patients chose numbered cards, then patients with sequential numbers were enrolled provided they are fulfilling inclusion and exclusion criteria. Patients signed an informed consent for the technical and scientific basis of the research, possible risks, expected effects and clinical photography. The study was conducted according to the Declaration of Helsinki Principles, and was approved by the research ethical committee of Faculty of Medicine, Ain Shams University.

Inclusion criteria were patients aged from 18 to 40-year-old who were diagnosed clinically to have FPHL with Ludwig's grade I or II. Included patients were free of other dermatologic or systemic diseases. Exclusion criteria included patients with other clinical forms of alopecia (eg, alopecia areata, cicatricial alopecia). Also patients with dermatological or systemic conditions known to cause hair loss, patients on medications known to cause hair loss, patients with a history of hematologic/coagulation disorders, patients who had received systemic or local treatments for FPHL in the previous 12 months before study enrollment, pregnant and lactating females were all excluded.

All patients were subjected to complete history taking including onset, course and duration of the disease, pattern of hair loss, other scalp symptoms (eg, pain, itching or erythema), other systemic or skin diseases, menstrual history, family history and drug history. Local scalp examination was performed (eg, for signs of inflammation or scarring). Investigations such as complete blood count, serum ferritin level, serum iron level, total iron binding capacity, thyroid function tests, total and free testosterone, dehydroepiandrosterone-sulphate, bleeding and clotting time, liver and kidney function tests were carried out to exclude systemic abnormalities and other causes of hair loss.

3 | TREATMENT PROTOCOL

Lyophilized growth factors (L-GF) used in this study were produced according to the European patency number issued in November 2016 by a patented method[22] and were prepared in Cairo Medical Centre Blood Bank from platelet concentrates obtained from different donors. According to manufacturer's information, the amount of growth factors present in each L-GF vial was adjusted to be equal to those derived from two million platelets/μl. Vials of L-GF contained a pale yellow round cake of L-GF and were stored between 2° and 8°C. Upon injection, the L-GF vial was allowed to reach ambient temperature, and a 2 ml of sterile physiological saline was injected by sterile syringe into the L-GF vial. The vial was gently swirled for 3 min then left to stand at ambient temperature for 5 min. Reconstituted vials remained viable for 1 h when stored between 15° and 22°C.

Patients were informed to avoid hair washing or applying hair emollients 2 days prior to the treatment session. Patients received the treatment in the frontal area of the scalp that was defined as the area extending between the frontal hairline till a vertical line drawn in front of the ears and bounded laterally by the parietal fringe.[23] After disinfection of the treatment area by isopropyl alcohol 70%, intralesional injection of L-GF was performed by point by point technique with a 1 cm distance between injection sites and 0.1 cc was injected per point at a depth of 4 mm using a 30-gauge 0.30 × 4 mm mesoneedle (Mesotry Co, Italy). Each patient received three treatment sessions of L-GF injection 4 weeks apart. Patients were instructed to avoid hair washing after each session for 24 h. Patients were followed-up for 3 months after the last session. Procedure related complications were reported by all patients at each visit.

4 | OUTCOME EVALUATION

Trichoscopic pretreatment and 3 months posttreatment images were taken from a fixed point in the scalp using DermLite DL4 dermoscope (3 Gen, California) for all patients. Defining a fixed point to be examined was done using a plastic headband and a tapeline. The "V" point (Kang's point) was chosen for examination which was located 1 to 1.3 cm in front of the point of intersection between the midsagittal line (represented by a tapeline) and the coronal line (represented by a headband) connecting the tips of the tragus.[24] Image capturing was performed by a single practitioner to avoid diversification during the procedure. The images were then analyzed using the software (TrichoScan,Tricholog GmbH, Freiburg, Germany) to identify the following measurements; terminal hair count (hair/cm^2), vellus hair count (hair/cm^2), T/V ratio, total hair count (hair/cm^2), and hair thickness (mm).

Top-view clinical photographs of the scalp were taken for each patient at baseline and 3 months after the last treatment session using Sony Cyber-shot DSC-W650 16.1 Megapixels digital Camera, (Sony Corp-Tokyo, Japan) by a single photographer with standardization of photography area, distance, illumination and angle of exposure. Clinical photos were assessed according to a predesigned photography score comparing the pretreatment with the posttreatment photos. The photography score was rated from 0 to 3; where 0 = no improvement, 1 = mild improvement, 2 = moderate improvement, and 3 = excellent improvement. Three blinded physicians regarding the patients and the treatment conducted the photography assessments and the mean photography score was calculated.

Patient's satisfaction score was conducted by comparing the pretreatment state to the results 3 months after the last session. The score was rated from 0 to 4; where 0 = no improvement, 1 = 1% to 25% improvement, 2 = 26% to 50% improvement, 3 = 51% to 75% improvement and 4 = 76% to 100% improvement.[25] The patients were asked to report any procedure related complications every

session (eg, pain, headache, itching, infection, ecchymosis, and puffed eyes).

5 | STATISTICAL ANALYSIS

Statistical analyses were performed using Statistical Package for Social Science (SPSS 20.0 for windows; Inc., Chicago, Illinois). Descriptive statistics were expressed as mean, SD and range for parametric quantitative data and frequency and percentage for qualitative data, while median and interquartile range (IQR) were used for nonparametric quantitative data. Statistical analysis was carried out using Mann-Whitney test (*U* test) to assess the statistical significance of the difference of a nonparametric variable between two study groups, Fisher's exact test to examine the relationship between two qualitative variables when the expected count is <5 in more than 20% of cells, Wilcoxon-signed rank test to assess the statistical significance of the difference of a nonparametric variable measured twice for the same study group, Kruskal-Wallis test to assess the statistical significance of the difference between more than two study groups nonparametric numerical variables, one-way analysis of variance (ANOVA) test to compare statistical significance of the difference between means of more than two study groups and Spearman's correlation analysis to assess the strength of association between two quantitative variables. The correlation coefficient denoted symbolically "*r*" defines the strength and direction of the linear relationship between two variables. *P* value ≤.05 was considered significant.

6 | RESULTS

This study included 20 female patients with FPHL with no dropouts. Their age ranged from 20 to 39 years with a mean of 28.95 years (±5.9 SD). Table 1 summarizes the demographic data of the patients.

Table 2 demonstrates the trichoscopic parameters in the studied patients and their comparisons before and after treatment. All the studied hair parameters except vellus hair count increased significantly after treatment ($P < .05$). On the other hand, a significant decrease ($P < .001$) in vellus hair count was detected after treatment as compared to baseline.

According to Ludwig's classification, there were six patients (30%) with grade I and 14 patients (70%) with grade II. Table 3 shows the studied trichoscopic parameters before and after treatment in the two grades and their intra- and intergrade comparisons (Figures 1 and 2). There were insignificant differences between the grades regarding all trichoscopic parameters before treatment except the total hair count median which was significantly higher in patients with grade I ($P = .011$). After treatment, no statistically significant differences were detected regarding all trichoscopic parameters between the two grades.

Regarding the photography score, 16 patients (80%) showed improvement as following; four patients (20%) showed no improvement, nine patients (45%) showed mild improvement, five patients (25%) showed moderate improvement and two patients (10%) showed excellent improvement. Table 4 shows the detailed improvement in photography scores in each Ludwig's grade and revealed a significant ($P = .037$) higher improvement score in Ludwig's grade II (n = 13; 92.86%) as compared to grade I (n = 3; 50%).

TABLE 1 The demographic data of the patients

Variable	Cases; n = 20 (%)
Age (years)	
Mean ± SD	28.95 ± 5.9
Range	20-39
Ludwig's grade	
Grade I	6 (30%)
Grade II	14 (70%)
Disease duration (years)	
Mean ± SD	3.2 ± 1.82
Range	1–7
Family history of FPHL	
Positive	13 (65%)
Negative	7 (35%)
History of previous treatment	
Yes	9 (45%)
No	11 (55%)

Abbreviations: FPHL, female pattern hair loss; n, number of cases, %, percentage.

Regarding the patient's satisfaction score, all patients (n = 20; 100%) showed various degrees of satisfaction. Both score 1 and score 2 was reported by two patients (10%). Score 3 was reported by nine patients (45%) and score 4 was reported by seven patients (35%). Table 4 shows the detailed improvement in patient's satisfaction scores in each Ludwig's grade with insignificant difference in the median satisfaction score ($P = .664$) between the two grades.

All patients (n = 20; 100%) reported mild injection pain that subsided within few hours. Mild headache occurred in five patients (25%) that lasted less than 24 h.

No significant associations were found between the percentage of change of the different trichoscopic parameters and the age, history of previous treatment and family history. Spearman's correlation showed significant negative correlation ($r = -0.549$, $P = .012$) between disease duration and the percentage of change in T/V ratio that decreased with increase in disease duration (Figure 3). There were insignificant differences ($P > .05$) in the demographic data studied among the different grades of either the photography score or the patient's satisfaction score.

7 | DISCUSSION

To the best of our knowledge, no previous studies had evaluated the therapeutic efficacy of intralesional injection of L-GF in FPHL. Kieb et al[21] suggested that L-GF could represent an alternative form of

TABLE 2 The trichoscopic parameters in the studied patients and their comparisons before and after treatment

Trichoscopic parameter	Assessment time	Cases (n = 20)		Test value[a]	*P* value
		Median	IQR		
Terminal hair count (hair/cm^2)	Baseline	89	65.25-142.25	−3.502	**<.001**
	After treatment	107.50	80.25-165.25		
	% of change	22.93	4.16-36.55		
Vellus hair count (hair/cm^2)	Baseline	39.50	35.50-53.50	−3.237	**.001**
	After treatment	33.50	26.25-41.75		
	% of change	−14.77	−27.61 to −1.53		
T/V ratio	Baseline	2.14	1.37-3.44	−3.833	**<.001**
	After treatment	3.22	2.51-4.66		
	% of change	41.07	19.28-81.77		
Total hair count (hair/cm^2)	Baseline	126	104.5-187.75	−2.633	**.008**
	After treatment	132.5	108.75-219.75		
	% of change	8.66	−0.52-29.09		
Hair thickness (mm)	Baseline	0.037	0.031-0.067	−3.174	**.002**
	After treatment	0.042	0.063-0.084		
	% of change	17.73	6.33-44.11		

Note: Significant *P* values are bold.
Abbreviations: IQR, interquartile range; n, number of cases; %, percentage,
[a]Wilcoxon-Signed rank test.

delivering growth factors with advantages over conventional autologous PRP due to standardization of the preparation process and the defined high concentrations of growth factors in L-GF. Additionally, in vitro stimulation of platelets during L-GF preparation permits avoiding the use of thrombin or calcium injections in vivo. Also L-GF has been suggested to have a much longer shelf life as compared to conventional autologous PRP (12-18 months vs 8 h) rendering it more practical to use.[26] Accordingly, it was theorized in this study that L-GF intralesional injection could have a valuable therapeutic influence on FPHL.

The outcome evaluation in this study was done based on objective trichoscopic assessment besides subjective photography score and patient's satisfaction score. The therapeutic effect of PRP in androgenetic alopecia has been evaluated in various studies by different methods including global photography, patient's assessment scale, trichograms, trichoscopy and histopathologic examination.[16]

TrichosScan is a system which combines dermoscopy and automated digital image analysis that allowed the objective estimation of various hair parameters in this study. Thus, based on this study, and in line with other endorsement,[1] it is recommended to use dermoscopy and TrichoScan for the objective evaluation of the therapeutic trials in FPHL.

After a monthly spaced three sessions of L-GF intralesional injection in this study, there was a significant increase in total hair count, terminal hair count, T/V ratio and hair thickness from the pretreatment values. Vellus hair count decreased significantly after treatment. Additionally, by the end of the follow-up, 80% of patients showed photography score improvement and all patients showed patient's satisfaction score improvement. This improvement could be attributable to the promoting effect of growth factors on hair growth.

Consistent with the results reported in this study, several studies have showed the positive therapeutic effect of conventional PRP treatment, on total hair count and density in androgenetic alopecia with a significant difference between the experimental and control groups in the number of total hairs count[6-8,10,12,27-30] and terminal hair count.[8,10,28]

Alternatively, Puig et al[31] did not report a significant improvement in hair count after one treatment of nonactivated PRP injection in FPHL. This could be due to insufficient effect of the single session or insufficient release of growth factors. Also, Mapar et al[17] assessed the number of terminal and vellus hairs 6 months after two PRP sessions 1-month apart using a magnifying glass in males androgenetic alopecia and found no significant difference after treatment from baseline. This could be due to insufficient subjective evaluation of the outcome and few numbers of sessions performed.

Studies that discussed the effect of PRP on vellus hair density considered the treatment incompetent with no major variations.[8-10,17,28] Alternatively, a decrease in vellus hair count was reported in this study with a consequent increase in T/V ratio. Based on the current study, it could be theorized that the high concentration of growth factors in L-GF could have promoted miniaturized vellus-like hair to re-grow to terminal hair decreasing evidently vellus hair count.

In line with the positive therapeutic effect of L-GF on hair thickness in this study, other studies showed that the hair thickness improved after PRP treatment.[12,15,27,32] Instead, Park et al[33] showed

TABLE 3 The trichoscopic parameters in the studied Ludwig's grades and their intra- and intergrade comparisons

		Grade I (n = 6)		Grade II (n = 14)		Intergrade comparison	
Trichoscopic parameter	**Assessment time**	**Median**	**IQR**	**Median**	**IQR**	**Test value[a]**	***P* value**
Terminal hair count (hair/cm^2)	Baseline	149.5	81.5-190.75	78	63.5-95.5	−1.899	.058
	After treatment	151.5	81-210.75	102.5	76-141.25	−1.179	.231
Intra-grade comparison before / after treatment							
Test value[b]		−1.219		−3.172			
P value		0.223		**0.002**			
Vellus hair count (hair/cm^2)	Baseline	47	36.75-63.25	37.5	34-50.5	−1.324	.185
	After treatment	41	26.5-47	29.5	25.25-39.5	−1.321	.187
Intra-grade comparison before/after treatment							
Test value[b]		−2.201		2.455			
P value		**.028**		**.014**			
T/V ratio	Baseline	3.43	1.48-3.99	1.78	1.31-2.96	−1.238	.216
	After treatment	4.13	1.86-4.93	3.11	2.55-4.61	−0.248	.804
Intra-grade comparison before/after treatment							
Test value[b]		−1.992		−3.297			
P value		**.046**		**.001**			
Total hair count (hair/cm^2)	Baseline	217	111.3-240.8	124	95-148	2.834	**.011**
	After treatment	217.5	121.5-250.25	129.5	103.5-200	−1.610	.107
Intra-grade comparison before/after treatment							
Test value[b]		−1.363		−2.136			
P value		.173		**.033**			
Hair thickness (mm)	Baseline	0.066	0.048-0.081	0.036	0.029–.058	−1.732	.083
	After treatment	0.067	0.055-0.092	0.049	0.04-0.078	−0.825	.409
Intra-grade comparison before/after treatment							
Test value[b]		−2.207		−2.480			
P value		**.027**		**.013**			

Note: Significant *P* values are bold.
Abbreviations: IQR, interquartile range, n, number of cases.
[a]Mann Whitney *U* test.
[b]Wilcoxon-Signed rank test.

that although PRP treatment in male androgenetic alopecia increased hair growth, no significant change was observed in hair thickness. This discrepancy could be related to the difference in gender, disease stage, duration and severity, besides the variation in evaluation methods. Few studies had measured hair thickness after PRP treatment in androgenetic alopecia demanding further investigation for this entity.

Going with the frequency and spacing of sessions used in this study, most PRP treatment protocols for androgenic alopecia consist of three injections of PRP with a 1-month interval.[19] Badran and Sand[11] considered three PRP treatment sessions are the minimum number of sessions required for treating hair loss. Though, some used two injections within a 3-month interval or one injection weekly for 1 month.[9,10,30] The least reported PRP injection frequency for androgenetic alopecia was a single injection[33] while the most reported frequency was six times.[15,33] The shortest treatment interval stated was 3 days,[33] whereas for most studies a 1 month interval was chosen.[16]

The therapeutic effect of L-GF in this study was assessed 3 months after the last treatment. Few studies extended follow-up beyond 3 months after the last session. Gkini et al[13] reported that the hair density increased after PRP treatment in androgenetic alopecia at 6 weeks and reached a peak at 3 months then decreased at 6 months and proposed a booster treatment at 6 months. Anitua et al[34] reported in his 12-months study, which evaluated three monthly PRP treatments, followed by boosters at 4 and 7 months, an increase of hair count at 12 months. Gentile et al[8] reported that patients who received three monthly PRP treatments showed a return of hair loss about 16 months from baseline. Schiavone et al[35] proposed the need for booster treatment at 10 to 12 months. Therefore, further studies with longer follow-up period are recommended.

Interestingly, patients with Ludwig's grade II FPHL in this study showed significant improvement in all trichoscopic parameters, while grade I showed significant decrease in vellus hair count with significant increase in T/V ratio and hair thickness. Comparably, the clinical

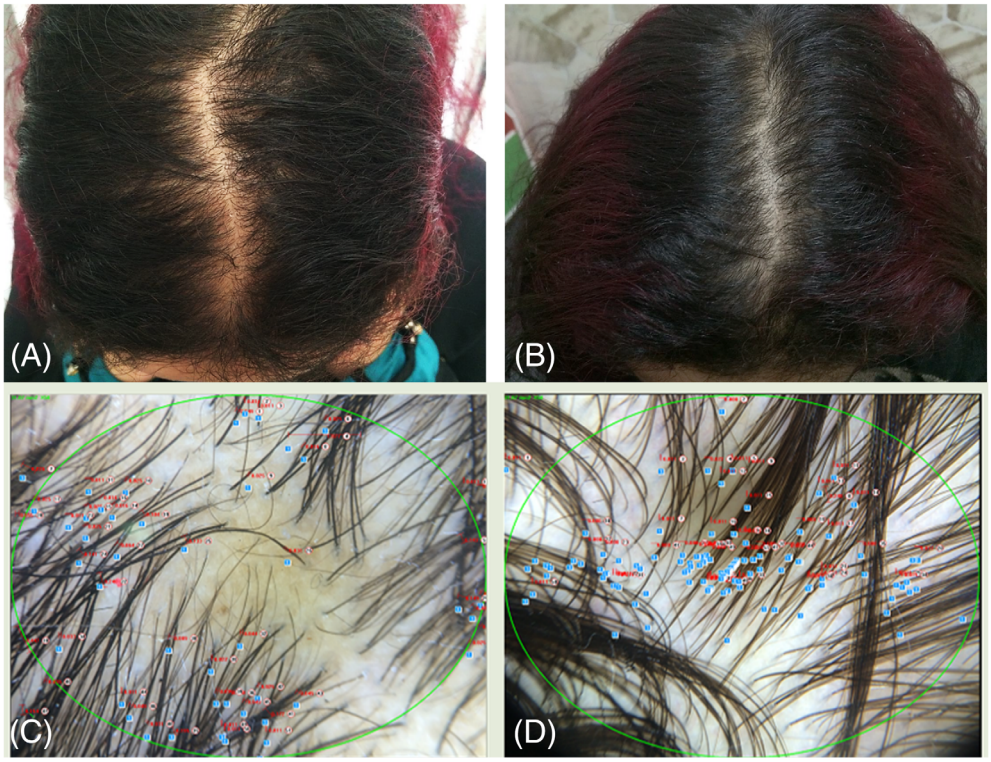

FIGURE 1 Clinical pictures of a 25-year-old female with FPHL grade I: A, at baseline, and B, 3 months after L-GF injection showing mildly increased hair density in the frontal area. Trichoscopic pictures (×100 magnification) showing C, hair diameter diversity and multiple vellus hairs at baseline and D, 3 months after L-GF injection showing increased terminal hair (blue boxes) and hair caliber (red numbers)

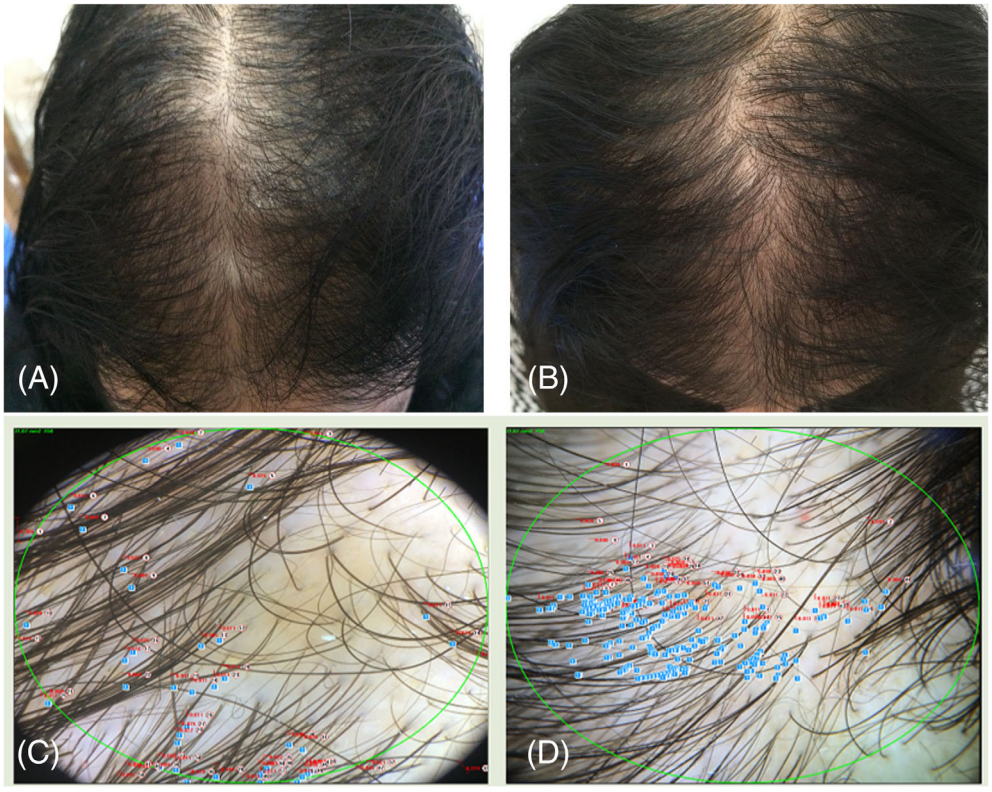

FIGURE 2 Clinical photos of a 32-year-old female with FPHL grade II: A, at baseline, and B, 3 months after L-GF injection showing moderately increased hair density in the frontal area. Trichoscopic pictures (×100 magnification) showing C, hair diameter diversity and multiple vellus hairs at baseline and D, 3 months after L-GF injection showing increased terminal hair (blue boxes) and hair caliber (red numbers)

scores showed more improvement in patients with grade II than grade I. This could be attributable to the small number of patients represented with grade I in this study with the resultant insignificant values as compared to grade II patients. There are limited published data regarding the specific correlation between the grade of androgenetic alopecia and PRP effect. In line with our results, two studies stated that the improvement of androgenetic alopecia increased with disease severity.[35,36] Alternatively, other studies recommended PRP for lower

TABLE 4 Comparison of photography score and patient's satisfaction score between the studied Ludwig's grades

	Grade I n = 6 (%)	Grade II n = 14 (%)	Test value[a]	*P* value
Photography score				
Score 0	3 (50%)	1 (7.1%)	8.479	**.037**
Score 1	3 (50%)	6 (42.9%)		
Score 2	0 (0%)	5 (37.7%)		
Score 3	0 (0%)	2 (14.3%)		
Total improvement	3 (50%)	13 (92.86)		
Patient's satisfaction score				
Score 0 (0%)	0 (0%)	0 (0%)	1.028	.794
Score 1 (1-25%)	1 (16.7%)	1 (7.1%)		
Score 2 (26-50%)	1 (16.7%)	1 (7.1%)		
Score 3 (51-75%)	2 (33.3%)	7 (50%)		
Score 4 (76-100%)	2 (33.3%)	5 (35.7%)		
Median % (IQR)	70% (35-92.5)	70% (70-90)	−0.434	.664

Note: Significant *P* value is bold.
Abbreviations: IQR, interquartile range; n, number of cases, %,percentage.
[a]Fischer's exact test.

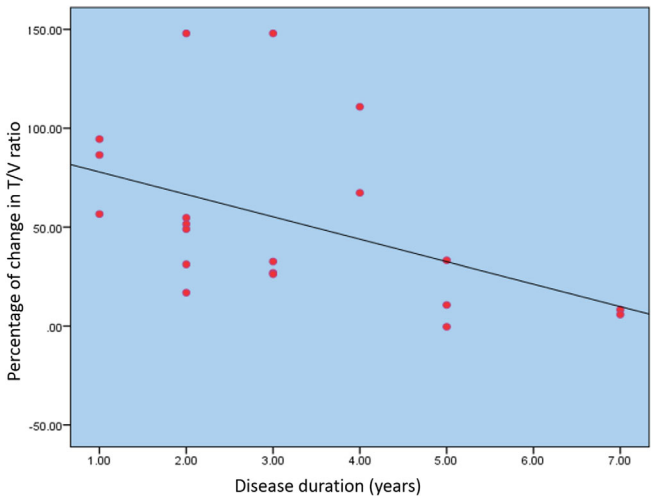

FIGURE 3 A graph showing the negative correlation between the disease duration and the percentage of change in T/V (terminal/vellus) ratio

grades of androgenetic alopecia.[14,37] Those studies linked the severity grade only with disease duration. Although FPHL is an evolution process over time, yet other factors, like the genetic background, could also play a role in FPHL severity. Further wider scale studies evaluating L-GF effect in various disease grades are advocated.

Current study revealed a significant negative correlation between the disease duration and the percentage of change in T/V ratio, signifying that the shorter the disease duration, the better was the treatment response. Comparable to this result, it was reported that the PRP efficacy was more evident in patients with shorter disease duration.[14] Reinforcing this connotation, transmission electron microscopy examination of scalp documented the presence of marked perifollicular fibrosis and follicular destruction in patients with androgenetic alopecia for long time.[38]

Using L-GF in this study was potentially safe with minimal side effects in the form of injection site pain and post procedure headache that last for few hours after the session. PRP had been reported to cause localized reactions (eg, inflammation) at the injection site[39] that was not encountered in this study. The inactivation of leukocytes during L-GF preparation was supposed to generate a less pro-inflammatory growth factors than conventional PRP.[20]

This study provided preliminary evidence that L-GF could be an effective, safe standardized option for treating FPHL of Ludwig's grade I and II. Limitations of this study included small sample size, and that the study was uncontrolled with relatively short follow-up period. Therefore, wider-scale, controlled studies with longer follow-up periods are endorsed. Additionally, comparative studies comparing the effect of L-GF and PRP in FPHL could further highlight the beneficial therapeutic outcome of L-GF in FPHL.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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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