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Stromal vascular fraction-enriched platelet-rich plasma therapy reverses the effects of androgenetic alopecia

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Abstract

Background: Since antiquity, humans have been trying to devise remedies to cure androgenetic alopecia (AGA). These efforts include use of oral and topical concoctions and hair transplant strategies. As AGA affects people of all colors and creed, there has been a continuous effort to find a magic bullet against AGA. Unfortunately, to date, all the strategies to negate AGA effects have limitations and thus require new treatment options.

Aim: To evaluate the efficacy of use of stromal vascular fraction (SVF) in androgenetic alopecia patients.

Methods: Stromal vascular fraction was obtained by enzymatic digestion of autologous adipose tissue. The patients were divided into two groups, that is, platelet-rich plasma (PRP) group and SVF-PRP group. In PRP group, only PRP was injected, while in SVF-PRP group a mixture of PRP and SVF was injected in affected scalp areas. After two sessions (4 weeks apart), the patients in both groups were assessed and analyzed using various parameters.

Results: Mean hair density in PRP group was increased from 52.44 hair/cm^2 to 63.72 hair/cm^2 (21.51% increase); while in SVF-PRP group, it was 37.66 hair/cm^2 before treatment and 57.11 hair/cm^2 after SVF-PRP therapy (51.64% increase). Percentage reduction in pull test was more significant in SVF-PRP group (80.78 ± 5.84) as compared to PRP group (34.01 ± 22.44). The physician and patient assessment scores also indicated a significant improvement in SVF-PRP group.

Conclusion: A combined SVF-PRP therapy reversed effects of AGA more efficiently as compared to PRP therapy alone.

KEYWORDS

adipose tissue, androgenetic alopecia, platelet-rich plasma, stromal vascular fraction

1 | INTRODUCTION

The progressive hair loss in androgenetic alopecia (AGA) is a distressing medical problem and is associated with low esteem and depression.[1] Current medicines for AGA have side effects and are not ideal as they can partially maintain the hair but cannot reverse it.[2] Similarly, surgical treatment options are expensive and are not available for completely bald patients.[3] AGA is similar to tissue damage, and repair processes are influenced by growth factors that in turn stimulate homing of cells and chemotaxis.[4] The repair of hair follicles in AGA thus could be improved using a combination of regenerative cells (i.e., SVF) and a source of growth factors (i.e.,

platelet-rich plasma [PRP]). PRP contains many growth factors that can activate the proliferative phase of hair.[5] PRP-derived growth factors can stimulate neovascularigenesis and development of follicular unit as they may affect dermal papilla and stem cells in the bulge area of follicles.[5-7] Although affective, PRP treatment needs to be repeated several times because the outcomes appear late and may be reversed if PRP use is stopped.[8] Adipose tissue-derived SVF contains noncultured regenerative cells (mesenchymal stem cells (MSCS) or adipose stem cells) that can "home" to the site of injury. Studies indicate that adipocyte lineage cells can affect the skin stem cells in order to drive hair cycle.[9] In the current study, we therefore used adipose tissue-derived SVF in combination with PRP to reverse the effects of AGA.

Androgenetic alopecia is characterized by progressive patterned hair loss and its prevalence increases significantly with age.[10,11]AGA becomes a medical problem when the hair loss is subjectively considered as excessive, premature, and distressing. Although AGA is hereditary, circulating androgen hormones may trigger its onset by miniaturization of hair follicles resulting in the conversion of terminal hair to vellus hair.[12] At cellular level, decrease in hair follicle size is due to loss of stem cells or progenitors at the bulge region of hair follicle.[12,13] The "bulge" is the main repository of these hair follicle stem cells (HFSCs).[14] New hair cycle begins when these HFSCs divide normally, however, due to their inactivation in AGA, vellus hair are produced instead of a terminal hair.[13] Apart from loss of stem cells, testosterone also plays an important role in the miniaturization of hair follicles.[15]

Conventional approaches for hair refurbishment include medications and hair follicle transplantation surgery.[2,3] However, these strategies are mostly ineffective due to drawbacks including high cost, several side effects, unsatisfactory results, and the requirement for long-lasting use of medicines and their efficacy is limited either to males or females. Therefore, contemporary therapies with promising results are required that should be effective in both sexes and outcomes should be long-lasting. Although PRP injections are frequently used for AGA patients and overall results suggest that it has a good therapeutic effect in AGA,[16] but the treatment has to be repeated several times leading to poor compliance of the patient.[8] Also studies indicate that stopping PRP treatment reverses its therapeutic effects.[8] So keeping in view these shortcomings of PRP use for AGA, the current study suggests the use of SVF along with PRP to reverse the effects of AGA.

Recently, stem cell-based therapies have proved effective in the treatment of several diseases and disorders. The current study has been designed for restoration of hair in AGA patients by using a combination of SVF and PRP. SVF was applied to restore hair growth because it contains several types of regenerative cells such as MSCs that are highly proliferative, have multi-lineage differentiation potential, are immunomodulatory and immunosuppressive.[17] Further, the cells in SVF also secrete various growth factors and proteins which can perform several functions including activation of hair follicle.[18] SVF can have multiple effects on miniaturized hair follicles by homing to the hair follicles and by their paracrine effects.[19]

Stromal vascular fraction-based treatment for AGA can open a new avenue for the development of therapies for hair restoration. In the current study, PRP and SVF were prepared using blood and autologous adipose tissue, respectively. In one group of AGA patients, only PRP was injected; while in other group, a combination of PRP and SVF was injected in the affected area of scalp. After two sessions of each type of therapy (PRP or SVF-PRP), the patients in both groups were assessed by physician and patient global assessment scores, pull test, trichoscan and photographs of affected areas. The results indicated an increase in hair density, decreased hair fall, and improvement in physician and patient global assessment score more in SVF-PRP group as compared to PRP group. The study will not only help the AGA patients by restoring and improving the hair quality but it seems to help patients with other hair loss conditions such as alopecia areata, telogen effluvium. The regenerative medicine field would benefit from additional large-scale double-blinded, randomized controlled studies treating both men and women, with standardized SVF-PRP preparation methods and administration protocol in different grades of AGA, and performing long-term follow-up.

2 | MATERIALS AND METHODS

Figure 1 shows overall study overview. The study was conducted in Tissue Engineering and Regenerative Medicine Laboratory, Department of Biomedical Sciences & Department of Dermatology, King Edward Medical University/Mayo Hospital Lahore from June 2016 to January 2018 using a sample size of 22 patients. Nonprobability convenient sampling was used with the inclusion and exclusion criteria given in Table 1. The study was approved by the Intuitional Review Board (IRB) at King Edward Medical University, Lahore, Pakistan. All the procedures used in the study were performed after written-informed consent of the patients.

2.1 | Isolation of platelet-rich plasma

Under sterilized conditions, 9 cc whole blood was taken from the antecubital vein of every patient. Blood was taken in commercially available PRP kit (Tray Life Tube Gel) containing preformed gel comprising a mixture of polymers that separated plasma and sodium citrate solution which acts as an anticoagulant and centrifuged at 650 *g* for 10 minutes. After centrifugation, red blood cells trapped under the gel, and the upper 1 mL of platelet-poor plasma was removed, and 5 mL PRP obtained. The whole process was performed in the processing laboratory under sterilized conditions.

2.2 | Isolation of stromal vascular fraction

Adipose tissue (47.26 ± 5.6 cc) was collected by tumescent liposuction as described by us previously.[20,21] Briefly, after sterilization of skin surface, tumescent solution (0.9% saline, lidocaine, epinephrine) was infiltrated into donor sites. Fat was harvested using a handheld cannula (3 mm) under sterilized conditions in the Plastic Surgery

FIGURE 1 Study overview

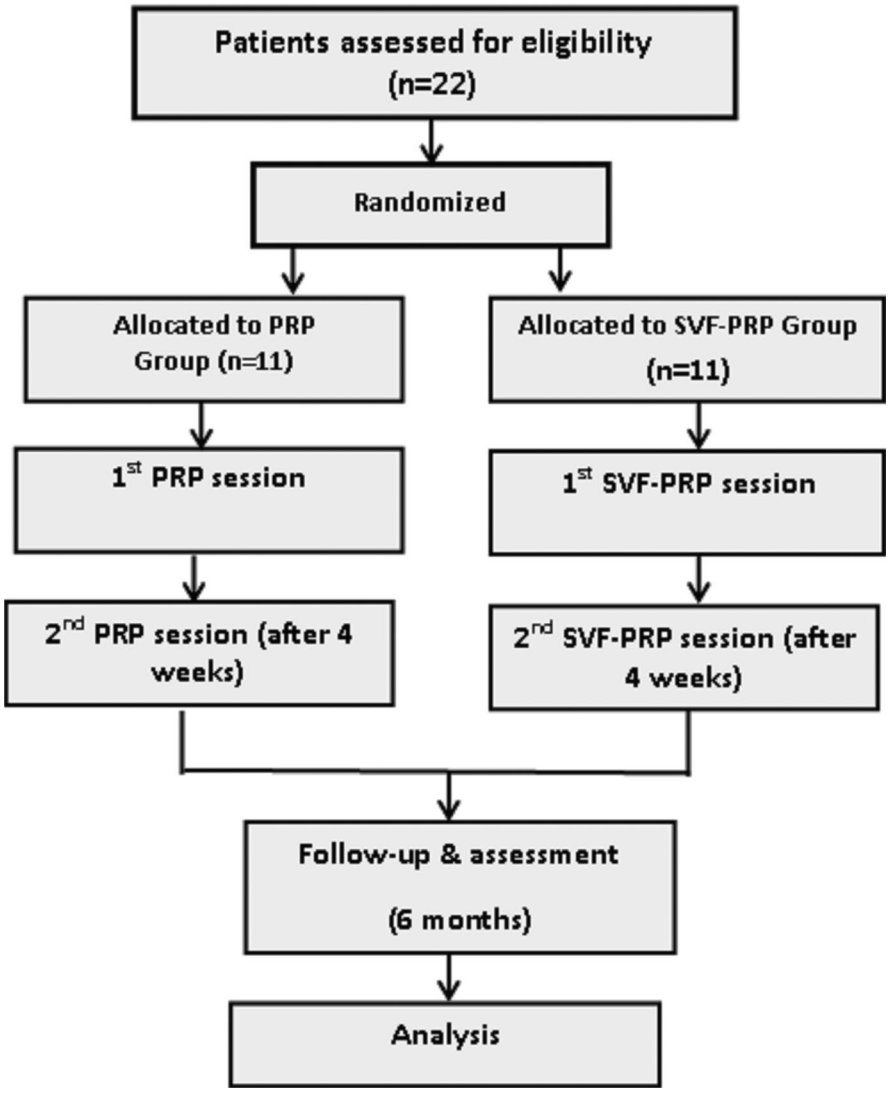

TABLE 1 Inclusion and exclusion criteria for enrollment of patients in the study

Inclusion criteria	Exclusion criteria
• Male and female patients with androgenetic alopecia • 15 y or older • Active hair loss within last 12 mo • Hamilton score III to VI in male patients and Ludwig score I-III in female patients • Patients receiving fitness certificate from fitness committee	• Patients with inflammation, infection, malignancy, allergic disease, autoimmune disease, pregnancy, and on current anticoagulant therapy. • Patient on chemotherapy during the last 5 y

Department of Mayo Hospital. Adipose tissue was processed within 2 hours under sterilized conditions to obtain SVF. Briefly, adipose tissue was washed repeatedly (3-5×) with an equal volume of phosphate buffer saline (PBS). Adipose tissue was then digested by adding an equal volume of collagenase type IV for 30 minutes at 37°C with periodic agitation. The digested tissue was passed through a 70 µm strainer and centrifuged at 650 *g* for 15 minutes, supernatant was discarded, and SVF was obtained as cell pellet that was mixed in 5 mL of PBS and centrifuged at 650 *g* for 5 minutes. In this cell pellet, PRP was added before injection in the affected areas of the scalp of AGA patients.

2.3 | Injection of stromal vascular fraction and platelet-rich plasma

The patients were divided into two groups: in group I (PRP group), only platelet-rich plasma (3 mL) was injected; in group II, (SVF-PRP group) adipose tissue-derived SVF mixed with PRP (3 mL at 20 µL/100 000 cells) was transplanted into the affected areas of scalp of AGA patients under sterilized conditions. Half an hour before injections, lignocaine gel was applied on scalp of patients of both groups. In each group, intra-dermal injections were given by using insulin syringes at 0.5 cm interval. Each patient underwent two

sessions (of injections/transplantations) each after 4 weeks and was followed up for 6 months after the last session.

2.4 | Assessment

After PRP and SVF-PRP therapy, the improvement was assessed using pre- and post-treatment photographs, patient global assessment (PaGAS) scores, physician global assessment (PhGAS) scores and pull test. Further, trichoscan was used to assess various hair parameters before and after PRP and SVF-PRP therapy.

Photographs were taken by the same photographer at the same distance each time using Nikon camera. Pull test was performed on each visit by the same evaluator. Approximately 20-60 hair were grasped between thumb, index, and middle fingers from hair base near scalp and firmly tugged away from scalp. More than 10% pulled hair from scalp constituted a positive pull test. Trichoscan comprised standard epiluminescence microscopy (ELM) with automatic digital image analysis for the measurement of hair density. PhGAS was performed by two independent evaluators using following criteria (0: poor; 1: satisfactory; 2: good; 3: excellent). Similarly, PaGAS was evaluated using the same criteria and scale as mentioned above for PhGAS.

2.5 | Data analysis

For data analysis, values were entered in SPSS version 23. Quantitative variables like age, number of hair/cm^2 number of hair pulled, PaGAS, and PhGAS were presented as mean ± SD. Qualitative variables like gender were presented as frequency and percentages. The normality of quantitative variables was tested by Kolmogorov-Smirnov test. For comparison of two groups, an independent sample *t* test was applied. *P*-value ≤ 0.05 was taken as significant.

3 | RESULTS

In the current study, twenty-two patients enrolled from June 2016 to January 2018 were divided into two groups: PRP group (n = 11) and SVF-PRP group (n = 11). Patients in each group underwent transplantation of either PRP alone (PRP group) or SVF mixed in PRP (SVF-PRP group). The mean age of patients was 26.45 ± 6.79 in PRP group and 33.27 ± 9.17 in SVF-PRP group. There were 77.27% males and 22.73% females in the study. A total of 13 (59.09%) patients had family history of AGA while remaining patients 18 (81.82%) although have no AGA history but were experiencing active hair loss within last 12 months. 4 females (80%) exhibited Ludwig scale type III and 1 (20%) had Ludwig scale II. Among males most patients (70.59%) were with Hamilton-Norwood scale type III. None of the patients in PRP group had lost to follow-up; however, two patients in SVF-PRP group underwent only one session. No side effects were observed in any enrolled patient.

3.1 | Hair density

Figure 2 summarizes the quantitative parameters measured using trichoscan. The trichoscan images were taken from the same affected area of the scalp at each visit in both groups, and number of hair/cm^2 was counted in each patient. Mean density of hair in PRP group on

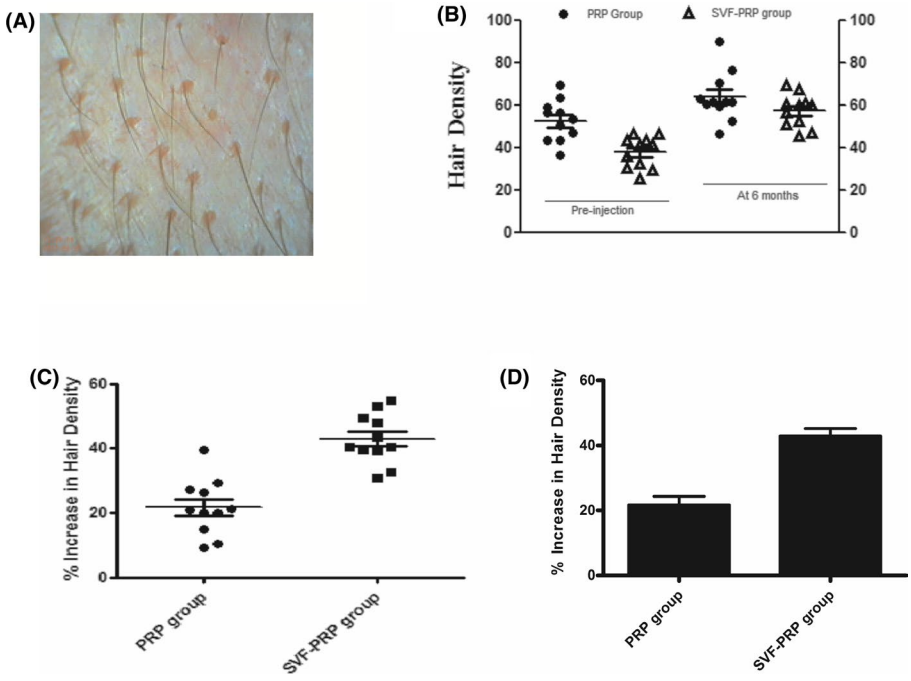

FIGURE 2 Hair density before and after PRP therapy and SVF-PRP therapy. A, showing representative trichoscan image. B, Hair density in both groups before injection and at 6 mo after injection of PRP or SVF-PRP. C, D, showing percentage increase in hair density 6 mo after injections of either PRP alone or a combination of SVF and PRP. PRP, platelet-rich plasma; SVF, stromal vascular fraction

first visit was 52.44 ± 9.66, and it was 37.66 ± 7.43 in SVF-PRP group. On final visit (after 6 months of last session), it was 63.72 ± 11.68 in PRP group and 57.11 ± 7.73 in the SVF-PRP group. Overall, the percentage increase in PRP group was 21.51% as compared to SVF-PRP group in which there was 51.64% increase (*P*-value .006).

3.2 | Pull test

The pull test was performed in patients of both groups (Figure 3). Before the injection of PRP mean number of hair pulled were 8.82 ± 1.83 in PRP group and 13.27 ± 3.93 in SVF-PRP group. After 6 months mean hair pulled were although decreased in both groups, percentage reduction was more significant in SVF-PRP group (80.78 ± 5.84) as compared to PRP group (34.01 ± 22.44).

3.3 | Global assessment scores

Table 2 summarizes the results of global assessment score in patients of both groups. Although majority of the patients in both groups showed improved physician and patient assessment scores, a more significant improvement in these scores was seen in SVF-PRP group as compared to PRP group. In PRP group majority of the patients fall in the "satisfactory" category of the physician and patient assessment scores while in SVF-PRP group patients came under category "good." One patient in SVF-PRP group also exhibited excellent "category" of score but no patient was in this category in PRP group.

Macrophotographs of patients in PRP group and in SVF-PRP group before start of treatment and 6 months after treatment have been depicted separately in Figure 4.

4 | DISCUSSION

The main finding of this study is that the use of a combination of SVF and PRP is more effective in reversing the AGA effects as compared to PRP alone. The therapeutic role of SVF-enriched PRP was assessed using parameters such as hair density, photographs, pull test, physician and patient global assessment scores. Eleven AGA patients were treated with SVF-enriched PRP, and eleven were given only PRP for comparison. A combination of SVF and PRP has already been used for various indications like nonhealing ulcers,radiation-induced damaged tissues, anti-aging treatment, osteoarthrosis [22] SVF-enriched therapy has recently been used in a single study for AGA patients[4] but without comparison. To the best of our knowledge, this is the first study in which the effect of SVF-enriched PRP was compared with the patients who were injected with PRP alone.

Adipose tissue being biologically active complex is important for tissue engineering and regenerative medicine applications. Adipose tissue can be used as intact, as SVF or SVF could be expanded ex vivo to obtain adipose-derived stem cells (ASCs). In the current study, we used SVF which is a mixture of several types of cells including ASCs. SVF obtained from adipose tissue can be prepared within 2 hours to be used clinically. In AGA patients, the basic concept of using SVF-enriched PRP is to replenish stem cell repository in the bulge region of hair follicles by homing and also to stimulate growth cycle of stem cells by paracrine effects.[23] These mechanisms seem plausible for a long-term benefit in reversing the AGA effects.

In this study, 80% of the patients were 21-31 years in PRP group and 27-39 years in the SVF-PRP group. Overall in both

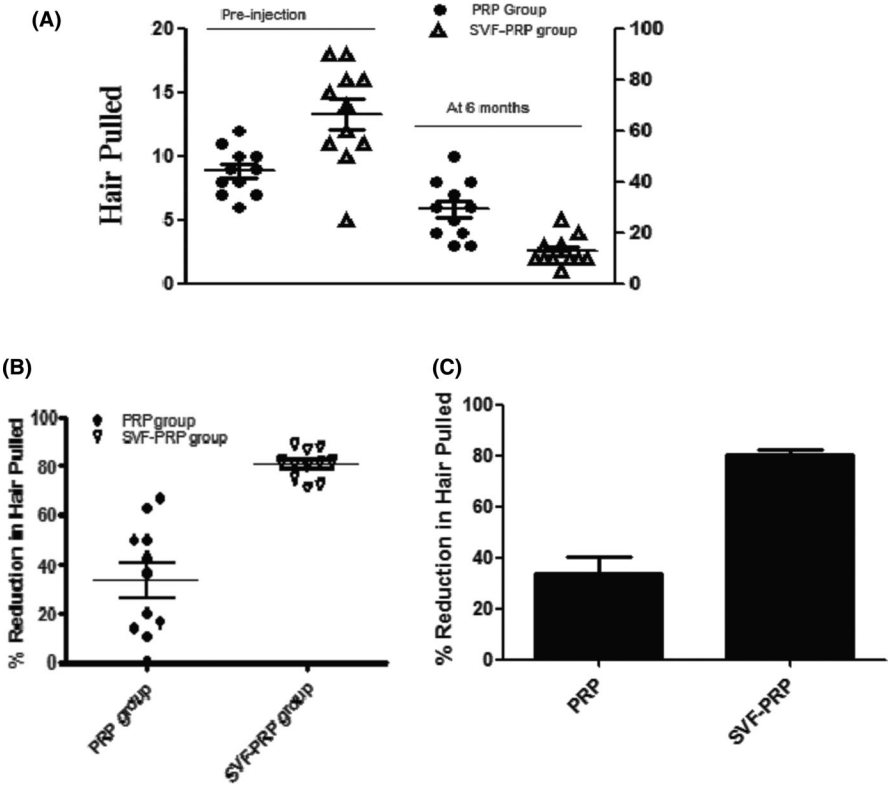

FIGURE 3 Results of pull test. Number of hair pulled before therapy in both groups (A). The number of hair pulled were significantly reduced in SVF-PRP group as compared to PRP group (B, C). PRP, platelet-rich plasma; SVF, stromal vascular fraction

TABLE 2 Global assessment score

	Physician global assessment (I)		Physician global assessment (II)		Patient global assessment	
	Groups		Groups		Groups	
	PRP	SVF-PRP	PRP	SVF-PRP	PRP	SVF-PRP
After 1st session						
Poor	5 (45.5%)	2 (18.2%)	2 (18.2%)	1 (9.1%)	3 (27.3%)	2 (18.2%)
Satisfactory	6 (54.5%)	8 (72.7%)	8 (72.7%)	8 (72.7%)	7 (63.3%)	6 (54.5%)
Good	0 (0%)	1 (9.1%)	0 (0%)	1 (9.1%)	1 (9.1%)	3 (27.3%)
Excellent	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
After 6 mo						
Poor	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Satisfactory	8 (72.7%)	4 (36.4%)	5 (45.5%)	2 (18.2%)	3 (27.3%)	3 (27.3%)
Good	3 (27.3%)	6 (54.5%)	6 (54.5%)	8 (72.2%)	8 (72.7%)	7 (63.3%)
Excellent	0 (0%)	1 (9.1%)	0 (0%)	1 (9.1%)	0 (0%)	1 (9.1%)

Note: The patient and physician 1 and physician 2 global assessment scores after first session and after 6 mo.

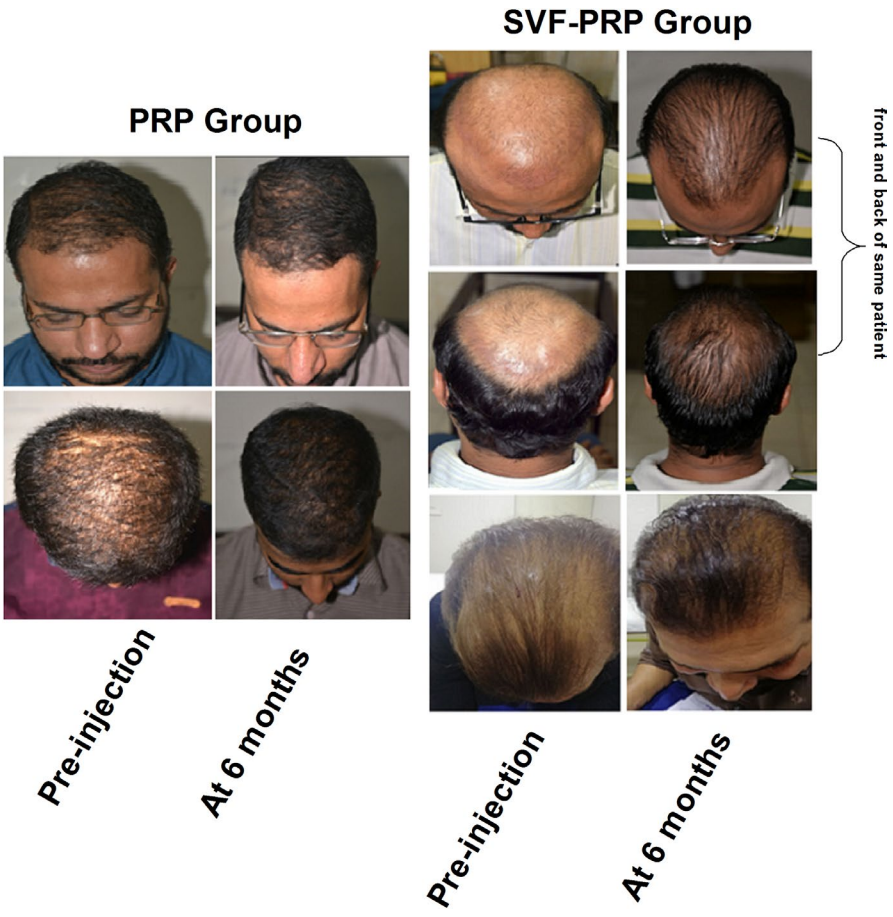

FIGURE 4 Representative picture of patients receiving either PRP and a combination of SVF and PRP. PRP, platelet-rich plasma; SVF, stromal vascular fraction

groups, 70% patients were having type III (Norwood Hamilton scale) AGA, 11.76% were having type IV (Norwood Hamilton scale) AGA. This shows that in our population, AGA occurrence is in early age and is comparable to other reports showing similar results.[24]

Mean hair density was significantly increased in SVF-enriched PRP group as compared to PRP group. In PRP group, hair density was increased from 52.44 hair/cm^2 to 63.72 hair/cm^2; while in SVF-PRP group, it was 37.66 hair/cm^2 before treatment and 57.11 hair/cm^2 after SVF-PRP therapy. Overall, increase in hair density in PRP

group was 21.51%; in the SVF-PRP group, it was 51.64%. Since use of PRP alone also increased hair density, we propose to use it in early hair loss in the patients of AGA. However, for severe cases of AGA, SVF-enriched PRP seems to enhance hair density many times. The results of our study are comparable to other studies indicating a mean increase in hair density after SVF use.[25] Results of pull test were also promising after use of SVF and PRP. Percentage reduction in pulled hair in PRP group was 34.01; in SVF-enriched PRP group, it was 80.78. The patients in both groups especially in SVF-enriched PRP group observed a decrease in hair fall along with increase in hair density. This was an additional finding as decrease in hair fall may be due to a microenvironment with an abundant blood supply for hair cells. SVF has been shown to support neovascularization.[4] The finding may be a major relief for those who are experiencing hair fall.

Both physician and patient global assessment scores further confirmed the efficacy of SVF-enriched PRP therapy, and it proved to be more efficacious than PRP alone. In PRP group, most of the patients gave satisfactory results, whereas SVF-enriched PRP group not only gave good results but also, in one of the patients, the results were excellent. Studies suggest that SVF-enriched therapies have a significant role in the treatment of alopecia.[4,25] In this current study, tolerability and safety of using SVF-enriched PRP were determined. We observed no adverse side effects in any patient. The procedure seems safe, well-tolerated by all the patients with encouraging response from these patients. There were certain limitations of the study like decrease number of sessions and a follow-up of only 6 months. However, despite these limitations SVF enrichment may prove to be a promising alternative approach for the treatment of AGA in both men and women. But further research is advised with increase number of sessions and prolonged follow-up.

The proposed strategy can provide not only a treatment for AGA patients but can also help in the development and success of tissue engineering and regenerative medicine applications in Pakistan. In addition, the results of this study will open a new avenue in dermatology for the treatment of patients with androgenetic alopecia.

The injection of SVF-enriched PRP helps reversing the effects of AGA by significantly increasing hair density in AGA patients. In addition hair-pull test, patient and physician assessment score were improved after use of a combination of SVF and PRP. The follow-up time of our study was limited (6 months), and further studies are required to determine the long-term effects of the use of SVF and PRP together.

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CONFLICT OF INTEREST

None.

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