Original Article

Platelet-rich Plasma by Single Spin Process in Male Pattern Androgenetic Alopecia: Is it an Effective Treatment?

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Abstract

Introduction and Objective: Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma. The platelet is a natural source of different growth factors and cytokines. These growth factors act on stem cells in the bulge area of the follicles and stimulate the development of new follicles, and promote neovascularization. The aim of this study was to investigate the efficacy and safety of PRP injections in androgenetic alopecia (AGA) in men. **Patients and Methods:** Fifteen male patients (mean age: 39 ± 9.7 years) with AGA grades III–VI were enrolled in the study. Five injections of 2–4 ml PRP (Regenlab PRP Kit-RegenACR®, Le Mont-sur-Lausanne Switzerland) by single spin process were administered every 2 weeks. Standard photographs, trichogram, and measurement of hair density and diameter in an area marked with a tattoo (with digital photographic hair analyzer) were done at baseline and 3 months after the last injection. In addition, patients completed a patient satisfaction questionnaire at each visit on a −2 to +2 score (−2: much worse, −1: slightly worse, 0: without change, +1: slightly better, +2: much better). **Results:** Thirteen patients completed the study. The number of hairs increased slightly from 149.62 ± 49.56 to 168.46 ± 43.703/cm^2, however, this increase was not statistically significant (P = 0.24). On the other hand, the thickness of hairs decreased from 0.051 ± 0.105 to 0.045 ± 0.011 mm, which was also not significant (P = 0.37). There was a significant decrease in anagen hairs and increase in telogen hairs, and anagen/telogen ratio decreased significantly from 6.38 ± 4.57 to 2.67 ± 1.87 (P = 0.003). **Conclusion:** Our study could not show any benefit from PRP injections in the treatment of male AGA. There is a strong need for well-designed, randomized controlled trials with large sample size, proper control group, standard treatment protocols (concerning the amount, number and interval of PRP injections, method of preparation and activation, etc.), and long follow-up periods to evaluate the safety and efficacy of PRP in the treatment of male AGA.

Keywords: *Androgenetic alopecia, male pattern, platelet-rich plasma*

Introduction

Androgenetic alopecia (AGA), a hereditary, androgen-dependent dermatological disorder, is the most common form of hair loss. AGA affects both males and females. Midfrontal hair loss affects nearly two-thirds of women over the age of 80 years and three quarters of men over 80 years.[1-3]

AGA has distinctive clinical presentations due to differences in the amount and distribution of androgen receptors and steroid-converting enzymes of the outer root sheath of the hair follicles, including cytochrome P-450-aromatase and 5α-reductase.[4]

In AGA, the terminal follicles transform into vellus-like hair, and the gradual nonscarring miniaturization of hair follicle develops. The duration of anagen phase is decreased progressively and the reduction of anagen to telogen ratio occurs.[5]

Although AGA is a benign condition, it can cause considerable psychoemotional stress and significant lowering of self-esteem.[6]

The only Food and Drug Administration (FDA) approved drugs for AGA in men are topical minoxidil and oral finasteride. Side effects such as scalp itching, burning, dryness, and scaling may be seen with the use of minoxidil, whereas finasteride intake may cause erectile dysfunction and decreased libido.[7-9]

Hence, the search for effective treatment modalities with less side effects is important. Recently, novel approaches such as application of platelet-rich plasma (PRP)

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have been introduced, and some studies have reported promising results.[10,11]

PRP is an autologous preparation of platelets concentrated in plasma. The platelet is a natural source of different growth factors and cytokines. Numerous proteins, including platelet-derived growth factor (PDGF), transforming growth factor (TGF), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), and interleukin (IL)-1 are released by the activation of alpha granules of platelets. The regenerative potential of PRP depends on these growth factors.[12]

It is postulated that platelet growth factors may act on stem cells in the bulge area of the follicles, where they bind to their respective receptors located in stem cells, stimulating the development of new follicles and promoting neovascularization.[13]

The aim of this study was to investigate the efficacy and safety of PRP injections in AGA in men.

Patients and Methods

Fifteen male patients referred to the outpatient skin clinic of Center for Research and Training in Skin Diseases and Leprosy who met the eligibility criteria and provided written informed consent were enrolled in this study conducted in 2014. The trial was approved by the Ethical Committee of Tehran University of Medical Sciences and was registered in Iran RCT Registry on 12/15/2014 as IRCT2014110919870N1.

A dermatologist made the clinical diagnosis of AGA in all participants. The inclusion criteria were age of 18–60 years, hair loss longer than 6 months, and Hamilton–Norwood grade of 3–6. Those with scalp infection, malignant disease, autoimmune diseases (Hashimoto, rheumatoid arthritis, lupus, etc.), history of vascular surgery in the past 3 months, any systemic disease interacting with hair growth, telogen effluvium, history of hematologic disorders, and addiction to oral or intravenous narcotics were excluded from study.

Other exclusion criteria were consumption of topical or systemic treatment for hair loss in the past 6 months, receiving chemotherapy or any immunosuppressive medication, use of any growth factors containing medications, hemoglobin level below 10, thrombocytopenia (or platelet count below 100,000), serum albumin below 2.5 g/dl, and participation in another clinical trial (within the last 3 months).

All volunteers were treated with RegenLab PRP Kit-RegenACR® followed by its instructions every 2 weeks for 5 sessions. In each session, 8 ml of participants' venous blood (as recommended by the company) was drawn manually using butterfly safety locks and emptied into 10-ml standard tubes (Regenlab PRP Kit-RegenACR®, Le Mont-sur-Lausanne, Switzerland) and were then put in RegenLab PRP Centrifuge. The centrifugation settings were set at 1500 g for 5 minutes. After centrifugation, the blood was fractionated; red blood cells were trapped under the gel, and cellular elements settled on the surface of the gel.

By gently inverting the Regen BCT tubes several times and processed for resuspension of the cellular deposit in the supernatant, approximately 2–4 ml of PRP was obtained from each tube. For each participant, we used one tube per session. Finally, the solution was immediately injected intradermally in the temporal and frontal areas, 0.05 ml per area in 1–2 cm intervals.

At the beginning of the study before the intervention and 3 months after the last treatment session standard photographs were taken with digital cameras (Nikon D300s®, Tokyo, Japan), trichogram was performed, and hair density and diameter were measured using the digital photographic hair analyzer (KC Triple Scope®, KC Technology Co, Korea) by a 150 × lens on an area marked with a tattoo. In addition, patients completed a patient satisfaction questionnaire at each visit on a −2 to +2 score (−2: much worse, −1: slightly worse, 0: without change, +1: slightly better, +2: much better).[14]

Percentage and frequency were used to describe qualitative data, and mean and standard deviation were used for description of quantitative data. Comparison of quantitative data before and after the test was performed by paired *t*-test (or nonparametric equivalent, depending on data distribution or categorized information). Estimation of all tests was done on significance level of 5%.

Results

The age range of patients was 24–60 years (mean: 39 ± 9.7). The Hamilton score was 3–6 (median: 4). The duration of hair loss was 12–360 months (median: 36 months). The range of hemoglobin, platelet count, and serum albumin were 14.6–17.9 g/dl (mean: 16.37 ± 0.94), 186–303 (mean: 236.13 ± 34.99) and 4.1–5.5 g/dl (mean: 4.41 ± 0.37), respectively.

Two patients were lost to follow-up and did not show up for the last assessment session, and hence, the study was conducted among 13 patients.

The number of hairs in the assessment area increased slightly, however, this increase was not statistically significant. On the other hand, the thickness of hairs decreased, although again this change was not significant. There was a significant decrease in anagen, increase in telogen, and decrease in anagen/telogen ratio [Table 1].

The mean of patient satisfaction scores for hair coverage (density, thickness) increased gradually to 1.46 (on −2 to +2 score) during the treatment sessions, however, decreased considerably to 0.15 after the treatment was stopped and during the follow-up period [Figure 1].

The Physician Global Assessment of before-after photos showed no change in 6 patients, mild improvement in 6 patients, and worsening in 1 patient [Figures 2-4].

Although all patients had tolerable pain during the procedure, serious immediate adverse effects, such as allergic reactions, postoperative pain or fever, and prolonged redness were not seen.

Discussion

In this study, we found that 5 injections of PRP (prepared by single spin process) 2 weeks apart did not change the density and thickness of hairs in 13 male patients with AGA. On the other hand, it induced a telogen effluvium in the 3 months follow up period.

Recent strategies for the treatment of AGA are predominantly focused on promoting cellular proliferation and differentiation during the hair growth cycle. Antiapoptotic effects of activated PRP have been proposed as one of the main contributing factors stimulating hair growth.[15-17]

PRP contains more than 20 different growth factors such as platelet-derived growth factor, vascular endothelial growth factor, transforming growth factor-β, epidermal growth factor, and connective tissue growth factor (FGF). The mentioned growth factors stimulate the proliferative phase and by acting on stem cells of follicle's bulge area, they stimulate the development of new follicles and promote neovascularization.[13,18] There are several studies that have evaluated the role of PRP in treating AGA. Most studies reported positive results such as increase in hair density or thickness. Greco *et al*. in 2009 showed that the average hair shaft diameter at 4 months and 8 months increased by 9.7% and 6.1%, respectively. There were 10 patients with AGA in their study; the treatment group was injected with 10 cc PRP and normal saline was injected into the scalp of the control group. Sixty cc of blood was drawn from each participant and 10 cc of PRP was processed. They did not mention the method of PRP preparation and the platelet concentration. They evaluated the patients' hair diameter 4 and 8 month after the injection session.[19]

In 2011, Rinaldi *et al*. analyzed PRP as an incubation medium in follicular unit transplantation (FUT) in their double-blind randomized controlled trial (RCT) among 100 AGA patients. Their *in-vitro* evaluation revealed that growth factors (GFs) from PRP prevented apoptosis in dermal papilla, prolonged anagen phase, and delayed catagen and telogen phases. Other notable results were reduction in diffuse hair loss and stimulation of hair regrowth in AGA. Also they showed that GFs have a significant effect on hair bulb both *in vitro* and *in vivo*, without any side effects during the treatment period and after 12 months from the end of the treatment.[20]

Takikawa showed that various GFs were concentrated in PRP, and that direct local administration of GFs in PRP may act on hair follicles and indirectly improve involution of the vascular plexus around each hair follicle. In the study by Takikawa *et al*. 26 volunteers with thin hair participated. They received five local treatments of 3 ml of PRP and dalteparin and protamine microparticles (D/P MPs) (13 participants) or PRP and saline (control, 13 participants) at 2- to 3-week intervals and were evaluated for 12 weeks.

For preparation of PRP solution 15 ml of blood was drawn into a tube containing 1.5 ml of 2% sodium citrate. The tubes were centrifuged for 15 minutes at 1700

Table 1: Evaluation of efficacy variables at baseline and 3 months after 5 PRP injections

	Baseline	End of follow-up	Changes	*P*
Anagen	84.31±7.341	66.38±14.245	(−) 17.92±12.12	<0.001
Telogen	15.69±7.341	33.62±14.245	(+) 17.92±12.12	<0.001
Anagen/ Telogen	6.38±4.57	2.67±1.87	−2.97	0.003
Hair thickness (mm)	0.051±0.105	0.045±0.011	(−) 0.006±0.01	0.37
Hair count (cm^2)	149.62±49.56	168.46±43.703	(+) 18.85±53.35	0.24

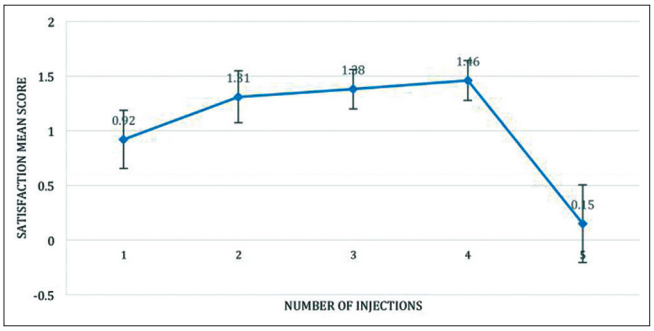

Figure 1: Change in patient's satisfaction score during the follow-up period

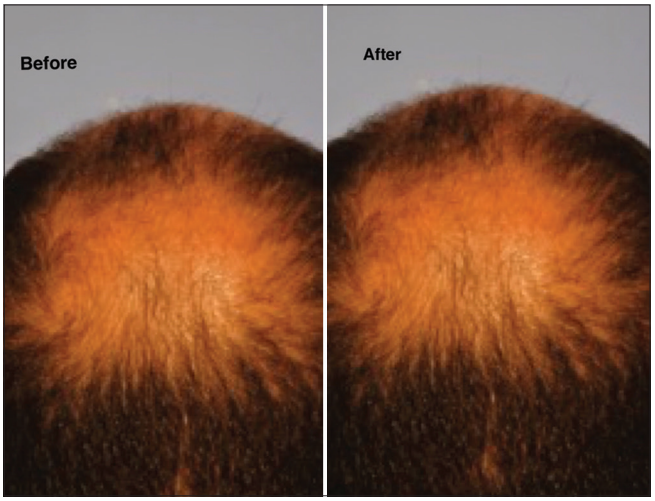

Figure 2: Patient with no change in hair growth/density after PRP injection

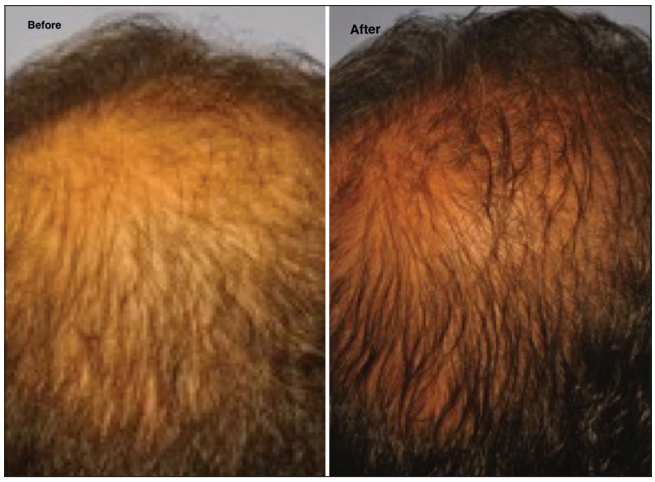

Figure 3: Patient with mild improvement (hair regrowth) after PRP injection

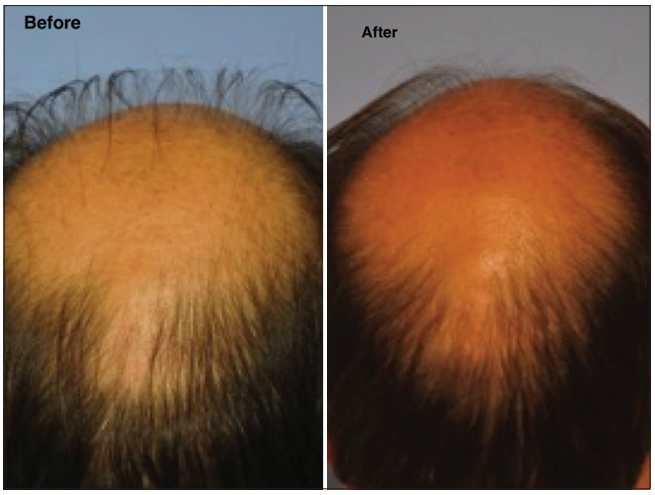

Figure 4: Patient with worsening condition (no hair regrowth/decreased his hair) after PRP injection

revolutions per minute (rpm). The PRP layer, which was the upper 1 cm of the red blood cell layer, was collected and centrifuged for 5 minutes at 3000 rpm to concentrate the platelets. Platelet concentration in PRP increased approximately 6 folds from the whole blood platelet concentration. Injected areas comprised frontal or parietal sites with lanugo-like hair. Experimental and control areas were photographed. Consenting participants underwent biopsies for histologic examination. Significant differences were seen after the fifth injection (at 12 weeks) between the control group and the PRP, and PRP and D/P MP groups ($P < 0.01$). Microscopic findings showed thickened epithelium, proliferation of collagen fibers and fibroblasts, and increased vessels around follicles in PRP and PRP and D/P MP groups.[11]

Kang *et al*. showed clinical improvement in mean hair count and mean hair thickness without remarkable major side effects 6 months after the interfollicular injection of autologous CD34+ cell-containing PRP preparation. Thirteen patients (7 males and 6 females with the mean age of 37.6 years) were treated with CD34+ cell containing PRP preparation. The 60 ml of obtained blood was transferred to tubes containing 8 ml of 4% sodium citrate solution, and then the blood was centrifuged with the SmartPReP2 platelet concentrate system (Harvest Technologies Corp.). Subsequently, 4 ml of CD34+ cell containing PRP preparation was injected on the frontal and parietal areas in each session. The injections were performed twice at 3 months interval. They did not mention the platelet concentration.

Additional 13 patients (8 males and 5 females with the mean age of 36.8 years) were treated with interfollicular injection of placental extracts. Two milliliter of placental extracts were injected on the frontal and parietal areas at weekly intervals for 6 months. At 6 months, the patients presented clinical improvement in mean hair count, 29.2 ± 17.8% ($P < 0.0001$) and mean hair thickness, 46.4 ± 37.5% ($P < 0.0001$) compared with baseline. The data revealed that CD34+ cell-containing PRP treatment presented a higher degree of improvement than placental extract treatment in hair thickness ($P = 0.027$) and overall clinical improvement ($P = 0.023$).[10]

Unlike the abovementioned studies, we found significant decrease in anagen hairs, as well as in the anagen/telogen ratio. Increase of telogen hairs was also observed. Nearly all our patients complained of increasing hair loss at the follow-up visit. Although the hair density and hair thickness increased slightly, the results were not statistically significant.

The patient's satisfaction scores during the treatment sessions was 1.46, which decreased to 0.15 during the follow-up period. In each treatment session, most of the patients were satisfied with the pervious injection and they claimed that their hair shedding was decreased after the injection; however, after 3 months in the follow-up session, they complained of increasing hair loss. The trauma of PRP injections might have induced this telogen effluvium in our study.

Although we did not evaluate PRP platelet count, Regen lab date showed that the PRP obtained after single spin process had a concentration factor of 1.6-fold. Different studies have shown that the therapeutically effective concentration of PRP platelet is more than 1 million/μL (approximately 4–7 times the mean levels).[15,21] Lower concentration of platelet that we obtained might be one of the possible explanations of our negative results.

Other limitations of our study were the small sample size, low volume of PRP injected in each session, and lack of control group.

In a recent systematic review, the role of PRP for treatment of nonscarring hair loss was evaluated among 18 studies. We can count on the fingers of one hand the numbers of published randomized controlled clinical trials (RCTs)

that are the gold standard for proving the efficacy of PRP treatments. In the mentioned systematic review due to the lack of accurate well-designed randomized controlled trials, the significant methodological deficiencies of nearly all reviewed studies, lack of approved scientific protocol for PRP preparation, lack of a reference protocol regarding the frequency of applications and the injected amount of PRP, heterogeneity in application methods, small sample size, lack of controls, and lack of meticulous reports in patients' characteristics, there was no definite possibility of having accurate judgment regarding the efficacy of PRP in treatment of nonscarring hair loss.[22]

In a meta-analysis recently published involving 6 studies, 4 RCTs encompassing a total of 177 patients with AGA, a significantly locally increased hair number per cm^2 and hair thickness cross section per 10×4 mm^2 ($P = 0.005$) was observed after PRP injections versus control ($P = 0.004$). Although PRP injection for AGA might increase hair numbers and hair thickness in the treated areas, the results of this meta-analysis should be interpreted with caution as it consists of pooling many small studies. Larger randomized studies are needed for verifying these results.[23]

Conclusion

Most of the available studies had some limitations such as the limited number of patients as well as lack of control group similar to our study. Hence, making any conclusion about the efficacy of PRP for treating hair loss in AGA in men is very difficult using the available data. There is a strong need for well-designed, randomized controlled trials with large sample size, proper control group, standard treatment protocols (concerning the amount, number and interval of PRP injections, method of preparation and activation, etc.), and long follow-up periods to evaluate the safety and efficacy of PRP in the treatment of male AGA.

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Conflicts of interest

There are no conflicts of interest.

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