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Exosomes: Myth or reality? Efficacy and safety of exosomes versus platelet rich plasma in patients of androgenetic alopecia

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Abstract

Background: Androgenetic alopecia (AGA) is terminal hair loss from scalp after puberty. Currently, FDA approved treatments topical minoxidil and oral finasteride are not favourable by patients due to their adverse effects. Platelet rich plasma (PRP) is a commonly used effective therapy. Exosomes is another emerging treatment modality, showing promising results in patients of AGA.

Objective: To compare the efficacy and safety of PRP and Exosomes in patients of androgenic alopecia areata.

Methods: This interventional study was conducted on AGA patients for one year. Patients were divided in two groups. Group A patients received injections exosomes whereas group B received PRP intradermally in scalp respectively, two sessions in exosomes group and 4 sessions in PRP group. In both groups patients were followed six months after the last procedure. The efficacy was assessed using trichoscopy, photographs, physician global assessment score and patient global assessment score.

Results: Among total 30 patients, mean age of patients was 37 years and 80% were males. At the end of 6 months, mean hair density, physician and patient global assessments showed significant improvement from baselines (p value < 0.05), more in Exosomes group as compared to PRP group. No significant adverse effect was observed in any patient.

Conclusion: It is concluded from this study that the Exosomes have better efficacy and comparable safety than PRP in AGA.

Keyword: Androgenetic alopecia; Exosomes; Platelet Rich plasma (PRP).

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Introduction

Alopecia is hair loss in areas where it is normally expected to grow like in AGA, there is continuous loss of terminal hair of scalp which starts after puberty. It is non-scarring alopecia, affecting 50% of male and female population.[1] It has a characteristic patterns, in males there is recession of frontotemporal hairline along with marked hair loss on vertex whereas in females there is diffuse hair thinning on crown giving a wider appearance of apex of scalp.[2] It has multifactorial pathogenesis, having complex interplay of genetic, hormonal and environmental factors. In

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genetically predisposed individuals, androgens cause shortening of anagen phase of hair growth cycle leading to hair follicle miniaturization. By progressive shortening of anagen, hair follicles become smaller leading to thin, short hairs.[3]

Currently FDA approved treatments are topical minoxidil and oral finasteride.[4] The common adverse effects of topical minoxidil are irritant and allergic contact dermatitis along with hypertrichosis.[5] The common adverse effects with oral finasteride are impaired sexual function, decreased libido, erectile dysfunction, decreased arousal and orgasm problems. Breast swelling and tenderness, headache, irregular menstruation, dizziness, and increased body hair have also been reported.[6] Due to these adverse effects most patients refuse to take this drug so PRP is currently an effective alternative therapy being used. It has obtained popularity because it is autologous, affordable, less invasive and has no crucial side effect in comparison to hair restoration surgery.[7] PRP is autologous concentration of platelets having multiple growth factors in their alpha granules.[7]

Literature review showed satisfactory results of PRP in androgenetic alopecia. A study in Pakistan, in 2018, showed that PRP is an effective treatment in androgenetic alopecia confirmed by increased hair density, increase in terminal to vellus hair ratio, and satisfactory physician and patient global assessment scores.[8] Multiple studies indicated that PRP has increased levels of various growth factors like Vascular Endothelial Growth Factors (VEGF) and Platelet Derived Growth Factors (PDGF) which induce angiogenesis at the site of injection and can prolong anagen phase of hair growth cycle.[9]

Exosome is another emerging treatment modality for AGA. Exosomes are 30-150 nm extracellular vesicles (EVs) derived from various mesenchymal stem cells (MSCs). Exosomes contain various proteins, nucleic acids and cell mediators like regulatory microRNAs that have role in cell signaling and gene expression.[10] They have same biological properties as that of their parent derived cells along with advantages of small size, easy penetration of biological membranes, low immunogenicity, easy storage, and no tumorization. Recently, exosomes are genetically modified so that they can exhibit better therapeutic properties such as enriching active ingredients, targeted delivery, and physiological barrier to penetration.[11] Literature review has shown that exosomes from DPCs increased proliferation of outer root sheath cells, hair matrix cells and DPCs. They also promote hair follicle stem cell differentiation and proliferation. Studies using ex vivo-cultured human hair follicles found that DP Cell derived exosomes not only prolong anagen phase of cell cycle but also increase elongation of hair shaft.[12,13]

Currently, very sparse literature is available on role of exosomes in humans in various diseases although multiple studies are in progress to assess their role. At present, no study is available to compare efficacy and safety of PRP versus Exosomes in androgenetic alopecia in our population, therefore, this study was planned to assess the clinical efficacy and safety of these therapies in our population.

Methods

It was randomized clinical intervention performed in Dermatology department of Services hospital, Lahore Pakistan for a duration of one year between October 2023 and September 2024. AGA was diagnosed clinically by history, clinical and dermoscopic examination. All patients of age range of 16-50 years of both gender having AGA of Male pattern, type III to VI, on Hamilton-Norwood scale and female pattern, type I to III AGA, on Ludwig classification were included.[14] Patients with platelet structural disorders, thrombocytopenia, anaemia, chronic diseases like hypertension, diabetes, malignancy, sepsis, inflammation or infection of scalp and patients on anticoagulant or antiplatelet medication or immunosuppressives were excluded from the study. Pregnant and lactating females as well as patients using any treatment for AGA in the last one year were also omitted.

After getting ethical approval from IRB and informed consent, patients were allocated into two equal groups. Group A received exosomes two sessions two months apart whereas group B received PRP four sessions monthly. All procedures were performed intradermally on scalp under local anaesthesia by gel. Injections were given under aseptic measures.

Exosomes used were in the form of freeze-dried lyophilized powder that contain high concentration of exosomes derived from Leuconostoc bacteria and a peptide-activating solution to enhance absorption and effectiveness. It was constituted at time of injection by combing powder and solution. Insulin needles (31G) were used to inject the exosomes at a dose of 0.1 ml/cm^2 into the affected areas of the scalp, 1 cm apart using Nappage technique;[15] in which injections were given at a depth of 1.5-2.5 mm in the dermis. A total volume of 3.5 ml was injected per patient per session.

PRP was prepared by collecting 10ml of fresh blood in sodium citrate containing vacutainers under aseptic conditions. The tubes were then rotated in a centrifuge machine at 1500 revolutions per minute for 10 minutes. The first centrifugation, called “soft spin”, allowed separation of blood into three layers with bottom red blood cells layer, topmost acellular plasma layer called platelet poor plasma (PPP) and an intermediate PRP layer known as “buffy coat”. Serum including buffy coat with PPP was collected with the help of Finn pipette in another test tube. This tube underwent a second centrifugation, hard spin, at 4000 revolution per minute for another 10 minutes. Whole serum was collected in an insulin syringe. Insulin needles were used for injections into scalp, which were given at dose of 0.1 ml/cm^2 into the affected areas of the scalp at 1 cm distance using Nappage technique in which injections were given at a depth of 1.5-2.5 mm in the dermis. A total volume of 3.5 ml was injected.

All patients were assessed for a period of one year. First follow up visit was done after one month of last session, second visit three months after last session, and then six months after the last session. The treatment outcomes were assessed by taking clinical photographs, trichoscopic photomicrographs, physician global assessment score (PGA), and patient global assessment score (PtGA). Photographs were taken by same photographer by the same camera at the same distance at each visit. In trichoscan, number of hairs were counted per centimeter square of scalp. Clinical response was assessed by PGA performed by same evaluator as satisfactory (<25% improvement in hair growth from baseline), good (25-50% improvement in hair growth from baseline) or excellent (>50% improvement in hair growth from baseline). Similarly, PtGA was evaluated using the same criteria and scale as mentioned above for PGA. Primary efficacy was >25% increase (good and excellent response) in hair density 6 months after the last session, whereas secondary efficacy was >25% increase in PGA and PtGA.

The study was performed as a pilot study to compare PRP and Exosomes therapy for AGA. Due to the sample size of this study, only large effect sizes (Cohen’s d: 1.12) could be detected with a sample size of 30 (power 80%, 2-sided significance level 5%). The primary outcome, hair number was summarized in each group using standard statistical measures. Differences between baseline and each of the follow-up measurements were calculated. Furthermore, differences between the groups regarding these calculated changes from baseline were determined. Intergroup comparison of baseline characteristics and their changes at week 16 of the trial were performed using the two-sample *t* test for continuous variables and the chi-square test for categorical variables.

Results

Total 30 patients were included in the study, mean age of the patients was 37.20±7.47 years. Age range was 25-53 years. A significant proportion of the study population fell within the age group of 30-40 years. Additionally, a strong familial link to AGA was observed, with 80% patients reporting a family history of AGA. While analyzing the types of AGA, 20% of

patients were females and exhibited either type II or Type III alopecia according to Ludwig scale in equal proportion. Among male patients, 46.7% were found to have type III alopecia, 26.7% had type IV and 6.7% had type VI alopecia according to the Norwood Scale, making type III the most commonly observed type of AGA in our study. In terms of disease duration, the majority of patients had this disease for 3-5 years meaning that patients seek delayed treatment.

In terms of primary efficacy, a significant difference was noted between the exosomes group and the PRP group. Thirteen out of fifteen (86.67%) patients in the Exosomes group showed significant improvement, whereas in the PRP group, only 7/15 (46.67%) of patients demonstrated good results. The secondary efficacy, evaluated through both the Physician Global Assessment (PGA) and the Patient Global Assessment (PtGA), further highlighted the difference between the two treatments. In the Exosomes group, more than 80% of patients achieved secondary efficacy, whereas in the PRP group, only 40% managed to do so **(Table 1)**.

Table 1 Comparison of demographic profile and efficacy of both groups.

	Treatment Given		*Total*	*p-value*
	Exosomes	*PRP*		
Mean Age	38.20 yrs	36.20 yrs		0.369
Gender				
Male	12	12		0.674
Female	3	3		
Age groups				
20-30 years	3	5		
31-40 years	7	7		0.160
41-50 years	5	1		
51-60 years	0	2		
Duration Of Disease				
<1 year	2	2		1.000
1-<3 years	4	4		
3-5 years	9	9		
Hair Density (hairs/cm^2)				
Satisfactory Response	2(13.3%)	8(53.3%)	10(33.3%)	
Good response	5(33.3%)	5(33.3%)	10(33.3%)	0.027
Excellent Response	8(53.3%)	2(13.3%)	10(33.3%)	
Total	15	15	30	
Physician Global Assessment				
Satisfactory Response	2(13.3%)	8(53.3%)	10(33.3%)	
Good response	5(33.3%)	5(33.3%)	10(33.3%)	0.027
Excellent Response	8(53.3%)	2(13.3%)	10(33.3%)	
Total	15	15	30	
Patient Global Assessment				
Satisfactory Response	2(13.3%)	7(46.6%)	9(30%)	
Good response	4(26.6%)	6(40%)	10(33.3%)	0.022
Excellent Response	9(60%)	2(13.3%)	11(36.6%)	
Total	15	15	30	

In terms of mean hair density, numbers of hairs per centimeter square area of scalp increased from 105.27±11.98 hairs/cm^2 to 165.87±19.48 hairs/cm^2 in patients receiving exosomes, whereas in patients receiving PRP mean hair density increased from 105.33±11.99 hairs/cm^2 to 132.13±13.59 hairs/cm^2 of scalp **(Figures 1,2)**. This showed significant improvement in both groups from baseline (p value <0.001). No adverse effect was observed in any patient in either treatment except mild transient pain during procedure.

Discussion

Androgenetic alopecia is a common hair loss disorder worldwide. Conventionally, several treatment options are available for this disease with variable results.[16] Based on our results we concluded that exosomes not only have a role in hair growth causing increased hair density but are also better tolerated than PRP in patients of AGA. Patients showed statistically significant improvement in hair density and satisfaction after treatment sessions with exosomes as well as PRP

Stevens et al.[17] assessed the efficacy of stromal vascular fraction (SVF) injections along with PRP in patients of androgenetic alopecia. He found that hair density notably improved from baseline of 157 hairs/cm^2 to 177 hairs/cm^2 ($p = 0.013$) and to 185 hairs/cm^2 ($p < 0.001$) at 12 weeks. He suggested that when platelet-rich stroma was injected in AGA patients scalp, there was significant increase in hair density within 6-12 weeks, these findings correspond to our study results that showed improvement in hairs density after exosomes treatment in scalp. SVF is a heterogeneous mix of cells, primarily mesenchymal stem cells (MSCs), endothelial cells, pericytes, macrophages, and other immune

cells, isolated from adipose tissue.[18] Whereas exosomes are extracellular vesicles (EVs) derived from various mesenchymal stem cells (MSCs).[10]

Figure 1 Cases of exosomes group**;** A: before treatment and B: 6 months after treatment of a male patient. C: before treatment and D: 6 months after treatment of a female patients. Trichoscopic pictures E: before treatment and F: 6 months after treatment of same male patient. G: before treatment and H: 6 months after treatment of same female patients.

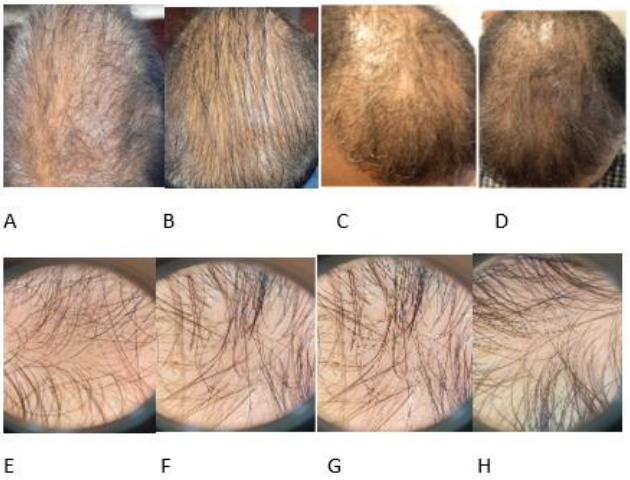

Figure 2 Cases of PRP group A: before treatment and B: 6 months after treatment of a male patient. C: before treatment and D: 6 months after treatment of a female patients. Trichoscopic pictures E: before treatment and F: 6 months after treatment of same male patient. G: before treatment and H: 6 months after treatment of same female patients.

Another randomized controlled trial was conducted by Tak et al.[12] in South Korea in which he included 38 AGA patients with age range of 18-59 years, in which he applied Adipocyte Derived Stem Cell (ADSC) constituent extract topically on the scalp and then analyzed hair count and thickness at 4 months. In this study, the hair density increased from 139.7±2.3 hairs/cm^2 to 153.6±16.8 hairs/cm^2 (p <0.05). They concluded that applying adipose-derived stem cell extracts solution topically increased hair density and thickness while keeping treatment safety. Their patients' demographic profile was same as that of study under discussion with comparable sample size. Regarding the research outcomes, these conclusions were similar to our results showing increased hair regrowth by stem cell constituents in adipose tissue.

Another metanalysis showed that exosomes were beneficial in hair growth.[19] It concluded that EVs hold great potential for advancing clinical treatments for alopecia in the future. EVs exert their effects by activating signaling pathways through ligand binding or by entering cells via membrane fusion or endocytosis. They offered several advantages as compared to traditional medications or stem cell therapies, like easy storage, high and sustained efficacy, biological safety, less immune rejection, and easy of transportation.

Ester Lee, et al.[20] used Human adipose tissue stem cells derived exosomes in AGA patients and concluded that the baseline mean hair density in the targeted area was 158.03/cm^2. After 12 weeks, the mean hair density was 161.90/cm^2 (p = 0.033), and after 24 weeks, it increased to 166.14/cm^2. Byung-Soon Park et al.[21] used Adipose-tissue derived exosomes and showed that mean hair density increased from 121.7±37.2 to 146.6±39.5 hairs/cm^2 (p <0.001), and mean hair thickness increased from 52.6±10.4 to 61.4±10.7 µm (p <0.001). Our study also demonstrated increase in hair density using Exosomes. Also there was a significant improvement in patient satisfaction achieved at the 12th week in comparison to the 4th week (p <0.05) in their study, similar to our observation.

Ersan M.[13] conducted a prospective study in turkey in 2024 in which he included 30 male patients, aged between 22 and 65, and with alopecia of Norwood-Hamilton classification III-IV. He assessed efficacy of exosomes in androgenetic alopecia by assessing hair density and thickness before starting treatment and 12 weeks after treatment. The mean hair density increased from 149.7±13.7 hairs/cm^2 at before treatment to 153.6±16.8 hairs/cm^2 at the 4th week ($p = 0.043$) and further to 157±18.3 hairs/cm^2 at the 12^{th} week ($p = 0.002$). Their sample size and age range is same as that of ours but they only included male patients. They concluded significant improvement in hair density from baseline to post treatment similar to ours. They too reported no adverse effects.

Studies on PRP therapy indicates that they can prolong anagen phase of hair growth cycle as it increases the levels of different growth factors like PDGF and VEGF and induces angiogenesis at the site of injection.[7] A study on role of PRP in androgenetic alopecia in Pakistan in 2018 concluded that hair density increased from 34.18/cm^2 at the first visit to 50.20/cm^2 at the last visit.[8] These finding were similar to our study showing increase in hair density from baseline (p value <0.001).

Another randomized trial was done by Qu Q et al.[22] in China on 52 patients of androgenetic alopecia to assess the therapeutic efficacy of PRP. It was a half head study, where they injected PRP on half of the head and placebo on the other half. They concluded that mean hair density, diameter, and anagen hair ratio showed marked improvement after 6 months in comparison to the control side. These findings are similar to our results. Another study done in 2020 involved 69 men having androgenetic alopecia. They were divided into 3 groups and received PRP therapy, minoxidil or their combination respectively. PRP treatment was more effective than minoxidil therapy (p=0.005). Combination therapy turned out to be more effective than minoxidil monotherapy (p <0.0001) and PRP monotherapy (p=0.007).[23] These findings were consistent with our results.

Shaipro et al.[24] performed a randomized controlled split-scalp study to find out the effects of PRP on hair regrowth and thickness. They marked two 7.6×7.6 cm area with tattoo on the scalps of 35 study participants with AGA. These areas were then randomly allocated to either intradermal injection with PRP or saline. All participants were given monthly treatment for 3 sessions, with evaluation after 3 months of the last treatment. Hair density in the PRP-treated area was remarkably improved as compared to baseline, at final assessment. Hair density in PRP-treated areas increased from 151±39.82 hairs/cm^2 to 170.96±37.14 hairs/cm^2 (P<.05). Our study results support the findings of this study. Similarly in another study, twenty-five healthy male patients with AGA were enrolled in a placebo-controlled, randomized crossover study with the treatments of PRP. They observed a significant improvement in hair density in the secondary endpoints after PRP treatment (p=0.014).[25] This study mirrored the results of our study.

Another study by Singh SK[26] in India, in 2020, concluded that significant improvement was found in patients given both PRP and minoxidil. Increase in hair density was observed maximally in PRP and minoxidil group, than with PRP-alone or minoxidil-alone, while a decrease in hair density was found in normal saline group, after 5 months. Similarly, the maximum patient satisfaction was found in PRP with minoxidil group followed by PRP-alone group, minoxidil-alone group, and normal saline group. These findings reinforced the findings of our study

Gordon H. Sasaki.[27] in 2022 conducted a retrospective open-label study among 22 female and 9 male patients who demonstrated early stages of alopecia. They injected exosomes intradermally same as technique done in current paper. They concluded that most growth was observed in older aged females and younger aged males, shorter history of alopecia, earlier stages of hair loss, larger and undiluted volumes of exosomes.

Jovian Wan.[28] carried out a prospective, open-label study including 16 male patients aged 36-45 years with mild to moderate AGA. They concluded that average increase in hair density was 35±6.5 hairs/cm^2 at end of 1 year. However, hair densities increased from 75 hairs/cm2 to 95 hairs/cm^2. 80% of participants were highly satisfied with hair regrowth. Mild side effects of scalp tenderness and slight irritation were reported.

Strength/ Limitation of study

The strength of this study is that it is a novel therapeutic comparison with prospective design which focuses on safety profile of patients. However, this study had some limitations like small sample size with short follow up. Thus, further studies are needed with longer follow up and with large sample size.

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