Abstract

Background: Platelet-rich plasma (PRP) is an autologous concentration of human platelets contained in a small volume of plasma with haemostatic and tissue repairing effects. Being enriched by various growth factors, PRP has become the focus of attention in numerous fields of medicine. Androgenic alopecia (AGA) is a common chronic hair loss disorder, characterised by progressive hair loss. Despite the therapeutic options available, there is low patient compliance and satisfaction rate. The topical and often systemic adverse effects of therapy has lead to the search of new treatment options for AGA. Recently, PRP has received growing attention as a potential therapeutic tool for hair loss. Aim: To compare the efficacy of placebo versus PRP injections in the treatment of male AGA. Patients and Methods: Fifty male patients with AGA (Grade III to VI) were enrolled in the study. PRP was prepared using the double-spin method and injected in the androgen-related areas of scalp on the left side. Normal saline was injected on the right side in a similar fashion. Treatment sessions were performed with an interval of 21 days, and six sittings were completed for every patient. Results: Hair loss reduced with evidence of new hair growth. Digital image analysis showed an overall improvement in hair density and quality as lanugo-like hair became thicker, normal hair. An improvement in hair density, quality and thickness on trichoscopy was noted. Conclusion: Our data suggest that PRP injections have therapeutic effect on male pattern hair loss with no major side effects and high patient satisfaction overall.

Keywords: Androgenic alopecia, growth factors, platelet-rich plasma

Introduction

Androgenic alopecia (AGA) is a common hair loss disorder and is characterised by androgen-related progressive thinning of the scalp hair in a defined pattern. It is a common dermatological disorder with effect on social and psychological well-being of the patient. It usually begins by 20 years of age and affects most of the men by the age of 50 years.[1,2] AGA may undergo significant impairment of quality of life since hair is considered to be an important feature of self-image.[3,4] Hair loss affects self-esteem, personal attractiveness and may even lead to depression and other negative effects. Its etiopathogenesis is androgen-dependent and orchestrated through the testosterone metabolite dihydrotestosterone, the expression of androgen receptor and genetic factors.

Hair follicle has a very complex biologic structure, and growth of the hair is regulated by specific growth cycles. The mature follicle undergoes successive transformation from anagen to catagen to telogen. Many growth factors play a fundamental role in the life-long cyclic transformation of the hair follicle, functioning as biologic switches that are turned on and off during the different phases. The main growth factors involved in the establishment of hair follicle are vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), insulin 1-like growth factor and fibroblast growth factor (FGF). Platelets release large amounts of platelet-derived growth factor (PDGFaa, PDGFbb and PDGFab), transforming growth factor beta β1 and β2, EGF and VEGF.

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the levels of growth factors released on activation; thus, it has emerged as an effective alternative in the management of AGA.

**Patients and Methods**

Fifty patients with AGA (Hamilton-Norwood Grade III to VI) were enrolled in the study. Between August 2015 and November 2015, male patients suffering from AGA and not on topical minoxidil and oral finasteride for at least 6 months were considered for PRP therapy.

Written informed consent was obtained. All included patients were tested for HIV, HBSAg and platelet count.

Exclusion criteria were as follows:
- Haematological clotting disorders and patients on anticoagulant therapy
- Thyroid dysfunction
- Malnutrition and other dermatological disorders contributing to hair loss
- Patients with unrealistic expectations
- Any inter-current infections.

A 1 cm × 1 cm square area was marked bilaterally over the parietal area in the mid-pupillary line, 10 cm proximal to eyebrow in each patient. Baseline follicular units were manually counted with the TrichoScan (Caslite Nova software (Catseye Systems and Solutions Private Limited, Mumbai). This tool allowed us to keep patient records with their images and hair thickness with hair density. The patients were examined at every follow-up session for hair thickness and hair density using the TrichoScan.

Before each session, the hair pull test was performed by the same clinician two times. A bundle of around fifty hairs was firmly grasped between the thumb, index and middle finger from the base close to the scalp. The hair were tugged away from the scalp, and the extracted hair were counted at every session. It was performed in a standardised manner by the two evaluators. Despite the fact that it is not an objective evaluation method, it gives a satisfactory general image of hair loss.

To evaluate overall hair growth, hair volume, hair quality and fullness, global pictures were taken in every session from front, vertex, lateral and back view.

PRP was prepared using a double-spin method for which the following materials were used:
- 10 ml syringes with 18-gauge needles
- 8.5 ml acid citrate dextrose (ACD) vacutainer tubes
- Insulin syringe
- Micropipette
- Digital centrifugation machine
- Sterile plastic tubes.

After obtaining informed consent, 16 ml blood sample was aspirated using 18-gauge needle and divided in two ACD vacutainer tubes, containing 8 ml each. The first centrifugation or ‘soft spin’ was carried out at 1200 rpm for 8 minutes, and the separated buffy coat with platelet-poor plasma (PPP) was collected with the help of a pipette in another test tube. This tube underwent a second centrifugation, a faster ‘hard spin’ at 2400 rpm for 4 minutes. The upper layer containing PPP was discarded, and the lower layer of PRP was taken for platelet count, after which it was loaded into an insulin syringe. One hour before the administration of PRP, local anaesthetic cream was applied over the area of the scalp to be treated and was cleansed with spirit and betadine. With the help of an insulin syringe, PRP was injected by intradermal technique (multiple small injections in a linear pattern 1 mm apart) under proper aseptic precaution in a minor operation theatre. A total volume of 1–2 cc was injected. The injected volume of PRP was considered satisfactory because the counts we obtained with our technique were spectacularly high making it easy for us to apply the limited amount in the area prefixed. PRP was injected in the androgen-related areas of scalp over the left half. Normal saline was loaded in another insulin syringe and injected on the right side in a similar fashion. Treatment sessions were performed with a 3-weekly interval. For each patient, six such sittings were done. At each visit, hair count was checked over the prefixed square area. Subjective improvements of patients were noted on a scale of worst (1) to best (10). All patients were advised to avoid head wash 1 day before the treatment and the patients were evaluated at the end of six sittings.

**Results**

A total of fifty patients got enrolled in the study. Nevertheless, there was a dropout of six patients who did not complete the therapy protocol. Therefore, 44 patients were finally included in the study. The mean age of the patients was 34 years (18–55). The demographic details of the patients have been graphically depicted in Figures 1 and 2.

We evaluated the number of hairs taken at the hair pull test done every session and depicted it graphically [Figure 3]. At baseline, the mean number of hairs pulled was eight while at sitting 3rd and 4th reached normal levels since less than three hairs pulled are considered to constitute normal hair loss. Macroscopic photographs showed an overall improvement in hair density and quality as lanugo-like hair became thicker, normal hair [Figures 4 and 5].

![Figure 1: Number of patients in various grades](image)
To test whether there was significant difference between hair thickness/density after T6, paired sample t-test was run in Microsoft Excel with 95% confidence interval, having null hypothesis as difference of mean in two-test sample is zero. We ran the paired t-test with the respective samples and observed the following results.

**Major insights in paired t-test:**
- There was significant difference in mean hair thickness/density after T1 and after T6. The graph showed that it has substantially increased after T6 [Figures 6 and 7].
- There was significant difference between left side and right side after T6 in respect of both hair density and thickness.
- As $P < 0.05$, we rejected the null hypothesis which implies that there is significant difference in mean hair thickness at left side after T6 than after T1 and the graph clearly showed that the thickness had increased significantly after T6.

Patients filled in the satisfaction questionnaire and reported adverse effects. They were satisfied with a mean result rating of 7.0 on a linear analogue scale of 1–10 (1 = no result, 10 = best result). Seventy per cent of patients reported an improvement in hair quality and thickness, while 55% reported an increase in hair density.

During application, almost all the patients felt pain, despite local anaesthesia, which subsided after 4 h. None reported any increase in hair shedding, any infection or ecchymosis.

**Discussion**

AGA remains the most common hair disorder with unsatisfactory treatment. Since AGA is characterised by anagen phase shortening and miniaturisation of terminal hair, the current therapeutic strategies target cellular proliferation/differentiation during the hair cycle.

Food and Drug Administration-approved drug therapies include oral finasteride and topical minoxidil, except transplantation which is a surgical treatment option. Minoxidil appears to prolong anagen phase, promotes survival of dermal papilla cells and increases hair follicle size. Finasteride also promotes hair growth of anagen hair leading to gradual increase in hair diameter and hair elongation.

However, there are several reported side effects such as headache and increase in other body hairs for minoxidil whereas loss of libido has been reported with oral finasteride. Finasteride also interferes with genital development in a male foetus and is contraindicated in pregnant women and those likely to become pregnant.

Activated PRP seems to promote differentiation of stem cells into hair follicle cells through the up-regulation of transcriptional activity of β-catenin. It also induces in vitro proliferation of dermal papilla cells and increases dermal papilla cell growth by activating extracellular signal-regulated kinase signalling. PRP prolongs the anagen phase through increased expression of FGF-7 and increases cell survival by inhibiting apoptosis (associated with increased Bcl-2 protein levels as well as activated Akt signalling). It appears to increase the perifollicular vascular plexus, through the increase of VEGF and PDGF levels, which have an angiogenic potential.
our study using non-invasive evaluation methods, such as trichoscopic photomicrographs [Figures 6 and 7], a significant increase in hair density was noted (from mild to significant improvement). Hair density followed an upward curve, peaked at 3 months, and patients are still in follow-up.

Patients with Grade III–IV alopecia according to the Norwood–Hamilton scale had better results compared to patients with more advanced alopecia.

Furthermore, patients with vellus hair had better results compared to those who had few but normal hair as PRP appeared to act on hair diameter causing thin hair to become thicker terminal hair.

Our protocol of PRP preparation and application made our results reproducible. The hair evaluation methods were not objective as hair pull test remained a subjective evaluation method. Digital images showed an overall picture of positive growth and hair density. Dermoscopic photomicrographs showed an improvement in hair density as number of hairs was counted manually thrice and was the most objective method. Trichogram, an objective evaluation method, was not performed as it was not available in our set-up. An adequate means of measuring hair growth over a span of time in a reproducible, cost-effective and non-invasive manner was unavailable.

A small cohort of patients restrained us from validating the results despite the fact that we had statistically significant results.

**CONCLUSION**

Hence, we can conclude PRP is an effective alternative modality in the treatment of AGA without remarkable adverse effects and is accompanied by a high satisfaction rate among patients. However, a randomised, double-blind study with larger cohort of patients and objective evaluation methods is needed.

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