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Evaluation of Efficacy of Platelet Rich Plasma in Androgenetic Alopecia Patients

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Abstract: ***Background:*** *Androgenetic alopecia (AGA) is a common disorder, with possible psychosocial implications. It affects around 80% of men and 50% of women throughout their lifetime. Platelet-rich plasma (PRP) treatment has gained popularity among different surgical specialities for improving various conditions.*
Aim: *In this prospective study efficacy of PRP in treating androgenetic alopecia is assessed*
Methods: *A total of 28 cases of clinically diagnosed male and female patients with androgenetic alopecia in the age group of 18-45 years constituted the present study. Detailed history and clinical examination of cases was done. All the routine investigations were carried out. PRP injections were given to alopecia areas once in every four weeks for 5 times.*
Results: *A total of 28 patients were studied. Large number belonged to the age group 21-30 years. Majority were from class 2 to 5, Hamilton Norwood classification of AGA. The family history was positive in 12.22patients showedimprovement of varying degrees ranging from single class to 2 classes, self-assessment questionnaire and global photographs of 78.5% patients showed improvement.*
Conclusion: *In a developing country like India, autologous PRP treatment is an affordable alternative therapy for androgenetic alopecia.As the demand for less invasive, highly effective cosmetic procedures is growing, PRP in androgenetic alopecia is found to be simple, safe and effective procedure.There is a high level of patient satisfaction with PRP treatment, minimal side effects and the treatment is cost effective to the patient.*
Keywords: *Androgenetic alopecia, platelet rich plasma*

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I. Introduction

Androgenetic alopecia (AGA) is considered to be the most common type of baldness characterized by progressive hair loss. AGA can affect all races, but the prevalence rates vary.[1]A study in an Indian population had type II and III as the commonest presentation[2].

Androgenetic alopecia (AGA) is characterized by stepwise miniaturization of the hair follicle, resulting from alteration in the hair cycle dynamics, leading to vellus transformation of terminal hair follicle. The normal hair cycle has an active growth phase (anagen) which can last from two years to six to seven years. This is followed by a brief stage of regression (catagen) which lasts one to two weeks and then a resting phase (telogen) lasting from five to six weeks to about 100 days. The catagen phase is a process of involution, where a burst of apoptosis occurs in a majority of follicular keratinocytes along with termination of pigment production and condensation of dermal papillae. The result is an upward movement of dermal papillae. In the telogen phase the hair shaft matures into a club (vellus) hair. In AGA, the duration of anagen phase gradually decreases and that of telogen phase increases. As the duration of anagen phase determines the hair length, the maximum length of the new anagen hair becomes shorter than that of its predecessor, leading to miniaturization and eventually a bald appearance[3].

The angiogenic role of PRP has recently caught the attention of dermatologists and plastic surgeons, to explore its usefulness in hair growth modality[8].

Mechanism of action of PRP in AGA

Activated PRP stimulates proliferation and differentiation of stem cells in the hair follicle bulge area via multiple molecular mechanisms[9]

Upregulation of transcriptional activity of β catenin →differentiation of stem cells into hair follicle cells

Increased bcl-2 levels →anti apoptotic → prolongs survival of dermal papilla cells. Activation of Akt and ERK signalling pathways→ prolongs survival of dermal papilla cells. Expression of FGF-7 in dermal papilla cells → prolongs anagen phase of hair follicles

Various modes of PRP therapy for AGA are as follows:
Inter-follicular injection of PRP of 0.05-0.1 ml/cm^2, in a retrograde fashion from deep to superficial, at every centimetre, throughout the treated site.

1. PRP mesotherapy: Scalp is punctured with microneedle roller of 1-mm fine needles followed by interfollicular injections of PRP (or by using mesogun) over the treated area
2. PRP can be used as an adjunct to hair transplantation:

a. The follicular grafts are dipped into PRP for about 15 minutes, before implantation, so as to increase their survival rate after implantation.
b. PRP is injected into the recipient area of scalp prior to or just after implantation of grafts.

II. Methods

The present study was an observational and interventional study undertaken to study the efficacy ofplatelet rich plasma(PRP) in the treatment of androgenetic alopecia patients. In this study PRP was given as a monotherapy without any concomitant treatment. A total of 28 cases of clinically diagnosed male and female patients with androgenetic alopecia constituted the subject material for present study. Prior approval for the study and the protocol was obtained from the ethical committee. The cases were selected on the basis of the following inclusion and exclusion criteria:

Inclusion criteria

 Patients whowere willing for the procedure having androgenetic alopecia.
 Patients in age group 18-45 years.

Exclusion criteria

Patients with active infections
Patients with Hb<10g/dl or platelet count <1,00,000µl/dl
Patients with keloid or hypertrophic scars.
Patients with bleeding disorders
Seropositive cases. (HIV, HCV, HBsag).

Patients presented with androgenetic alopecia both males and females, irrespective of their initial grades were included in the study. A detailed history of symptoms, duration, site, history of previous treatment, family history, history of smoking and alcohol is taken.

The pattern of hair loss and the degree of alopecia were thoroughly examined and graded according to Norwood Hamilton classification for males and Ludwig scale for females

Global photographs were taken before starting and after completion of PRP treatment. PRP injections were advised monthly once for a total of 5 sittings.

Procedure for PRP preparation

1.20 ml autologous whole blood was drawn and collected in sterile disposable test tubes 10ml in each test tube.
2. 3.8%sodium citrate(anticoagulant) was added to blood in the ratio of 9:1(9-blood,1-anticoagulant)
3.The collected mixture (blood with anticoagulant) was subjected to centrifugation (soft spin) at a speed of 1500rpm for 10 minutes.
4. After the first centrifugation 3 zones can be seen in the test tube where upper layer is platelet poor plasma (PPP), middle buffy coat containing 70% leukocytes and 90% platelets and lower zone constituting 90%Red blood cells (RBC).
5.PPP and buffy coat were collected into the second test tube.
6.The serum in the second test tube was subjected to second centrifugation (hard spin) at 3000 rpm for 10 minutes.
7. From the obtained serum upper $2/3^{rd}$ was discarded and lower $1/3^{rd}$ was PRP which was mixed thoroughly and was collected in insulin syringes for use.

Procedure

Patients were clearly explained about the procedure, area to be treated is cleaned with betadine. Prepared PRP was injected with the insulin syringes. Side effects, grading and self-assessment scoring were noted at each session.

III. Statistical Methods

Significance isassessed at 5 % level of significance.Chi-square test has been used to find the significance ofstudy parameters on categorical scale between two or moregroups. The confidence interval is calculated according to therecommended method given by Altman et al. (2000).

Statistical Software

The Statistical software namely SPSS 21.0, were used for theanalysis of the data and Microsoft word and Excel have beenused to generate graphs, tables etc.

IV. Results

Distribution of Androgenetic Alopecia based on age

Age distribution among the androgenetic alopecia patients were 7% (2) 18-20 years, 75% (21) in 21-30 years, 14% (4) in 31–40 years and 3% (1) in >40 years.

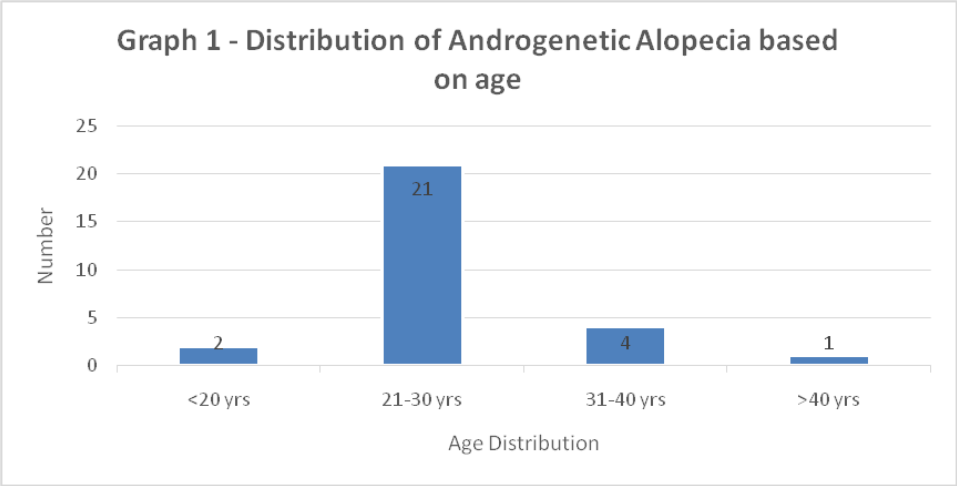


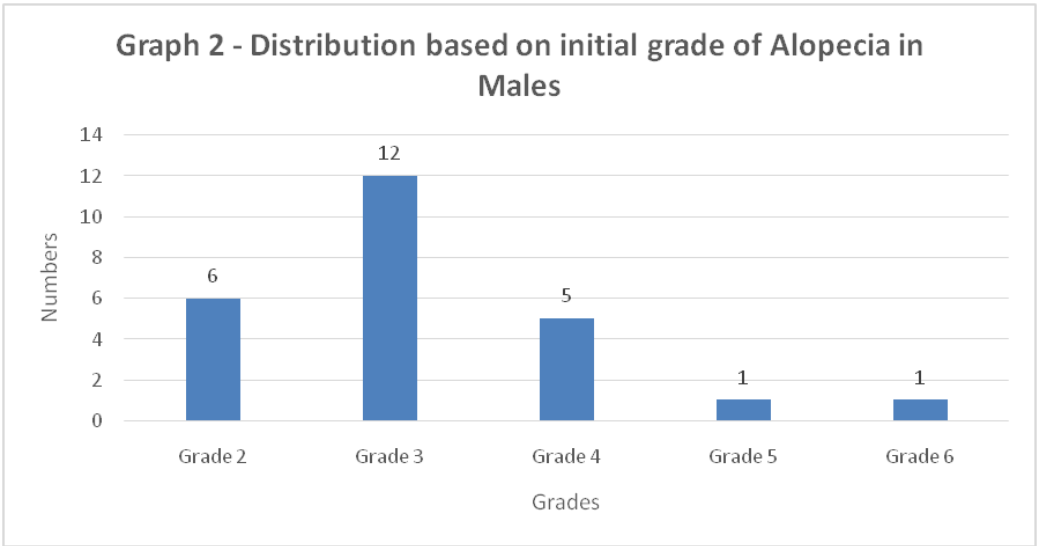
Table 1: Distribution of Androgenetic Alopecia based on age

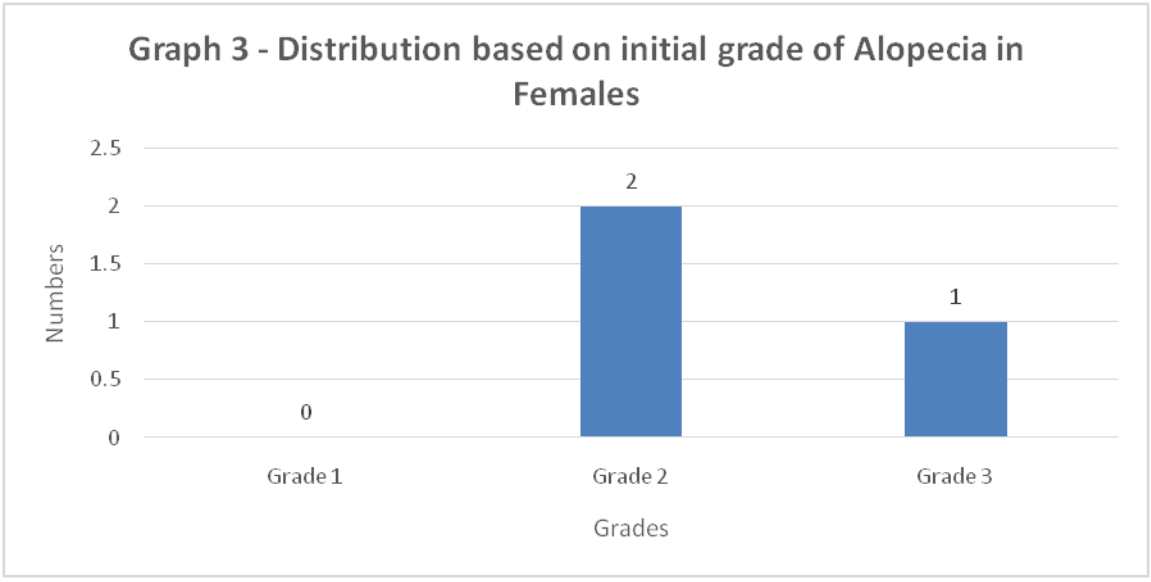
Age	Number of persons	Percentage %
<20	2	7
21-30	21	76
31-40	4	14
>40	1	3
Total	28	100

Distribution of androgenetic alopecia patients based on the initial grade of alopecia in males by Norwood Hamilton Classification and females by Ludwig Scale

Distribution of male androgenetic alopecia patients based on the initial grade of alopecia by Norwood Hamilton Classification showed 24% (6) –Grade 2, 48% (12)- Grade 3, 20% (5) – Grade 4, 4% (1) – Grade 5, 4% (1) – Grade 6.

Distribution of female androgenetic alopecia patients based on the initial grade of alopecia by Ludwig Scale showed 66.6% (2) – Grade 2 and 33.3% (1) – Grade 3.

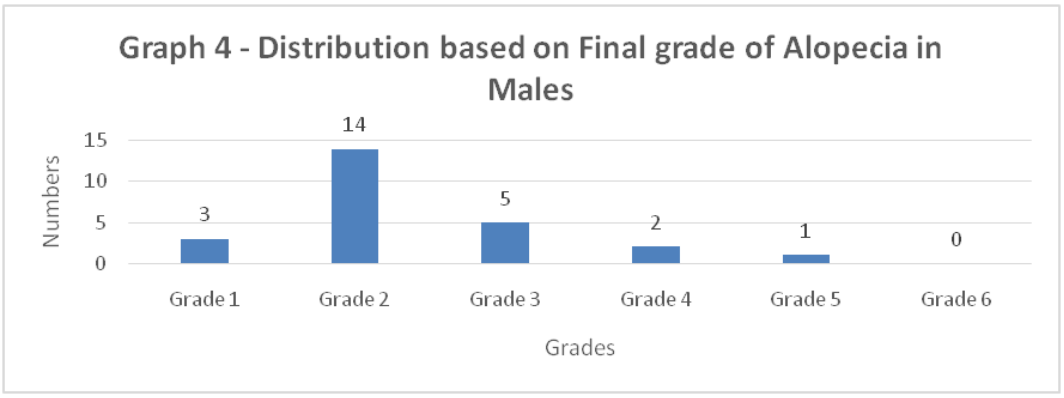




Distribution of androgenetic alopecia patients based on the Final grade of alopecia in males by Norwood Hamilton Classification and females by Ludwig Scale

Distribution of male androgenetic alopecia patients based on the final grade (outcome of PRP treatment) of alopecia by Norwood Hamilton Classification showed 50%(3) – persons of grade 2 showing improvement to grade 1 , 91.6%(11) persons of Grade 3 improved to grade 2, 80% (4) persons of grade 4 improved to grade 3, One person with grade 5 and grade 6 in initial group improved to grade 4 and grade 5 respectively.

Distribution of female androgenetic alopecia patients based on the final grade of alopecia by Ludwig Scale showed improvement of one patient with grade 2 to grade 1 (50%), one patient with grade 3 improved to grade 1 (100%).



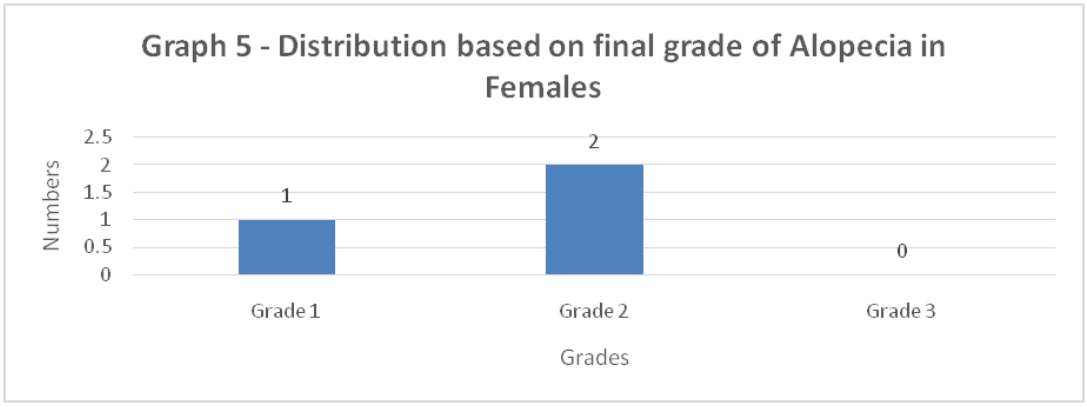


Table 2: Distribution based on age and final outcome

Age	No. of patients who showed improvement	No. of patients who did not show improvement
<20	2	0
20-30	16	5
31-40	3	1
>40	1	0

In our study among the 2 patients belonging to the age group of <20 years, 2(100%) showed improvement. Among the 21 patients belonging to the age group of 21-30 years, 16(76.1%) showed improvement and 5(23.9%) showed no improvement. Among the 4 patients belonging to the age group of 31-40 years, 3(75%) showed improvement and 1(25%%) showed no improvement. Only one patient belonged to the age group of 41-50 years and he showed improvement. This is not a statistically significant (p=0.09/>0.05)

Table 3- Distribution of androgenic alopecia patients based on occupation and final outcome.

OCCUPATION	No. of patients who showed improvement	No. of patients who did not show improvement
Doctors	10	1
Manual worker(labourer)	3	0
Student	6	3
Teacher	3	1
Driver	0	1

In our study among the 11 doctors, 10 (90.9%) showed improvement and 1(9.1%) showed no improvement. Among the student group of 9, 6(66.6%) showed improvement and 3(33.3%) showed no improvement. Among the 3 manual workers, 3(100%) showed improvement. Among the 4 teachers, 3(75%) showed improvement and 1(25%) showed no improvement. Among the 1 driver, 100% improvement was not seen. Occupation does not seem to affect the outcome. This is not a statistically significant (p=0.09/>0.05)

Graph 6- Distribution of androgenic alopecia patients based on family history and final outcome.

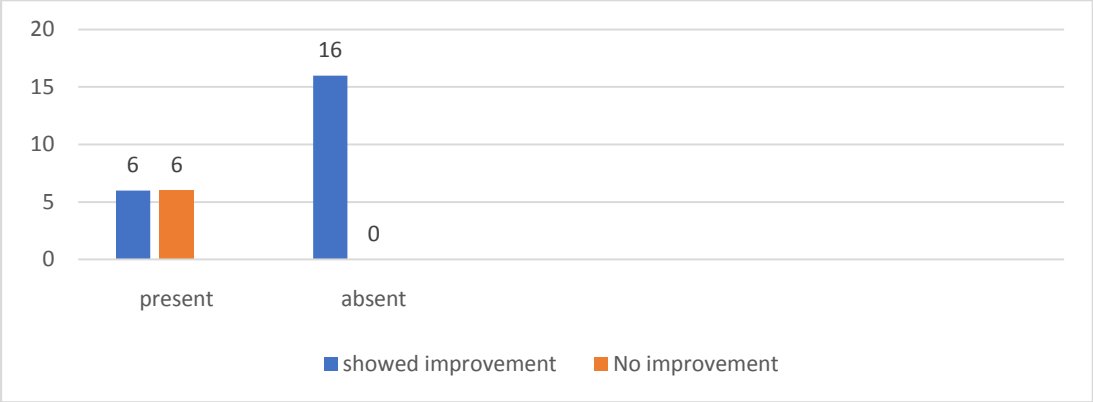


Table 4 - Distribution of androgenic alopecia patients based on family history and final outcome.

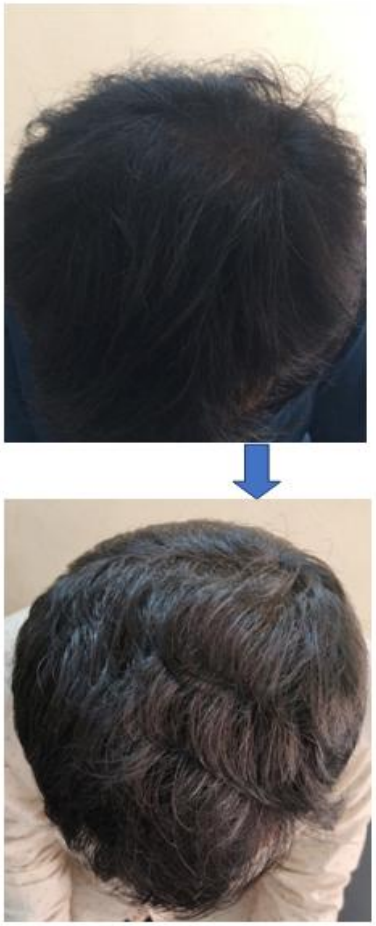
Family history	No. of patients who showed improvement	No. of patients who did not show improvement
Present	6	6
Absent	16	0

In our study among the 12 patients who had family history of alopecia, 6(50%) showed improvement and 6(50%) showed no improvement. Among the 16 patients who had no family history of alopecia, 16(100%) showed improvement. Family history of alopecia seem to affect the final outcome and the p=0.0025 which is <0.05, it is a significant.

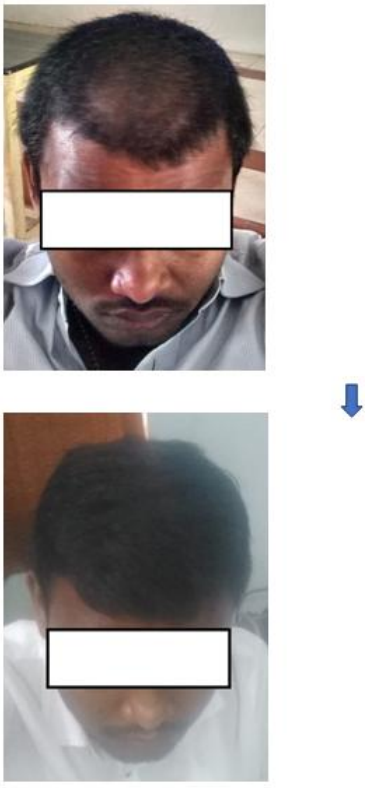
.92.9% reported complete cessation of hair fall by 3 months.

.89.3% reported increase in hair growth.

.78.6% reported increase in hair texture.



Response of Androgenic alopecia before and after 4 sessions of treatment with PRP



Response of Androgenic alopecia before and after 5 sessions of treatment with PRP

Age

In our study of 28 patients, the median age for males was 27 and females was 25. Gkini et al[4]. study included 20 patients in their study and the mean age of patients was 34 years. In Schiavone et al[5]. study total number of patients included were 64, 42 males and 22 females and the median age for males was 28 and females was 32. In our study among the 25 male patients, 20(80%) showed improvement and 5(20%) showed no improvement. Among the 3 female patients, 2(67%) showed improvement and 1(33%) showed no improvement. In our study among the 2 patients belonging to the age group of <20 years,2(100%) showed improvement. Among the 21 patients belonging to the age group of 20-30 years, 16(76%) showed improvement and 5(24%) showed no improvement. Among the 4 patients belonging to the age group of 31-40 years, 3(75%) showed improvement and 1(25%) showed no improvement. Only one patient belonged to the age group of 41-50 years and he showed improvement. In our study and Schiavone et al[5]. study, age and gender does not seem to influence the outcome. In Vasconcelos RCF et al[7]. study, 18 patients showed a clearer and moresatisfactory response in the female group (mean = 42.9%) as compared to themale group (mean = 25.6%).

Occupation

In our study among the 11 doctors, 10 (90.9%) showed improvement and 1(9.1%) showed no improvement. Among the student group of 9, 6(66.6%) showed improvement and 3(33.3%) showed no improvement. Among the 3 labourers, 3(100%) showed improvement. Among the 4 teachers, 3(75%) showed improvement and 1(25%) showed no improvement. Among the 1 driver, 100% improvement was seen. Occupation does not seem to affect the final outcome.

Family history of Androgenic alopecia

In our study among the 12 patients who had family history of androgenic alopecia, 6(50%) showed improvement and 6(50%) showed no improvement. Among the 16 patients who had no family history of alopecia, 16(100%) showed improvement. Family history of alopecia seem to adversely affect the final outcome. P value is statistically significant.

Grading

In our study, males were classified according to Norwood Hamilton classification. 50% (3) persons of grade 2 showing improvement to grade 1, 66%(8) persons of Grade 3 improved to grade 2, 80% (4) persons of grade 4 improved to grade 3, One person each with grade 5 and grade 6 improved to grade 4 and grade 5 respectively. In our study higher grades of androgenic alopecia showed better improvement in contrast to KhatuS et al[6]. study which showed improvement in lesser grade of alopecia.

Treatment protocol.

Throughout the literature different studies have used different time intervals between initial and subsequent PRP treatment sessions. In our study PRP injections were given monthly once for 5 months and at the end of 5months treatment outcome was studied. Gkini et al[4]. study the treatment protocol was to give three sessions with an interval of 21 days and a booster session at 6 months. In Schiavone et al. study two treatment plans were followed with 3 months interval. During the first session they used PRP prepared by single spin technique using the commercial device GPS III platelet separation system. To this they added plasmatic protein concentrate (platelet poor plasma filtered through mini hem concentrator). After inducing a cutaneous

inflammatory response through gentle pressure of a 1.0 mm scalp roller, the mixture was injected. During the second session after 3 months PRP was prepared by double spin centrifugation method and injected the same way after mixing it with plasma protein concentrate. In KhatuS et al[6]. study PRP was prepared by double spin technique and injected every 2 weeks for 4 sessions and evaluated after 12 weeks. In Vasconcelos RCF et al[7] study 3 treatment sessions with 21days interval was followed. In our study the PRP was prepared by double spin centrifugation method.

Subjective assessment.

In our study based on self-assessment questionnaire[10] and global photographs of 78.5% patients showed improvement. In Schiavone et al. study, improvement difference was assessed by two evaluators, which was 40.6% and 54.7% respectively, based on global photographs. In our study, the mean self-assessment satisfaction score was 9.64. Gkini et al. study also reported increase in hair density significantly and patient mean satisfaction rate was 7.1 on a scale of 1-10. Khatu S et al. showed improvement in hair volume and coverage by global pictures.

In our study patient self-assessment questionnaire, 92.9% reported complete cessation of hair fall by 3 months. 89.3% reported increase in hair growth. 78.6% reported increase in hair texturewhereas Gupta et al.

study group reported 93.3% complete cessation of hair fall by 2 months, 66.7% reported increase in hair growth,36.7% reported increase in hair texture.

Side effects

In our study pain was the most common side effect noted during the procedure with a downtime of 6 hours. No other major side effects were noted during and post procedure period. In similar to Khatu S et al. study who reported no remarkable adverse effects.

ANALYSIS OF PRP USAGE IN ANDROGENIC ALOPECIA- COMPARISON WITH OTHER STUDIES

	Our study	Gkini et al	Schiavone et al	Vasconcelos RCF et al	KhatuS et al
Place of study	Tirupati, India	Greece	Italy	Brazil	India
Year of study	November 2017-november 2018	October 2012-september 2013	2012-2013	2015	August 2013-November 2013
Number of patients	28	20	64	16	11
Method of PRP preparation	Injection in scalp	Injection in scalp	Injection in scalp	Injection in scalp	Injection in scalp
Treatment session	Monthly once for 5 months	3 sessions with an interval of 21 days and a booster session at 6 months	1st injection single spin ,2nd injection after 3 months double spin	3 injections at the interval of 21 days	4 sessions at 2 weeks interval
Alopecia grading	Male- Norwood Hamilton grading Female -ludwig scale	Male-Norwood Hamilton grading Female -Ludwig scale	Male-Norwood Hamilton grading Female -ludwig scale	Male- Norwood Hamilton grading Female -Ludwig scale	Male- Norwood Hamilton grading
Outcome	Macroscopic photograph showed overall improvement, hair loss reduced at 3 months, the mean patient self-assessment questionnaire was 9.64	Macroscopic photograph showed overall improvement, hair loss reduced at 3 months, patient satisfaction rate on linear analogue scale of 1-10 was 7.1	Macroscopic photograph jaeschke rating of clinical change by 2 evaluators were 40.6% and 54.7% respectively.	Macroscopic photographs and dermoscopy. In dermoscopy-thickening of hair and increase number of hair follicles	Macroscopic photograph showed moderate improvement in hair growth tricoscoposy follicle density increased by 15%
Adverse effects	No remarkable adverse effects	No remarkable adverse effects	No remarkable adverse effects	No remarkable adverse effects	No remarkable adverse effects

V. Conclusion

In a developing country like India, autologous PRP treatment is an affordable alternative therapy for androgenic alopecia.

There is a high level of patient satisfaction with PRP treatment, minimal side effects and the treatment is cost effective to the patient.

However, further studies on large number of patients with long term follow up are essential to exactly assess the treatment outcome

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest
No conflict of interest

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