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Platelet Rich Plasma as Monotherapy in Androgenetic Alopecia

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ABSTRACT

Introduction. We aimed to know the effect of PRP therapy in androgenetic alopecia and to determine the degree of response to platelet-Rich plasma in various grades of androgenetic alopecia.

Methods. This hospital-based pilot study was conducted in Department of Dermatology, Venereology and Leprosy Shri Guru Ram Rai Institute of Medical and Health Sciences, Patel Nagar, Dehradun during November 2014 to May 2017. Subjects with in the age 18-40 years, under Hamilton-Norwood classification 1-4 for male pattern baldness, Ludwig alopecia score 1-2 for female pattern baldness were included in the study. After taking history and general examination and routine blood tests and serology was done in all subjects. PRP was prepared by collecting 20 ml of patient's venous blood in Sodium citrate vial and centrifuged at 3000 rpm for 15 minutes to obtain plasma, buffy coat and RBC layer. The plasma and buffy coat were aspirated and again centrifuged at 2000 RPM for next 10 minutes to further obtain reduced volume of PRP by ¼ settled at the bottom**.** PRP was activated by addition of calcium gluconate by 9:1 and injected by insulin syringes two units intradermal 1 cm apart at 0.5 cm depth in alopecic area monthly for 6 months.

Results.The mean of hair pull test before study was 5.44 ± 2.75 which reduced to 0.38±0.62 after completion of 6 sessions. Further post hoc analysis showed significant improvement was noted between hair pull test results before therapy and at 3 months ($p<0.0001$) and at 6 months ($p=0.007$). The mean hair density before treatment was 96.54±24.99 which increased to 106.92±28.04/cm^2. Further post hoc analysis showed significant improvement was noted between hair density results before therapy and at 3 months ($p<0.0001$) and at 6 months ($p<0.0001$). Also, significant improvement was noted at 6 months of follow up compared to 3 months of follow up ($p<0.0001$).

Conclusion. Role of PRP in androgenetic alopecia is an emerging treatment but there is lack of data about its efficacy. We lack a comparative group with placebo or with some other treatment like PRP with minoxidil or finasteride.

1. INTRODUCTION

Androgenetic alopecia (AGA) is one of the most prevalent and most diagnosed hair loss dysfunctions characterized by follicular miniaturization in a patterned hair loss occurring due to systemic androgen and genetic factors [1,2,3,4]. The AGA incidences increase with advancing age and can affect a variety of psychological and social experiences thereby impacting quality life of patients [5,6].

Pathophysiological features of AGA include an alteration in the hair cycle via reduction of the anagen (growth) phase, inflammation, and follicular miniaturization [7]. Hormone influences and genetics are particularly the determinants for AGA. The key hormone is dihydrotestosterone (DHT), a metabolite of testosterone, which activates androgen receptors. In men, testosterone is converted to DHT by 5α-reductase, while dehydroepiandrosterone and other weaker androgens are the precursors of DHT in women. Increased density of androgen receptors is found in hair follicles in the scalp vertex and frontotemporal areas; hence, they exhibit a greater response to DHT and experience increased hair loss in AGA [8].

As part of the wound healing process, activated platelets are understood to release numerous growth factors and cytokines from their alpha granules. Platelet-rich plasma (PRP) is a treatment modality that has gained popularity for AGA due to its autologous nature, minimal invasiveness, absence of major side effects, and more affordable cost compared with hair

restoration surgery. PRP is an autologous preparation of platelets in concentrated plasma (usually > 1,000,000 platelets/μL or 2-7 times the native concentration of whole blood) [9]. Because of its autologous origin and minimally invasive collection technique, the risk of infection and immune rejection is minimized [10].

Despite the therapeutic options available, low patient compliance and satisfaction rates as well as the plethora of topical and often important systematic adverse effects has led to the search of new treatment options for AGA [11]. Hair transplant is found to give promising results but due to its high cost and invasive procedure it is not readily adopted. However, PRP therapy in hair loss is a newly emerging treatment that requires exploration, which is done and discussed in this study. The use of PRP to treat AGA is promising based on the results of the reviewed clinical studies. Safety issues, side effects, and downtime seem to be minimal. Although PRP does appear to be beneficial, the preparation, dosage, number, and interval of treatment sessions, as well as injection technique, vary between the studies due to a lack of standardization of PRP preparation. Thus, we aimed to know the effect of PRP therapy in AGA and to determine the degree of response to platelet-Rich plasma in various grades of androgenetic alopecia.

2. METHODS

This hospital-based pilot study was conducted in Department of Dermatology, Venereology and Leprosy, Shri Guru Ram Rai Institute of Medical and Health Sciences, Patel Nagar, Dehradun, during November 2014 to May 2017. Subjects with in the age 18-40 years, those good in general health, and under Hamilton-Norwood classification I-IV for male pattern baldness, Ludwig alopecia score I-II for female pattern baldness were included in the study. Exclusion criteria were hematological disorders, thyroid function, malnutrition, any kinds of infection or disorder, pregnant or breastfeeding females or those with known case of HIV, Hepatitis B,HCV or any immune compromised disease. Ethical approval was obtained from the institution and written informed consent was obtained from all the subjects in the study.

After taking history and general examination in subjects; CBC, platelet count, RBS, thyroid profile, serum iron/ferritin and BT,CT, serology was done in all included patients. PRP was prepared by collecting 20 ml of patient's venous blood in Sodium citrate vial and centrifuged at 3000 rpm for 15 minutes to obtain plasma, buffy coat and RBC layer. The plasma and buffy coat were aspirated and again centrifuged at 2000 RPM for next 10 minutes to further obtain reduced volume of PRP by ¼ settled at the bottom**.** PRP was filled in insulin syringes. After cleaning the scalp with betadine and sprit, adequate local anesthesia with 2% Lignocaine subcutaneous infiltration was given. PRP was activated by addition of calcium gluconate by 9:1. and injected by insulin syringes two units intradermal 1 cm apart at 0.5 cm depth in alopecic area. A total of six such sittings at an interval of around 30 days was given. No other therapy was given in between session. Patients were instructed not to dye or change their hair style in study period.

Subjective and objective improvement, Global photography at every session, Hair pull test before study after 3 months and at the end of 6 months, Examination and counting of hair by tricoscope with 1.3 mega pixel camera and up to 400X zoom were performed for appropriate assessment. Data was expressed as percentage and mean ± S.D.P value <0.05 was considered as statistically significant. SPSS© for windows™ Vs 17, IBM™ Corp NY and Microsoft excel™ 2007, Microsoft® Inc USA was used perform the statistical analysis.

3. RESULTS

Table 1: Baseline characteristics in study subjects

		Frequency	Percent
Age (Years)	</=20	14	9.9
	21-25	38	27.0
	26-30	25	17.7
	31-35	27	19.1
	36-40	37	26.2
Gender	Female	25	17.7
	Male	116	82.3
Family history		105	74.5
Grade of alopecia (Hemilton Norwood for Male	I	14	12
	II	35	30

	III	30	26
	IV	37	32
Grade of alopecia (Ludwig scale for female)	I	10	40
	II	15	60

Baseline characteristics in study subjects were observed and noted. 38 (27.0%) subjects were found to be 21-25 years of age. This was followed by 37 (26.2%) in 36-40 years, 27 (19.1%) in 31-35 years, 25 (17.7%) subjects in 26-30 years of age and 14 (9.9%) subjects were found to be </=20 years of age. Out of 141 subjects 116 (82.3%) subjects were males while 25 (17.7%) subjects were females. Family history of androgenetic alopecia in study subjects showed 74.5% cases had a history of androgenetic alopecia. Hemilton Norwood grading was used for males. Maximum number of subjects, i.e., 37(32%) belonged to grade IV followed by 35 (30%) in II, 30(26%) in III and 14 (12%) subjects in I. Ludwig scale grading system was followed in females and it was found that 15 (60%) subjects were in grade II while, 10(40%) subjects were in grade I (table 1).

Table 2: Comparison of Hair pull test and hair density in study subjects at different interval of treatment.

	Mean	S.D.	Minimum	Maximum	P value
Hair pull test (before therapy)	$5.44^{a,b}$	2.75	1	13	<0.0001
Hair pull test (at 3 months)	1.52^{c}	1.26	0	5	
Hairpull test (at 6 months)	0.38	0.62	0	2	
Hair density before treatment (/cm^2)	$96.54^{x,y}$	24.99	39.00	143.00	<0.0001
Hair density after 3 months follow up (/cm^2)	105.06^{z}	27.35	40.00	151.00	
Hair density after 6 months follow up (/cm^2)	106.92	28.04	32.00	150.00	

Hair pull test: a p<0.05 Vs 3 months, b p<0.05 Vs 6 months, c p<0.05 Vs 6 months

Hair density: x p<0.05 Vs 3 months follow up, b p<0.05 Vs 6 months follow up, c p<0.05 Vs 6 months follow up

The mean of hair pull test before study was 5.44 ± 2.75 which reduced to 0.38±0.62 after completion of 6 sessions. Comparison of Hair pull test in study subjects at different interval of treatment was performed using Friedman test significant difference was noted between the results of hair pull test at different interval (p<0.0001). Further post hoc analysis was performed to assess the significance of difference between results of hair pull test at different interval of therapy, significant improvement was noted between hair pull test results before therapy and at 3 months (p<0.0001) and at 6 months (p=0.007). Also, significant improvement was noted at 6 months of follow up compared to 3 months of follow up (p=0.008).

The mean hair density before treatment was 96.54±24.99 which increased to 106.92±28.04/cm^2.Comparison of hair density at different interval of therapy was performed using Friedman test significant difference was noted between the results of

hair pull test at different interval (p<0.0001). Further post hoc analysis was performed to assess the significance of difference between results of hair density test at different interval of therapy, significant improvement was noted between hair density results before therapy and at 3 months (p<0.0001) and at 6 months (p<0.0001). Also, significant improvement was noted at 6 months of follow up compared to 3 months of follow up (p<0.0001) (table 2,figure 1).

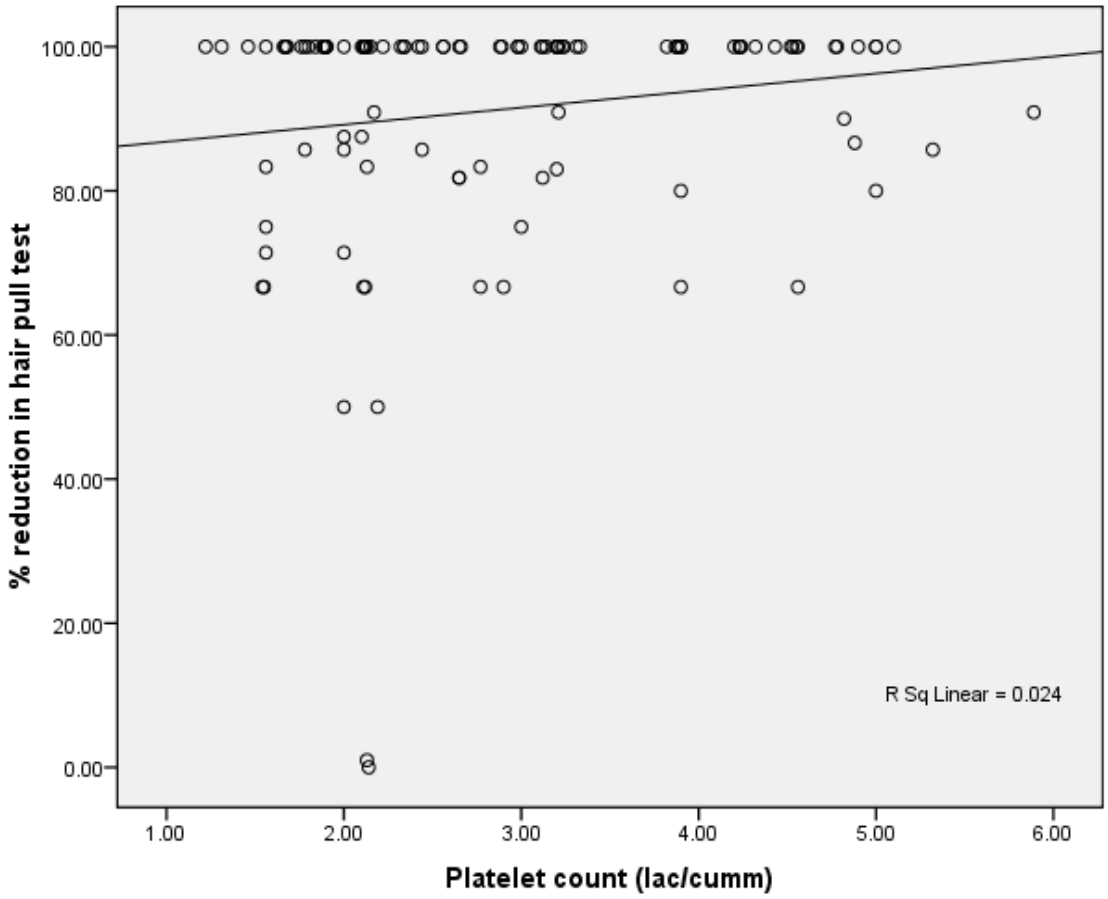


Figure 1: Correlation analysis of Platelet count with % reduction in hair pull test.

Correlation analysis was performed between platelet count and % reduction in hair pull test. A non-significant (p=0.104) weak correlation (r=0.155) was found to exist between two parameters.

Table 3: time of hair growth, global photography changes, subjective and objective improvement, pain erythema and interruption in between

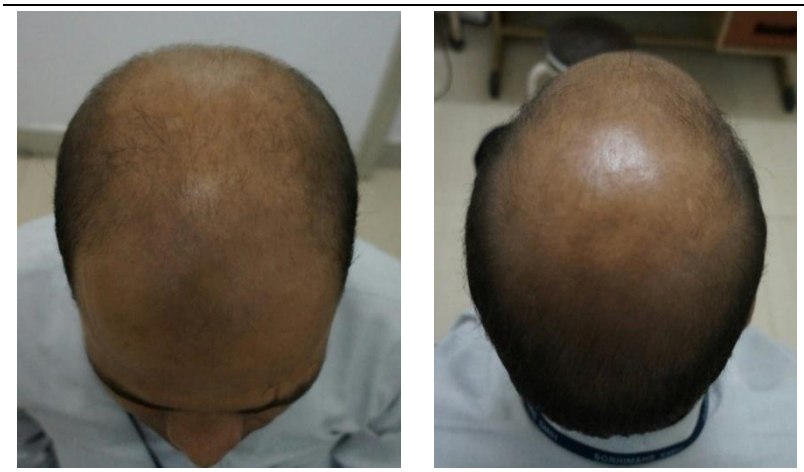
		Frequency	Percent
Time for first visible hair growth (Weeks)	Interrupted therapy	29	20.6
	</=4	44	31.2
	5-8	61	43.3
	>8	7	5.0
Improvement in global photography	Interrupted therapy	29	20.6
	Yes	83	58.9
Improvement in hair density (on observation by patients)		64	45.4

Improvement in hair density (on observation by doctor)		82	73
Persistence of pain (Days)	1	96	68.1
	2	25	17.7
	3	9	6.4
	4	5	3.5
	5	4	2.8
	6	1	.7
	7	1	.7
Persistence of erythema (Days)	1	94	66.7
	2	34	24.1
	3	8	5.7
	4	4	2.8
	5	1	.7
Reason for interruption of therapy	Pain	13	59.09
	Headache	3	13.64
	Others	6	27.27

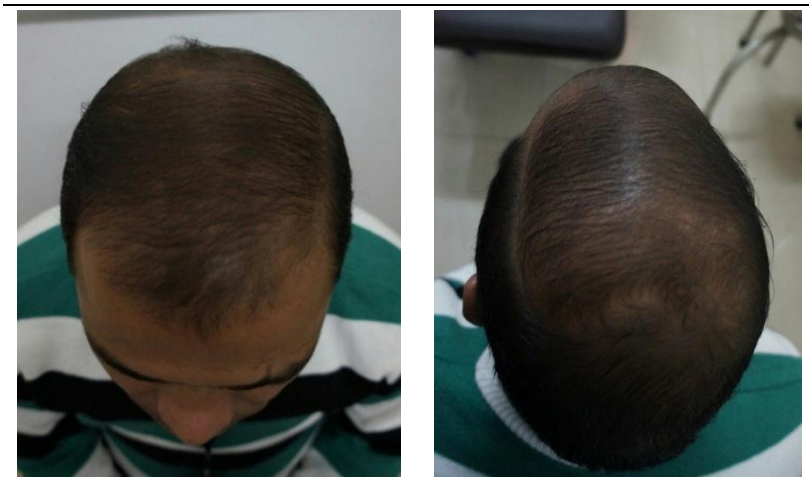
Time for first visible hair growth was observed in study subjects was observed. In 61 (43.3%) subjects it was observed to be 5-8 weeks. This was followed by 44 (31.2%) subjects in </=4 weeks, 29 (20.6%) while, >8 weeks was seen in case of 7 (5.0%) subjects. In 83 (58.9%) subjects improved in global photography was observed (figure 2). Improvement in hair density was reported by 64 (45.4%) patients while, 82 (73.3%) doctors reported the same. In maximum number of subjects i.e., 96 (68.1%) subjects pain persisted for only one day while, persistence of erythema was seen in 94 (66.7%) subjects for one day. Reasons for interruption in therapy was noted in the subjects. Pain was reported by 13 (59.09%) subjects, headache was reported by 3 (13.64%) subjects and 6 (27.27%) subjects had other reasons (table 3).

Figure 2: Images of few subjects before PRP and after 6 sessions

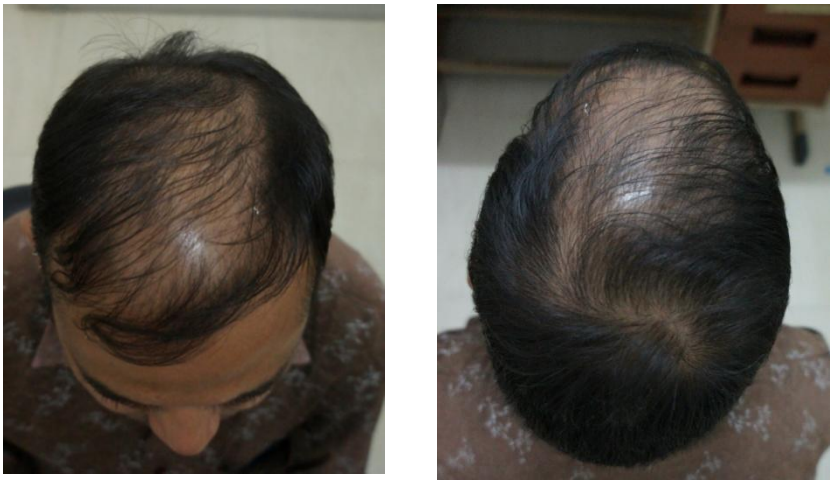
Before PRP



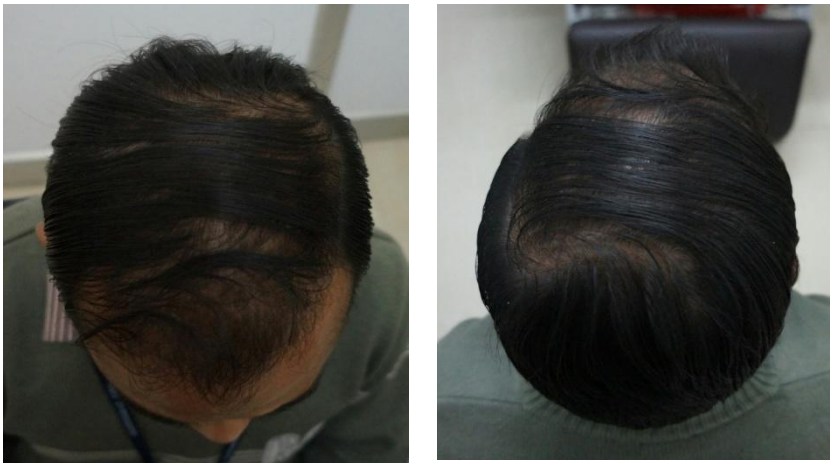
After 6 sessions



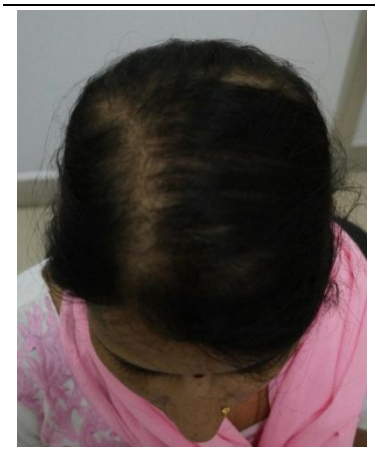
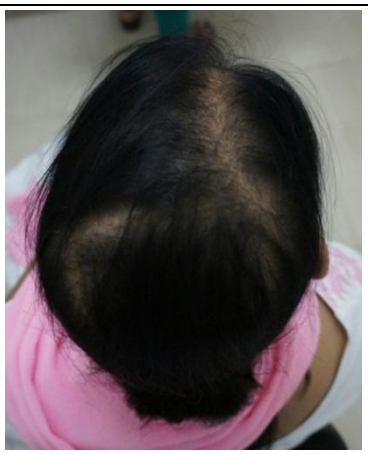
Before PRP



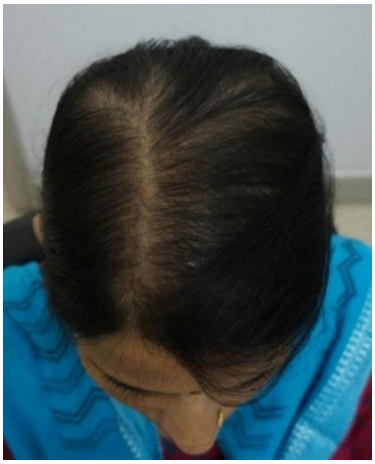
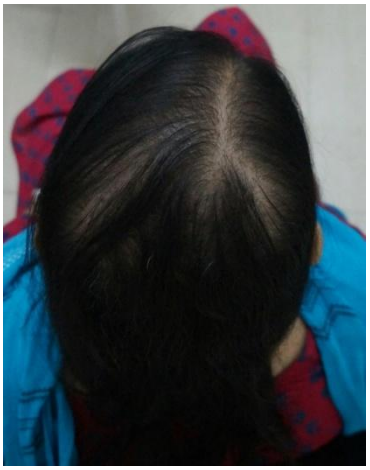
After 6 sessions



Before PRP

After 6 sessions

4. DISCUSSION

In the present study sample size was 141 patients (116 males and 25 females) of age between 18-40, and grade of alopecia was between grade 1-4 for male and grade 1-2 for females. Schiavone *et al*. studied role of PRP on a sample size of 64 patients whereas Betsiet al and Trinket al evaluate the same in 42 and 45 patients [13,14]. The age group was more or less same in all studies. In present study sample size was more than double/triple as it was conducted at tertiary care center where OPD patients are in a good number. As androgenetic alopecia is more common in males so number of male patients is more in these studies but still, we had 25 female patients which were more than enough to see the results.

In present study out of 141 cases 74.5 % cases had family history of androgenetic alopecia up to 3 generation whereas 24.5% cases are denovo in hair loss. Grover S. stated that in the Indian population had type II as the commonest presentation of AGA and according to Sehgal VN type II and III are the commonest presentation in female patient grade-II was around 60% grade-III was not taken as it is only 1% and rare [14,15]. Enough data is not available in other studies to compare the grading of female patients.

In a study conducted by Betsi et al, before treatment, 90.5 % of the patients had a positive pull test with a mean number of eight hair [13]. After the third session, the pull test was negative in all patients with an average number of three hairs. A significant decrease of hair loss (25%) was observed between the first and last injection. Gkini et al. concluded that before starting PRP session hair loss was 8 hairs in hair pull test and after the end of 6 sessions it was 6 hairs [16]. Despite the fact that it is not an objective evaluation method, it gives a satisfactory general image of hair loss.

In present study out of 141 patients 74.5 percent had a visual hair growth between 4-8 weeks after first session, 20 % patient showed hair growth in first month after a single session whereas 7% patient took more than 8 weeks.

Gkini et al. did a four-session treatment with an interval between session of 3 weeks and the last session at 6months increase in hair density was from143.10±31.07 cm^2 toa peak at 3 months (170.70 ± 37.81, $P < 0.001$) [16]. At 6 months and at 1 year, it was significantly increased, 156.25 ± 37.75 ($P < 0.001$) and 153.70 ± 39.92 ($P < 0.001$) respectively, comparing to baseline. Patients were satisfied with a mean result rating of 7.1 on a scale of 1-10. No remarkable adverse effects were noted. Khatu

et al. studied 11 patients with 4 sessions at 2-week interval moderate improvement was seen in hair volume and coverage [17]. Mean increase in hair density: 22.09 follicular units/cm^{2}was observed by Kang et al [18].

Global photography is an easy and effective tool to see the results in hair loss treatment as it shows the views which we see by naked eyes in daily life. Four views were taken –frontal, right and left partial top. Out of 141 patients 83 patients accepted and observed good improvement in global photography and 9 patients were unable to improve rest 29 patients were interrupted due to various causes. No documented data or parameter except yes/no was seen in any study of PRP.

Most common complication of PRP therapy were pain and erythema in present study. PRP therapy is a time taking treatment and we cannot say is painless,22 patient left therapy in between due to various reasons. Out of 22, 13 patient left treatments as they cannot bear pain of intralesional injection. 3 patients opted out of session as they developed headache after therapy. One patient developed tonic clonic seizures during intralesional PRP injection though he did not have any personal or family history related to seizure disorder and one patient developed serum sickness like reaction on 1st day of therapy.

Thus, we conclude that Platelet rich plasma therapy leads to significant improvement in androgenic alopecia. Improvement in alopecia associated with Platelet rich plasma therapy is further with prolonged duration (greater if therapy is continued for 6 months compared to 3 months) and this improvement is irrespective of initial platelet count or gender of subjects.

5. LIMITATIONS AND SUGGESTIONS

Role of PRP in androgenetic alopecia is an emerging treatment but there is lack of data about its efficacy. There is no documentation regarding dose and duration of PRP therapy. Neither we have a documented plan about at which dose or duration we have to give PRP therapy. We lack a comparative group with placebo or with some other treatment like PRP with minoxidil or finasteride. A better parameter of pictogram should be there so the exact number of hairs can be counted. Frontal block can also be opted for local anesthesia.

PRP therapy for androgenetic alopecia is still in experimental process with variety of results. Rather using PRP as a mono-therapy in AGA management it can be used as adjuvant with other FDA approved treatment i.e., with topical minoxidil or oral finasteride for better results.

Data Availability

Data will be deposited in a repository, or they can also be obtained from the corresponding author on request.

Conflicts of Interest

The authors declare no conflicts of interest.

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