

Comparing Single Spin versus Double Spin Techniques of Platelet Rich Plasma Preparation in Treating Male Androgenetic Alopecia: A Prospective Study

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ABSTRACT

Introduction: Platelet Rich Plasma (PRP), a source of autologous growth factors, has been in use in many fields of medicine with different spin techniques but a standardised method is yet to be established. In this study, the authors have tried to compare the results of single spin vs double spin technique of PRP preparation on treating Androgenetic Alopecia (AGA).

Aim: To compare the clinical outcome obtained with single spin versus double spin technique of PRP preparation in treating male AGA.

Materials and Methods: The present prospective study was conducted in the department of Dermatology at Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India, with a total of 28 participants who were divided into two groups of 14 each and treated with PRP prepared either by single spin or double spin method and observed for a period of six months. This study was started in September 2022 and completed in April 2024. Males aged 18 years and above, with Grade III and Grade IV AGA as per the Hamilton-Norwood Scale were included in the study. They were regularly recalled and evaluated for hair count and also with the Merck Research Laboratories questionnaire.

In addition, demographic details such as age, duration of onset of AGA were also collected. Chi-square test and One-way Anova test were used for categorical and continuous variables respectively.

Results: While both methods of PRP preparations showed significant improvement in hair count post PRP administration, there was no statistical difference between the two groups (p -value=0.98). Patient satisfaction increased in both groups post treatment. Patients belonging to the age group of 20-24 years had a better response when compared to those belonging to the age group of 25-29 years. Patients with duration of AGA less than one year showed marked improvement in hair counts when compared with other groups.

Conclusion: The present study concludes that both single spin and double spin techniques of PRP preparation are effective in AGA and as such, PRP is a good therapeutic option for the treatment of AGA irrespective of the method of preparation since both the methods showed significant improvement in hair growth. Single spin method is an easier, less time consuming and also a cheaper alternative to the commercial gel tubes and hence can be considered as a standard method.

Keywords: Growth factors, Male pattern baldness, Spin techniques

INTRODUCTION

The AGA is a genetic disorder characterised by gradual thinning of the scalp hair due to an excessive androgen response. It affects around fifty percent of males and twenty-five percent of females. In males, hair loss is prominent in the vertex and frontotemporal regions, while females retain their frontal hairline but experience diffuse thinning on the top of the scalp [1]. By the time they are 70-year-old, fifty to sixty percent of men have progressive hair loss that results in bald spots on their scalps [1]. AGA is thought to be caused by both environmental and genetic causes. It is known to cause increased perifollicular 5- α reductase. In androgen-dependent regions of the scalp, testosterone is converted by 5- α reductase to dihydrotestosterone, which binds to androgen receptors and activates genes that cause healthy terminal follicles to become vellus-like hairs.

Recently, there has been an increase in the usage of PRP for the treatment of AGA. PRP is an autologous preparation of platelets in concentrated plasma, often with a concentration of $>1,000,000$ platelets/ μ L or two to seven times that of whole blood. The danger of infection and immunological rejection is reduced because of its autologous origin and minimally intrusive collecting method [2].

The single spin suspension and buffy coat PRP systems that were accessible in 2006 served as the foundation for Mishra's

classification system [3]. According to Mautner K et al., the double spin process was created to manufacture platelets with little to no red blood cells and neutrophils and a concentration that is greater than five times that of whole-blood platelets [4,5]. Commercially accessible systems were grouped into four basic families: Pure PRP (P-PRP), Leukocyte and PRP (L-PRP), P-PRF (Pure Platelet-Rich Fibrin), and L-PRF (Leukocyte and Platelet-Rich Fibrin). Preparations without leukocytes and with a low-density fibrin network following activation are referred to as pure P-PRP or leukocyte-poor PRP; preparations including leukocytes and a low-density fibrin network following activation are referred to as L-PRP; Leukocyte-poor platelet-rich fibrin, or P-PRF, refers to preparations that have a high-density fibrin network but no leukocytes; L-PRF products, on the other hand, have both a high-density fibrin network and leukocytes [6].

Growth factors are extracted from a platelet concentration by centrifugation and used to make PRP [7]. PRP's healing properties stem from the abundance of growth factors secreted by platelets, including VEGF, TGF- β 1, Transforming Growth Factor beta-1 (TGF- β 1), Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor AB (PDGF-AB), and many more, which work in concert with a variety of cytokines. These elements support tissue repair through angiogenesis, migration, and proliferation of cells, and they are

essential in the early stages of wound healing. Furthermore, PRP helps in haemostasis and quicker regeneration of bone [8].

For the preparation of PRP, Kramer ME and Keaney TC recommended a mean spin rate of 2,480 rpm and a mean spin time of twelve minutes [9]. Cervantes J et al., proposed a double spin centrifugation method, with the first spin occurring at 1,500-1,700 rpm for six to ten minutes and the second spin occurring at 2,500 rpm for ten to fifteen minutes [10]. Favourable results were also found in numerous investigations that used a single spin method.

Contrasting results were obtained in various studies regarding the efficacy of single spin and double spin technique in treatment of AGA. Using a single spin centrifugation approach, a study by Gkini MA et al., found that at three months, hair density rose and hair loss significantly decreased without any notable serious negative effects [11]. However, Kurita M et al., discovered that the double spin approach is recommended for PRP production because it produces larger thrombocyte concentrations than single spin centrifugation [12]. Single spin centrifugation is not advised for the preparation of PRP; rather, it should only be used for diagnostic purposes. Using the double spin centrifugation method, Jo CH et al., study found that after the second centrifugation, the thrombocyte count increased more than the thrombocyte concentration following the first centrifugation [13].

The present study was designed to evaluate the clinical outcome obtained with single spin technique versus double spin technique of PRP preparation in treating male AGA in a metropolitan city in South India. There is a strong need for this study as double spin technique is more time consuming and expensive compared to the single spin technique of PRP preparation. In addition, this study also brings about the fact that single spin is effective in hair growth and that it could be a preferred alternative to the newer commercially available tubes. The primary objective was to compare the clinical outcome obtained with single spin versus double spin technique of PRP preparation in treating male AGA. The secondary objective was to find out if the single spin and double spin techniques of PRP preparation were association with any adverse effects.

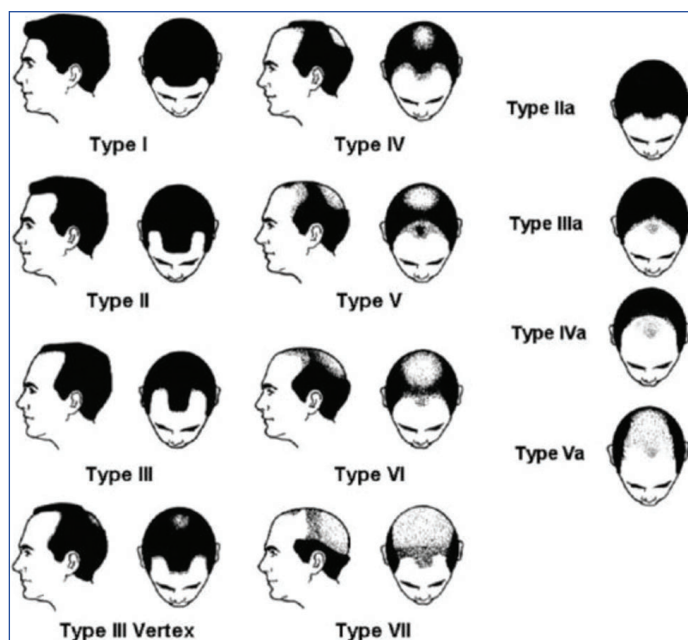
MATERIALS AND METHODS

The present prospective study was conducted in the Skin Outpatient Department at Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India, from September 2022 to April 2024, after obtaining ethical approval from the Institutional Human Ethical Committee (Ref. No. 002/ IHEC/2022/1780, Date: 22/09/2022) and informed consent from the patients.

Inclusion and Exclusion criteria: A total of 28 male patients above 18 years, who had Grade III and IV AGA based on the Hamilton-Norwood scale were included in the study and divided into two groups of 14 each [Table/Fig-1] [14]. It was a convenience sampling where 30 patients were included, but two patients were lost to follow-up. A brief history was recorded after which the treatment procedure was explained to the subject and informed consent was obtained for the same. Subjects with any other kind of alopecia, a history of keloid or blood coagulation abnormalities, and use of Non-steroidal Anti-Inflammatory Drugs (NSAIDs) within seven days of the start of the trial were excluded from the study. Those undergoing treatments involving growth factors, such PRP and microneedling, performed within the six months before the trial were likewise disqualified.

Study Procedure

Around 9 mL of venous blood were drawn and placed into a conical bottom plastic tube with 1 mL of 3.2% sodium citrate as an anticoagulant to create PRP. The single spin centrifugation was



[Table/Fig-1]: Hamilton-Norwood Scale [14].

performed at 3,500 rpm for ten minutes and PRP was obtained. The PRP that was obtained via the double spin centrifugation method was prepared by first spin of blood centrifugation at 750 rpm for ten minutes after which, a two-cc syringe was used with caution to aspirate the upper PRP layer, the aspirated PRP layers were centrifuged a second time in a plain tube at 3,000 rpm for ten minutes [5]. A one-cc insulin syringe was then used to aspirate the obtained PRP and injected intradermally over scalp. The methodology has been explained in detail in [Table/Fig-2].

Parameters	Single spin technique	Double spin technique
Blood collection	9 mL of venous blood was collected and placed in a conical bottom plastic tube with 1 mL of 3.2% sodium citrate (anticoagulant)	
Spin details	Single spin at 3500 rpm (revolutions per minute) for 10 minutes.	1 st Spin: 750 rpm for 10 minutes upper platelet rich layer along with buffy coat is transferred to a 2 nd plain tube using a 2 mL syringe. 2 nd Spin: 3000rpm for 10 minutes.
Total time taken	10 minutes	20 minutes
Pros	Less time consuming less equipment cheaper	Reduced risk of contamination from white and red blood cells
Cons	Higher risk of contamination from white and red blood cells	More time consuming more equipment expensive compared to single spin

[Table/Fig-2]: Methodology of single spin and double spin techniques of PRP preparation.

The objective response to treatment was carried out using dermoscopy. A metric tape measure, graded in centimetres, was used to locate and mark one square cm area over a standard location over scalp at about 15 centimetres from the glabella [Table/Fig-3]. The hair over this area was trimmed, the image was then amplified using a dermoscope and was transferred to the microsoft program (paint version) and was further amplified to permit manual counting.

The subjective response to treatment was carried out using Merck Research Laboratories questionnaire [Table/Fig-4] at day 60 and day 180 [15]. Patients assessed their scalp hair by choosing the answer for these questions on treatment efficacy and satisfaction with appearance and scoring was done accordingly. Response to treatment is reflected by a fall in the scoring from baseline as per the Merck research Laboratories questionnaire in [Table/Fig-4].

Each patient underwent six sittings of PRP, one month apart, with a final follow-up four weeks after the 6th sitting. The objective



[Table/Fig-3]: Site where dermoscopy with manual hair count was done. a) A metric tape measure, graded in centimetres, was used to locate and mark one square centimetre area over a standard location over scalp at about 15 centimetres from the glabella; b) The white square indicates the area which was trimmed and subjected to a manual hair count via a dermoscope.

assessment using the dermoscope with manual counting of hair per sq.cm and the subjective assessment using the Merck Research Laboratories Questionnaire which is prevalidated and involves the patient answering certain questions pertaining to treatment and hair growth. The responses have certain fixed scores which are added to get a total score as seen in [Table/Fig-4]. The questionnaire scoring was done at third visit (day 60) and four weeks after the sixth sitting of PRP (day 180) [15].

Check the appropriate boxes below. Please check only one box for each question.

1. Since the start of the study, I can see my bald spot getting smaller

Strongly agree	☐ 1	Disagree	☐ 4
Agree	☐ 2	Strongly disagree	☐ 5
No opinion either way	☐ 3		

2. Because of the treatment I have received since the start of the study, the appearance of my hair is:

A lot better	☐ 1	A little worse	☐ 5
Somewhat better	☐ 2	Somewhat worse	☐ 6
A little better	☐ 3	A lot worse	☐ 7
Same	☐ 4		

3. Since the start of the study, how would you describe the growth of your hair?

Greatly increased	☐ 1	Slightly decreased	☐ 5
Moderately increased	☐ 2	Moderately decreased	☐ 6
Slightly increased	☐ 3	Greatly decreased	☐ 7
No change	☐ 4		

4. Since the start of the study, how effective do you think the treatment has been in slowing down your hair loss?

Very effective	☐ 1	Not very effective	☐ 3
Somewhat effective	☐ 2	Not effective at all	☐ 4

5. Compared to the beginning of the study, which statement best describes your satisfaction with the appearance of:

(Check the best response)

	I am very satisfied	I am satisfied	I am neutral (neither satisfied nor dissatisfied)	I am dissatisfied	I am very dissatisfied
a) the hairline at the front of your head?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
b) the hair on top of your head?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5
c) your hair overall?	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5

[Table/Fig-4]: Merck research laboratories Scoring [15].

STATISTICAL ANALYSIS

Data was collected in MS Excel and represented in the form of tables and graphs wherever necessary. Data was analysed using statistical software Statistical Package for Social Sciences (SPSS) version 20.0. Association between the variables such as age, duration of the disease, grade of AGA, family history of AGA were calculated using Chi-square test and One-way Anova test for categorical and continuous variables respectively, with a level of significance of 0.05.

RESULTS

Majority of the participants in both the groups were between 21-25 years with 6 (42.9%) in the single spin group and 8 (57.1%) in the double spin group. A 10 (71.4%) subjects in both the groups experienced onset of AGA between the ages of 21-25 years. The mean age of AGA onset in case of double spin (25.8±3.1) was lower than that of single spin (26.2±2.1). A 5 (35.7%) patients in single spin group had a family history of AGA out of which 2 (14.3%) patients

each reported a maternal and paternal history. In the double spin group, 12 (85.7%) patients had a family history of AGA out of which 7 (50%) reported a paternal history. Grade III AGA was commoner than Grade IV AGA, 9 (64.3%) patients in the single spin technique and 10 (71.4%) patients in double spin technique. Of the nine patients with Grade IV AGA, there were 5 (35.7%) patients in the single spin technique and 4 (28.6%) in the double spin technique [Table/Fig-5].

Grades	Single spin n (%)	Double spin n (%)	p-value	Chi-square value
Grade III	9 (64.3)	10 (71.4)	0.68	0.167
Grade IV	5 (35.7)	4 (28.6)		
Total	14 (100)	14 (100)		

[Table/Fig-5]: Distribution of patients based on Grading of AGA.

The hair count increased from day 0 to day 180 in both single as well as double spin method. The hair count for single spin and double spin method at baseline was 178.1±21.2 and 183.9±24.5, respectively. Similarly, it was 198.1±20.7 and 198.2±25.5, respectively for single and double spin method at day 180 [Table/Fig-6]. Though the scoring at day 60 was lower in the single spin group, at day 180 it was similar in both groups (9.3±1.6). There was no significant statistical difference between both groups at the end of treatment [Table/Fig-6].

Hair count and scoring	Single spin mean (SD)	Double spin mean (SD)	p-value
Hair count at day 0/Sq.cm	178.1 (21.2)	183.9 (24.5)	0.51
Hair count at day 60/Sq.cm	189.1 (24.6)	185.4 (25.9)	0.69
Hair count at day 180/Sq.cm	198.1 (20.7)	198.2 (25.5)	0.98
Scoring at day 60	12.7 (1.8)	13.0 (1.4)	0.64
Scoring at day 180	9.3 (1.6)	9.3 (1.6)	0.71

[Table/Fig-6]: Comparison of method of spin with hair count per one square centimetre of scalp (/Sq.cm) and Merck Research Laboratories scoring.

While the hair count in all the age groups increased on day 180 when compared to day 0, the maximum growth was seen in the 21-25 year age group (203.5±20.1) and the least was seen in the 31-35 year age group (180±43.8). At day 180, the scoring was highest for the 31-35 year age group (10±1.4) followed by the 26-30 year group (9.9±1.2) indicating that patients in the age group of 21-25 years showed better improvement with the lowest scores [Table/Fig-7].

	21 to 25 years (n=14) Mean (SD)	26 to 30 years (n=12) Mean (SD)	31 to 35 years (n=2) Mean (SD)	p-value
Hair count				
Hair count at day 0/ Sq.cm	180.7 (25.4)	182.6 (11.1)	173.0 (63.6)	0.86
Hair count at day 60/ Sq.cm	193.1 (28.7)	184.1 (14.4)	165.5 (46.0)	0.29
Hair count at day 180/ Sq.cm	203.5 (20.1)	194.9 (22.6)	180.0 (43.8)	0.33
Merck research laboratories questionnaire scoring				
Scoring at day 60	12.4 (1.7)	13.4 (1.4)	12.5 (0.7)	0.28
Scoring at day 180	8.9 (1.7)	9.9 (1.2)	10.0 (1.4)	0.17

[Table/Fig-7]: Comparison of age with the hair count and Merck Research Laboratories Questionnaire Scoring.

But based on the age of onset, patients who developed AGA between the age of 20-24 years had a higher hair count at day 0, day 60 and on day 180 (182.3±21.7, 192.2±24.1 and 204.2±19.8) when compared to those who developed it during the age of 25-29 years (177.6±26.3, 175±23.1 and 182.8±23.6).

Based on the duration of onset, the hair count at day 0 was highest in the <1 year group (187.4 ± 20.0) and least in the four to five years group (167.6 ± 37.2). Similarly, the hair count at day 180 was highest in the less than one year group (198.4 ± 28.7) and least in the four to five years group (197.4 ± 33.6). When comparing the duration of onset and scoring, it was seen that scoring at day 60 was almost similar in all three groups, however the scoring at day 180, it was highest in the four to five years group (9.6 ± 1.7) and lowest in the less than one year group (9.4 ± 2.3) indicating a better response in the latter group. There was no significant statistical difference between both groups at the end of treatment [Table/Fig-8].

Hair count	<1 year (n=5) Mean (SD)	2 to 3 years (n=18) Mean (SD)	4 to 5 years (n=5) Mean (SD)	p-value
Hair count at day 0/ Sq.cm	187.4 (20.0)	182.9 (18.2)	167.6 (37.2)	0.33
Hair count at day 60/ Sq.cm	184.4 (40.1)	190.0 (19.9)	180.2 (27.5)	0.72
Hair count at day 180/ Sq.cm	198.4 (28.7)	198.3 (19.1)	197.4 (33.6)	0.99
Merck research laboratories questionnaire scoring				
Scoring at day 60	12.8 (1.9)	12.9 (1.7)	12.8 (1.3)	0.99
Scoring at day 180	9.4 (2.3)	9.3 (1.3)	9.6 (1.7)	0.94

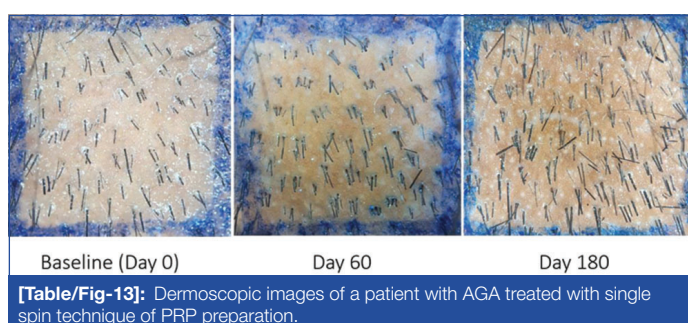
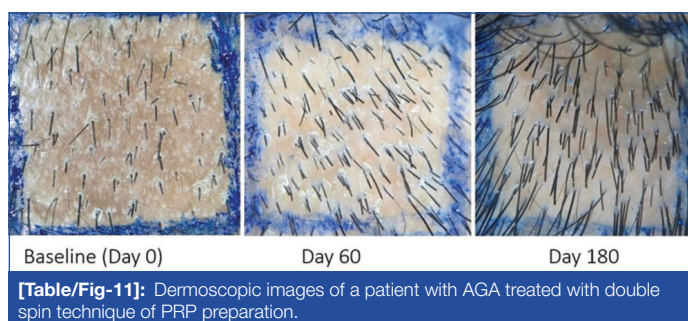
[Table/Fig-8]: Comparison of duration of the disease with the hair count and Merck Research Laboratories Questionnaire Scoring.

No significant difference was observed between family history of AGA with hair count and scoring. Although both the groups showed an increase in hair count from day 0 to day 180, those without a family history had a greater increase compared to those who had.

In terms of grading of AGA, when comparing hair count at all visits, irrespective of the technique used, the hair count was more in Grade III when compared to Grade IV. In case of Grade III, the hair count on day 180 in case of single spin technique was 201.6 ± 23.1 which was slightly less compared to that of double spin technique (205.2 ± 21.8). However, in case of Grade IV, the hair count at day 180 for single spin technique (191.8 ± 15.6) was higher compared to that of double spin technique (180.7 ± 28.6). While there was a fall in scoring in both grades by both techniques, the fall in scoring was slightly higher in case of Grade IV single spin technique as compared to grade double spin technique [Table/Fig-9]. There were no adverse effects noted or reported by the patient to either single spin or double spin techniques of PRP preparation. The outcome of both single spin and double spin techniques of PRP preparation have been depicted clinically and through the dermoscope in [Table/Fig-10-13].

Spin technique	Single spin			Double spin		
Hair count	Grade III Mean (SD)	Grade IV Mean (SD)	p-value	Grade III Mean (SD)	Grade IV Mean (SD)	p-value
Hair count at day 0/Sq.cm	177.8 (25.2)	178.4 (13.9)	0.96	190.5 (20.7)	167.2 (28.4)	0.11
Hair count at day 60/Sq.cm	195.8 (25.3)	177.2 (19.8)	0.18	193.5 (22.7)	165.0 (23.8)	0.06
Hair count at day 180/Sq.cm	201.6 (23.1)	191.8 (15.6)	0.42	205.2 (21.8)	180.7 (28.6)	0.1
Merck research laboratories questionnaire scoring						
Scoring at day 60	12.4 (2.1)	13.2 (1.0)	0.47	12.7 (1.3)	13.7 (1.5)	0.22
Scoring at day 180	9.2 (1.9)	9.4 (1.1)	0.85	9.2 (1.0)	10.2 (2.2)	0.23

[Table/Fig-9]: Comparison of Grade of AGA with hair count and Merck Research Laboratories Questionnaire scoring.



DISCUSSION

Male pattern baldness lowers one's self-esteem and can cause melancholy, depression, tension, and a reduced quality of life in addition to its effects on appearance. In younger individuals with more advanced disease, these issues are more noticeable. The development of an effective therapeutic approach is therefore imperative. Many oral and topical drugs have been developed to treat this illness.

The mean age of the subjects in case of double spin (25.8 ± 3.0) in this study was lower than the single spin (26.1 ± 2.0). Legiawati L et al., reported almost similar mean age for both the groups in their study [5]. In a study by Gupta AK and Carviel JL the mean age of the study participants was observed to be 25.4 ± 3.5 years [16].

According to Hamilton JB, 30 to 50% of men develop AGA by the time they are in the fifth decade [17].

In the present study, a comparison was done between the single and double spin method of PRP preparation and its effectiveness in hair growth in AGA patients. The hair count increased from day 0 to day 180 in both single as well as double spin method. This could be attributed to the fact that platelets produced by single spinning centrifugation can reach three times the baseline level, while platelets produced by double spinning centrifugation can reach eight times the baseline level [18]. Although similar results were obtained in a study by Legiawati L et al., subjects in the single spin group showed better results compared to double spin group [5].

Subjects belonging to the age group of 20-24 years had a higher hair count on all the visits when compared to those belonging to the age group of 25-29 years. When comparing the duration of onset of alopecia with the hair count, it was observed that the hair count at day 0 as well as that at day 180 was highest in the less than one year group and least in the four to five years group [Table/Fig-4]. A study by Gupta AK and Carviel JL showed a higher increase in hair density in the individuals with onset of AGA within five years possibly indicating that a decreasing response to treatment as the duration of AGA increases [16].

According to a comprehensive review by Picard F et al., PRP injections appear to be useful in helping AGA patients restore hair that has been lost, reduce hair loss, and increase hair thickness [19]. Growth factors from PRP have been shown by Rinaldi F et al., to be able to delay the catagen and telogen phases, lengthen the anagen phase, and inhibit dermal papilla death [20]. These results are corroborated by histological evidence [21]. James R et al., study revealed that patients showed clinical improvement in hair counts, hair thickness, hair root strength, and overall alopecia three months following PRP treatment [22]. In a study, Garg S et al., found that at six months, all participants in the PRP group had more than 75% hair regrowth [23]. According to Singhal P et al., the patients showed clinical improvement in terms of hair counts, hair thickness, hair root strength, and overall alopecia three months following the autologous PRP treatment [24]. All the above studies validate the findings of the present study. The authors observed that single spin and double spin PRP preparation techniques for AGA therapy were equally effective in improving clinical AGA. Both techniques had a beneficial effect on AGA and increased hair count.

There was improvement of patient satisfaction across both the groups as evident by the scoring in the questionnaire. Patient's innate expectations for treatment results and the qualitative characteristics of their scalp hair naturally influence their self-assessments of hair growth, appearance, and happiness with appearance. A more comprehensive picture of the effectiveness of therapy can be obtained by combining information about the amount of scalp hair growth with patient self-perception of that growth and the level of patient satisfaction with hair appearance. A higher hair count does not always indicate the patient's satisfaction with the amount of hair growth attained, how thick or dense the hair is, or whether the rise has reached cosmetic importance. This validated male hair growth Merck Research Laboratories questionnaire can be used to augment clinical measures of hair growth in clinical studies by giving patients a quick and easy way to express their perceptions of their hair growth and level of satisfaction with their look. Single spin method, an easier and less time-consuming technique can be considered as a standard method of PRP preparation in day-to-day practice. In addition, single spin technique is a cheaper alternative to the commercial gel tubes.

Limitation(s)

A bigger cohort research is required to validate the findings of this study.

CONCLUSION(S)

While, there was a notable increase in overall hair growth in response to PRP therapy, there was no significant difference in hair growth between single spin and double spin techniques indicating that PRP was beneficial in this trial, irrespective of the method of spin. Single spin method, an easier and less time-consuming technique can be considered as a standard method of PRP preparation in day-to-day practice. PRP has become an attractive therapeutic option for AGA because it is autologous, has minimal side effects, and is less expensive than hair restoration surgery.

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