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Efficacy of Platelet-Rich Plasma Injection for The Treatment of Male Androgenetic Alopecia – A Systematic Review and Meta-Analysis of Changes in Hair Density

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ABSTRACT

Background: Androgenetic alopecia (AGA) is the most common form of hair loss in men, wherein systemic androgen disorders and genetic factors lead to progressive miniaturization of hair follicles due to changes in the duration of hair growth cycle, characterized by shorter anagen phase and longer telogen phase, resulting in smaller, thinner, and less pigmented terminal hair. The main goal of treatment in AGA is to prevent the miniaturization process. The therapeutic options commonly used in the treatment of male AGA, such as topical minoxidil and oral finasteride, have yet to produce satisfactory results. Currently, many new therapeutic modalities are being developed for AGA, one of which is platelet-rich plasma (PRP). Multiple growth factors secreted by PRP are thought to support hair growth by prolonging the anagen phase, preventing apoptosis, delaying induction of the catagen phase, triggering angiogenesis and increasing follicular vascularization.

Methods: A systematic literature search was performed using PubMed-MEDLINE, Cochrane library, Embase, AMED, Clinicaltrials.gov, dan WHO ICTRP with the search terms “platelet rich plasma” and (“male androgenetic alopecia” or “male pattern hair loss”). Eight RCT were included in the qualitative synthesis and seven of them were included in the meta-analysis.

Results: The overall SMD in hair density was -0.889 (95% CI: -1.630 – -0.147, $p<0.05$) in favor of PRP treatment, indicating that PRP has significantly greater efficacy over placebo treatments in improving hair density among male patients with AGA.

Conclusion: Platelet-rich plasma injection is effective in improving hair density. The addition of PRP might be considered to help improve treatment outcomes.

Keywords: platelet-rich plasma, male androgenetic alopecia, hair density

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Introduction

Androgenetic alopecia (AGA) is the most common form of hair loss in men, characterized by miniaturization of hair follicles due to systemic androgen and genetic factors.[1] Systemic androgen disorders will cause progressive miniaturization of hair follicles due to changes in the duration of hair growth cycle, characterized by shorter anagen phase and longer telogen phase, resulting in smaller, thinner, and less pigmented terminal hair. [2-5]

The main goal of treatment in AGA is to prevent the miniaturization process. Treatment options should be evaluated in terms of efficacy, practicality, risk, and cost. Treatment options that are currently approved by the Food and Drug Administration (FDA) for the treatment of male patients with AGA are limited to topical minoxidil and oral finasteride. These two therapeutic options commonly used in the treatment of male AGA have yet to produce satisfactory results, and they were also known to have several side effects such as hypertrichosis, leg edema, decreased libido, erectile dysfunction, risk of prostate cancer and depression, especially in long term use, so that patients may hesitate to continue using them. Consequently, many new therapeutic modalities are being developed for AGA, one of which is platelet-rich plasma (PRP). [3,6]

Platelet-rich plasma (PRP) is an autologous concentrate of human platelets produced by centrifugation of the patient's own venous blood and administered by intradermal injection. Platelet-rich plasma contains a number of major growth factors secreted by platelets, particularly platelet-derived growth factor (PDGF), transforming growth factors (TGF) TGFβ-1 and TGFβ-2, vascular endothelial growth factor (VEGF), basic fibroblastic growth factor, endothelial growth factor and insulin-like growth factor. These cytokines are involved in cell proliferation and stimulated hair growth through upregulation of fibroblastic growth factor expression, β-catenin expression, extracellular signal-regulated kinase (ERK), and protein kinase B (PKB) signaling. VEGF-mediated angiogenesis can control hair growth and follicle size. Platelet-rich plasma also acts on dermal papilla cells by increasing the expression of β-catenin, which induces follicular stem cell differentiation, fibroblast growth factor 7 (FGF-7) which prolongs the anagen phase, and B-cell lymphoma protein (Bcl-2) which protects cells from apoptosis. [4,7-9]

Several randomized controlled trials (RCT) had evaluated the use of PRP injection for hair loss treatment in male patients with AGA, but the size of these studies were small, thus insufficiently powered to fully assess the efficacy of this modality. The objective of this literature review and meta-analysis was to evaluate the efficacy of PRP injection in improving hair density of patients with male androgenetic alopecia based on literatures that has been published regarding this topic.

Material and Methods

Literature Search

A systematic literature search was performed using PubMed-MEDLINE, Cochrane library, Embase, AMED, Clinicaltrials.gov, dan WHO ICTRP with the search terms “platelet rich plasma” and (“male androgenetic alopecia” or “male pattern hair loss”).

Study Selection

Included studies were placebo-controlled randomized trial that evaluated the efficacy of PRP injection in male patients ≥18 years old that was diagnosed with AGA (Norwood-Hamilton I-VII), and had reported changes in hair density (hairs/cm^2) after at least 3 months of follow up period. Studies were excluded if they were not published in English or had subjects with coagulation disorders, immunosuppression, malignancy, history of keloids, local infections, and subjects that were taking anticoagulant or were previously receiving several other treatments for AGA including minoxidil, finasteride, and laser.

Data Analysis

Statistical analysis was performed using Comprehensive Meta-Analysis software Version 3.0. Differences in continuous variables were expressed as standardized mean difference (SMD) with 95% CI. Heterogeneity was assessed by using I2 statistic, which describes the percentage of total variation across studies. Meta-analysis with random effect model was performed because I2 was greater than 50% (not homogenous). A *P* value of <0.05 was considered statistically significant.

Results

The literature search was conducted based on the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) flowchart (Figure 1). [10]

Eight research articles that met the criteria were included in qualitative data analysis (systematic review) and 7 of them were used in quantitative data analysis (meta-analysis) to determine the efficacy of PRP injection in improving hair density in men suffering from AGA when compared with placebo.

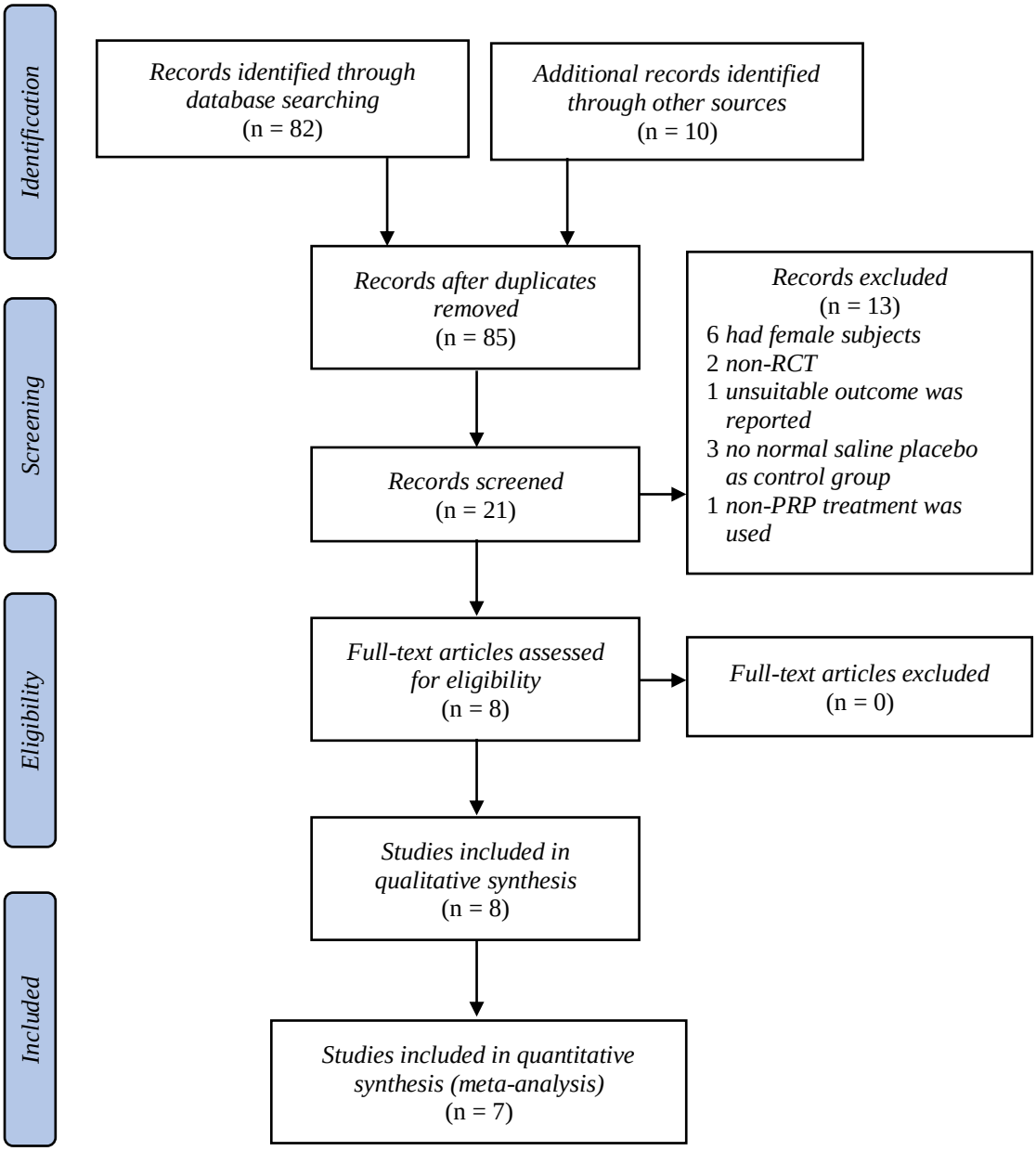

Figure 1. Flowchart of identification and selection of research literature in systematic review and meta-analysis.

Table 1. Characteristics of Included Studies

No	Researcher, year, and country of origin	Study design	Final sample size	Treatment Protocol		Duration
				Treatment group	Control group	
1.	Cervelli, 2014, in Italy[11]	Half-head, placebo controlled RCT	10	Manual injection of AA-PRP (0.1 mL/cm^2), 3 sessions, 1 month interval.	Physiological solution injection as placebo.	3 months
2.	Gentile, 2015, in Italy[12]	Half-head, placebo controlled RCT	20	Manual PRP injection on treatment area (0.1 mL/cm^2) using Luer lock 1 ml syringe (30G needle), 3 sessions, 1 month interval.	Physiological solution injection as placebo.	3 months
3.	Gentile, 2017, in Italy[13]	Half-head, placebo controlled RCT	18	Interfolicular A-PRP injection (0.2 mL/cm^2, 5 mm deep), using Luer lock 10 ml syringe (30G needle) mounted on Ultim Gun, 3 sessions, 1 month interval.	Physiological saline solution injection as placebo.	3 months
4.	Rodrigues, 2018, in Brazil[14]	Double-blind, placebo controlled RCT	Treatment: 15 Control: 11	Subcutaneous injection of 100 µl PRP (20 times up to a total of 2 ml) using 1 ml syringe (32G needle), 4 sessions, 15 days interval.	Physiological saline injection as placebo.	2 months
5.	Dicle, 2019, in Turkey[15]	Prospective, randomized, placebo controlled, crossover study	Treatment: 10 Control: 15	Manual PRP injection using Luer lock 1 ml syringe (30G needle, 3 mm deep), without local anesthesia, in linear pattern 5 mm apart, up to a total of 5 cc, 3 sessions, 1 month interval.	NaCl 0.9% injection as placebo.	3 months
6.	Singh, 2019, in India[16]	Double-blind, placebo controlled RCT	Treatment: 19 Control: 19	Manual PRP injection (0.05-0.1 mL/cm^2) using Beckton Dickinson 1 ml syringe, 3 sessions, 1 month interval.	Physiological saline injection as placebo.	3 months
7.	Gressenberger, 2020, in Austria[17]	Placebo controlled RCT	Treatment: 19 Control: 9	Manual PRP injection (0.1 ml) using 30G needle, 1 cm intervals in a grid-like pattern, up to a total of 3-4 ml, 5 sessions, 4-6 weeks interval.	Physiological saline injection as placebo.	20-30 weeks
8.	Zhou, 2020, in China[18]	Placebo controlled RCT	10	Manual subcutaneous PRP injection (0.1 mL/cm^2), 1.5-2.5 mm deep, 3 sessions, 1 month interval.	Physiological saline injection as placebo.	3 months

Study Characteristics

The studies included in the analysis were randomized controlled trials published between 2014–2020, of which 3 studies (37.5%) were conducted in Italy, while the rest were each conducted in Brazil, Turkey, India, Austria, and China, respectively. The number of patients who were used as subjects in each of these studies seemed to vary between 10–38 patients. Six studies evaluated outcomes using TrichoScan (75%), while one used dermoscopy (12.5%), and one used the DinoLite system (12.5%).

All studies provided PRP injection for the treatment group and physiological saline injection as placebo for the control group. Four studies (50%) injected half-head with PRP and the other half-head of the same patients with placebo, while the remaining 4 studies (50%) used two different groups of patients. Six studies (75%) used the single spin PRP method and the remaining 2 (25%) used the double spin method. Seven studies (87.5%) used PRP preparation that had been activated using different type of activators, while one study used an inactivated autologous PRP preparation (12.5%). Six studies (75%) gave 3 PRP injections at 1 month intervals, while one study (12.5%) gave 4 PRP injections at 15 day intervals, and one study (12.5%) gave 5 PRP injections at 4-6 weeks intervals. Characteristics of included studies are summarized in Table 1.

Result of Qualitative Data Analysis

1. Cervelli et al, 2014[11]

A randomized, placebo-controlled trial in 10 male patients with male pattern hair loss (MPHL) types IIa, III, III vertex, and IV according to the Norwood-Hamilton classification. The study subjects were between 22-60 years old and had

not been receiving any other treatment (topical or systemic) during the last 12 months. AA-PRP preparations were made using the Cascade-Selphyl-Esforax system by centri-fugation at 1100 G for 10 minutes, and then activated with Ca^{2+}. Evaluation of treatment results was carried out by blinded independent evaluators without knowing the study group assignment using photo-trichogram and TrichoScan.

The treatment area showed a significantly greater increase in mean hair density compared with the control area ($p < 0.0001$) after receiving treatment for 3 months, wherein the treatment area showed an average increase of 27.7 hairs/cm^2 (from 103.6 ± 30.9 to 121.6 ± 34.1 hairs/cm^2) while the control area showed an average decrease of 3.0 hairs/cm^2 (from 111.3 ± 28.9 to 109.3 ± 28.2 hairs/cm^2). Based on the results obtained, the authors conclude that AA-PRP injection has a positive therapeutic effect in MPHL cases without causing significant side effects.

2. Gentile et al, 2015[12]

A randomized, placebo-controlled trial in 20 male patients with MPHL types IIa, III, III vertex, and IV according to the Norwood-Hamilton classification. The study subjects were between 19-63 years old and had not been receiving any other treatment (topical or systemic) during the last 12 months. PRP preparations were made using the Cascade-Selphyl-Esforax system with centrifugation at 1100 G for 10 minutes, and then activated with Ca^{2+}. Evaluation of treatment results was carried out by blinded independent evaluators without knowing the study group assignment using phototrichogram and TrichoScan.

The treatment area showed a significantly greater increase in mean hair density compared with the control area after 3 months ($p < 0.0001$), wherein the treatment area showed an average increase of 45.9 hairs/cm^2 (from 161.2 ± 41.9 to 207.1 ± 56.1 hair/cm^2), while the control area showed an average decrease in hair density of 3.8 hairs/cm^2 (from 166.5 ± 45.6 to 170.3 ± 42.1 hair/cm^2). The recurrence of androgenetic alopecia were evaluated in all patients within 12 months after the last treatment session, wherein 4 patients reported a progressive hair loss that seemed to get worse at 16 months after the last treatment and then underwent re-treatment. The results obtained in the study showed a positive effect of PRP injection in MPHL cases without causing significant side effects, so it was concluded that PRP could be a safe and effective alternative treatment option for hair loss.

3. Gentile et al, 2017[13]

A randomized, placebo-controlled trial in 18 male patients with MPHL types II-IV according to the Norwood-Hamilton classification. The study subjects were between 19-63 years old and had not been receiving any other treatment (topical or systemic) during the last 12 months. A-PRP preparations were made using the CpunT Preparation System with centri-fugation at 1200 rpm for 10 minutes, and then the PRP was injected without being activated. Evaluation of treatment results was carried out by blinded independent evaluators without knowing the study group assignment using phototrichogram and TrichoScan.

Evaluation that was carried out within 12 weeks after treatment found a statistically significant increase in mean total hair density at the treatment area (an average of 65 ± 5 hairs/cm^2 increase [31 ± 2%], from 218 ± 17 to 282 ± 20 hairs/cm^2), while the placebo area only showed an insignificant increase (an average of 1.9 ± 2 hair/cm^2 increase [1%], from 225 ± 15 to 227 ± 16 hair/cm^2). Based on these results, the authors concluded that injection treatment using A-PRP that was prepared using CPunT Preparation System can be considered as an alternative treatment option for male androgenetic alopecia.

4. Rodrigues et al, 2018[14]

A randomized double-blind, placebo-controlled trial was conducted between

August 2014 and October 2016. The study was conducted on 26 male patients between the age of 18-50 years who had suffered from type III-vertex AGA according to the Norwood-Hamilton classification for more than 2 years. A-PRP preparations were made using the single spin method with centrifugation at 1258 G for 15 minutes, and then activated using autologous serum. Evaluation of treatment results was carried out using TrichoScan.

The PRP treatment group were found to have a statistically significant increase in hair density within 3 months after the last treatment (p = 0.012), while the control group did not show a significant change in hair density (p = 0.206). The researcher concluded that the result of their study supported the use of PRP as an alternative treatment option for male androgenetic alopecia.

5. Dicle et al, 2019[15]

A prospective, randomized, placebo-controlled trial with a crossover study design in 30 male patients with type III, III-vertex, IV and V AGA according to the Norwood-Hamilton classification. The study subjects were between 21-48 years old and had not been receiving any other treatment (topical or systemic) during the last 6 months. PRP preparations were made using the Genesis PRP Kit by centrifugation at 1700 G for 10 minutes, then physically activated with a bio-activator tube. Evaluation of the treatment results was carried out by two blinded independent evaluators without knowing the study group assignment using TrichoScan. Five patients in the treatment group were not included in the final analysis because they dropped out after randomization and the first treatment session.

There was no significant change in the mean hair density between baseline and after receiving treatment for 3 months, neither in the group receiving PRP injection (275.4 ± 54.5 vs 277.7 ± 47.5 hairs/cm^2, p = 0.703) nor the placebo control group (251.8 ± 39.0 vs 247.5 ± 45.4 hairs/ cm^2, p = 0.999). Overall, patients with milder alopecia reported a greater increase in hair density, so the results obtained in this study may be influenced by the severity of alopecia, especially since the proportion of patients with mild alopecia in the control group appears to be larger than those in the treatment group (53.3% vs 30%). This study only reported mild side effects such as temporary pain and edema at the injection site, so it was concluded that PRP therapy was relatively safe to use, although it had not been proven to be effective.

6. Singh et al, 2020[16]

A randomized double-blind, placebo-controlled trial conducted between February 2017 and February 2018. The study recruited 40 male patients between the age of 18-60 years with male type baldness type II-V according to the Norwood-Hamilton classification who had not been receiving any other treatment (topical or systemic) for the past 6 months. PRP preparations were made using the double spin method, with the first centrifugation at 2200 rpm for 12 minutes and the second centrifugation at 3000 rpm for 6 minutes. The preparation was then activated using 10% calcium gluconate. Both study groups were also given a topical placebo containing a mixture of 58 mL of normal saline and 2 mL of spirit applied to dry scalp twice daily during the study period. Evaluation of treatment results was carried out by blinded independent evaluators without knowing the study group assignment using dermoscopic images on an area of 1 cm^2 located at 10 cm above and 3 cm in front of the upper border of the right tragus. One patient in the treatment group and one patient in the control group were excluded because they refused to continue treatment, so that only 19 patients in each group were included in the final data analysis.

The treatment group showed a significant increase in hair density after receiving treatment for 3 months (93.75 ± 32.49 [baseline] vs 138.75 ± 31.70 hairs/cm^2,

p<0.001). The greatest increase in hair density appeared to be obtained at 4 month follow up which was 55.46%, in comparison with 49.97% at 3 month follow up and 54.91% at 5 month follow up. Meanwhile, the placebo group showed no statistically significant change in hair density after receiving treatment for 3 months (90.05 ± 39.02 [baseline] vs 89.45 ± 37.92 hairs/cm^2, *p* = 0.337), as well as at 4 and 5 months follow-up (*p* = 0.468 and *p* = 0.411, respectively). Overall, the study concluded that PRP treatment had been proven to be more effective than placebo without causing significant side effects, so the results of the study were considered to support the use of PRP as a safe and effective therapeutic method for male pattern baldness.

7. Gressenberger et al, 2020[17]

A randomized, placebo-controlled randomized trial was conducted from 2016 to 2019. The study recruited 30 male patients between the age of 18-52 years with AGA type III according to the Norwood-Hamilton classification who had never been treated before. PRP preparations were made using the Yes PRP Kit (single spin procedure) by centrifugation at 2800 rpm for 9 minutes. Evaluation of treatment results was carried out by blinded independent evaluators without knowing the study group assignment using TrichoScan. One patient from the treatment group and 1 patient from the control group had dropped out and were not included in the final analysis.

The median change in hair density at 4 weeks after the last treatment when compared to baseline value was -6.5 (-38.0 – 4.0) hair/cm^2 in the treatment group and -9.0 (-15.0 – 2.0) hair/cm^2 in the placebo group. The difference in median hair density changes between treatment and placebo groups at 4 weeks after the last treatment did not appear to be statistically significant (*p* = 0.817). The second follow-up conducted at 6 months after the last treatment also did not find any significant changes, wherein the median change in hair density was -9.0 (-27.0 – 8.0) hair/cm^2 in the treatment group and -12.0 (-30.0 – 3.0) hairs/cm^2 in the placebo group (*p* = 0.366). Based on the results obtained, the researchers concluded that the use of PRP injection as monotherapy could not improve hair growth in men with AGA.

8. Zhou et al, 2020[18]

A randomized, placebo-controlled trial in 10 male patients with type IIa-V AGA according to the Norwood-Hamilton classification. The study subjects were between the age of 18-65 years and had not been receiving any other treatment (topical or systemic) during the last 6 months. PRP preparations were made using the double spin method with the first centrifugation at 200 G for 15 minutes and the second centrifugation at 1200 G for 15 minutes. The preparation was then activated with 10% calcium chloride. Evaluation of treatment results was carried out using digital photographs which were analyzed using the DinoLite system.

The PRP injection side showed a significant increase in mean hair density at 3 month follow up (145.20 ± 26.76 [baseline] vs 151.80 ± 27.81 hairs/cm^2, *p* = 0.031) and 4 month follow up (154.20 ± 23.75 hairs/cm^2, *p* = 0.048), but there was no statistically significant increase at 6 month follow up (*p* = 0.066) and 9 month follow up (*p* = 0.253). However, the overall mean hair density seemed to reach the highest peak at 6 month follow up (157 ± 17.33 hairs/cm^2, with an average increase of 6 ± 17 hairs/cm^2), and the effect of PRP injection started to decrease after 9 month (152 ± 27.06 hair/cm^2). Meanwhile, the control side showed a non-significant decrease in mean hair density at 3 month follow up (150.30 ± 26.68 [baseline] vs 144.30 ± 30.16 hair/cm^2, *p* = 0.137) and 6 month follow up (142.50 ± 16.20 hairs/cm^2, *p* = 0.171), while there was a significant decrease at 4 month follow up (128.80 ± 24.52 hair/cm^2, *p* = 0.003) and 9 month follow up

(median 125.80 [min-max, 117.25–138.00] hairs/cm^2, p = 0.007).

There was no significant difference in mean hair density changes between the treatment and control groups at 3 month (p = 0.570), 6 month (p = 0.078) and 9 month

follow up (p = 0.075), but there was a statistically significant difference at 4 month follow up (p = 0.030). Based on the results of the clinical efficacy evaluation, the researchers concluded that PRP injection could be beneficial in increasing hair density, so it can be considered as an additional treatment option for male patients with AGA who cannot use traditional treatment options.

Quantitative Data Result (Meta-Analysis)

The difference in mean hair density between baseline and after final treatment are presented in Table 2, and the result of meta-analysis evaluating the efficacy of PRP injection treatment compared with placebo in improving hair density in male patients with androgenetic alopecia are presented in Figure 2.

Overall test of heterogeneity obtained an I2 value that was greater than 50%, thus indicating that the data was non-homogeneous, and a random-effect meta-analysis model was utilized. Analysis of the mean change in hair density per cm^2 from 7 studies involving a total of 150 patients obtained a pooled SMD value of -0.889 with a 95% CI between -1.630 to -0.147 (p < 0.05). This overall results indicated that PRP injection treatment was significantly better than placebo in improving hair density in male patients with androgenetic alopecia.

Table 2. The difference in mean hair density (number of hairs/cm^2) between baseline and after treatment in the PRP treatment and placebo control group.

No	Study	Treatment	Control	Treatment		Control	
				Mean ± SD	n	Mean ± SD	n
1.	Cervelli, 2014	AA-PRP injection	NS injection	27.7 ± 5.6	10	15.9 ± 4.1	10
2.	Dicle, 2018	PRP injection	NS injection	9.3 ± 10.0	10	17.4 ± 8.7	15
3.	Gentile, 2015	PRP injection	NS injection	45.9 ± 15.8	20	3.8 ± 7.1	20
4.	Gentile, 2017	A-PRP injection	NS injection	64.0 ± 3.9	18	2.0 ± 2.4	18
5.	Zhou, 2020	PRP injection	NS injection	11.3 ± 3.9	10	-7.8 ± 5.3	10
6.	Singh, 2019	PRP injection	NS injection	49.5 ± 4.6	19	-1.1 ± 5.6	19
7.	Gressenberger, 2020	PRP injection	NS injection	-3.3 ± 4.2*	19	-8.0 ± 8.2*	9

PRP, platelet-rich plasma; NS, normal saline; SD, standard deviation. *The original data was presented as median (min – max), it was then converted to mean ± SB using the conversion method by Wan et al.[19], the difference in means was calculated based on the converted data.

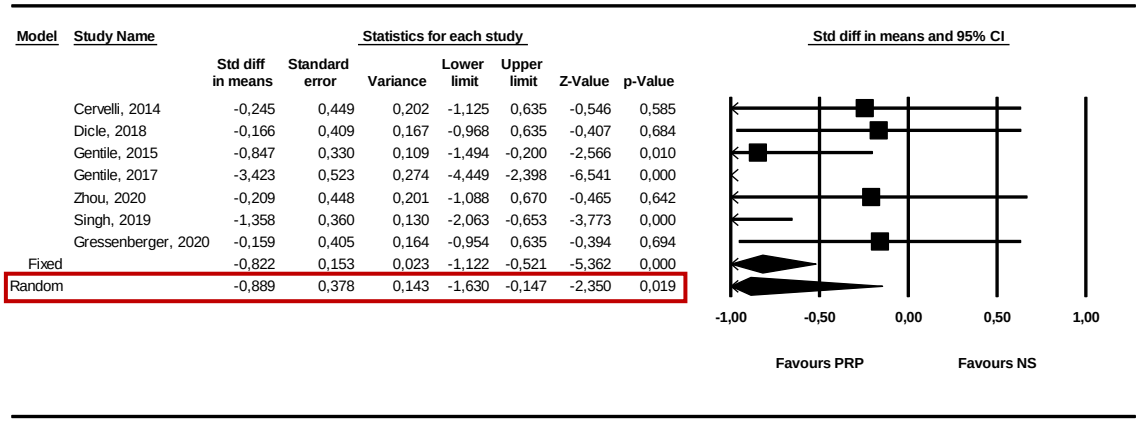

Figure 2. Forest plot showing the efficacy of PRP injection treatment compared with placebo from all RCT that were evaluated in the meta-analysis. 95% CI, 95% Confidence Interval; NS, normal saline.

Risk of Bias in Included Studies

The quality of evidence was assessed using the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) system. [20] Randomized controlled trial began as high-quality evidence, but it had been rated down due to the risk of serious bias, in which two studies did not describe the method used for randomization, three studies did not describe the method used for masking of group allocation, one study did not explain whether blinding was used in the outcome assessment, and one study showed a significant drop out (>20%) in the PRP injection treatment group. Overall, the result of meta-analysis evaluating the efficacy of PRP injection treatment compared with placebo in improving hair density was considered to be of moderate-quality (⊕⊕⊕⊖).

Moderate-quality evidence indicated that there was a moderate level of confidence in the estimated effect size from the meta-analysis, where the actual effect size was likely to be close to the estimated value, although there was a possibility that the actual effect size would be substantially different. Further studies are likely to have an important impact on the estimated effect size and the level of confidence in the estimated effect size. [20]

Discussion

This was a meta-analytic observational study, systematic review and meta-analysis evaluating the efficacy of PRP injection in improving hair density in male patients with androgenetic alopecia. Eight studies were included in the qualitative review (systematic review) and 7 of them could be reviewed quantitatively (meta-analysis) to determine the effect size of PRP injection in improving hair density in male patients with androgenetic alopecia. Study by Rodrigues et al. (2018) could not be included in the meta-analysis because the data required in the analysis was not described in the manuscript.

The studies that were included in both the systematic reviews and meta-analyses had evaluated subjects with different age range. The subjects were between the age of 22-60 years in the study by Cervelli et al, 21-48 years in the study by Dicle et al, 19-63 years in the study by Gentile et al, 18-50 years in the study by Rodrigues et al, 18-60 years in the study by Singh et al, 18-52 years in the study by Gressenberger et al, and 18-65 years in the study by Zhou et al. The stages of MPHL according to the Norwood-Hamilton classification also varied between II-VII. European-American men in the United States was reported to have a prevalence of 12% for frontal hair loss type A variant and 16% for Type III MPHL between the age of 18-29 years, which would be increasing progressively to 53% by the age of 40-49 years. The type of MPHL that was most often reported in Norwegian men between the age of 20-50 years were type I (31%), type II (26%) and type V (20%). Wang et al. reported that Caucasian men was reported to have a prevalence of 2.8% for MPHL at the age of 18–29 years, 13.3% at 30-39 years, 21.4% at 40–49 years, 31.9% at 50– 59 years, 36.2% at 60-69 years and 41.4% at 70 years and over. The incidence increases gradually with age and occurs after puberty. [2,21,22]

The evaluated studies also used different PRP preparation methods. Six studies used single spin method, including Cerveli et al. who performed centri-fugation at 1100 g for 10 minutes with Ca^{2+} as activator, Gentile et al. in 2015 who performed centrifugation at 1100 g for 10 minutes with Ca^{2+} as activator, Gentile et al. in 2017 who performed centrifugation at 1200 rpm for 10 minutes without activator, Rodrigues et al. who performed centrifugation at 1258 g for 15 minutes with autologous serum as activator, Dicle et al. who performed centrifugation at 1700 g for 5 minutes with physical activation using a bio activator tube, and Gressenberger et al. who performed centrifugation at 2800 rpm for 9 minutes, but had not reported whether or not an activator was utilized. Meanwhile, two studies used double spin method, including Singh et al. who

performed the first centrifugation at 2200 rpm for 12 minutes and the second centrifugation at 3000 rpm for 6 with 10% calcium gluconate as activator, and Zhou et al. who performed the first centrifugation at 200 g for 15 minutes and the second centrifugation at 1200 g for 15 minutes with 10% calcium chloride as activator.

Single spin method will theoretically resulted in lower platelet concentration in comparison with the double spin method. However, studies have reported that the single spin method was effective in producing adequate platelet concentra-tions for PRP treatment. Platelet con-centration was proportionally related to the centrifugal force and TGF-β1 concen-tration will increase with centrifugal force above 800 × g. Centrifugal force (g) can be calculated by the formula RCF= (RPM)2 × 1.118 × 10-5 × radius (cm). The activator that is often used is calcium gluconate or calcium chloride. Once activated, PRP must be used immediately to maintain its viability. If PRP was injected immediately after activation, there shoul be no difference in results between the use of activated and non-activated preparations. [23,24]

Patients in the PRP treatment group were expected to have greater improvement in hair density compared with placebo. Six studies gave 3 PRP injection with an interval of 1 month, and they all reported significant increase in hair density at 3 month follow up. Cervelli et al. reported a significantly greater increase in mean hair density in comparison with control (p < 0.0001), wherein the treatment area showed an average increase of 27.7 hairs/cm^2 (from 103.6 ± 30.9 to 121.6 ± 34.1 hairs/cm^2). Gentile et al. in 2015 reported an average increase of 45.9 hairs/cm^2 in the treatment area (from 161.2 ± 41.9 to 207.1 ± 56.1 hair/cm^2, p < 0.0001), while in 2017 Gentile et al. also reported a statistically significant increase in mean total hair density at the treatment area (an average of 65 ± 5 hairs/cm^2 increase [31±2%], from 218 ± 17 to 282 ± 20 hairs/cm^2). Rodrigues et al. reported a statistically significant increase in hair density in the treatment group (p = 0.012). Singh et al. reported a significant increase in hair density in the treatment group (93.75 ± 32.49 [baseline] vs 138.75 ± 31.70 hairs/cm^2, p<0.001). Last of all, Zhou et al. reported a significant increase in mean hair density at 3 month follow up (145.20 ± 26.76 [baseline] vs 151.80 ± 27.81 hairs/cm^2, p = 0.031). The decision to evaluate hair growth after 3 months could be influenced by the fact that the telogen phase of the hair growth cycle in the hair follicle usually lasts for about 3 months, while the dermal papilla remains in a resting phase. At the end of this stage, the hair will fall out (exogenous phase) a few weeks later, and the hair follicle re-enters the anagen phase by stimulating stem cells from the bulb area. Therefore, hair growth evaluation after 3 months of treatment was expected to produce a valid data that can accurately describe the efficacy of PRP treatment in improving hair density. [5,25]

Among all studies included in the analysis, two reported further follow up results beyond the first 3 months. Singh et al. reported the results from 4 and 5 month after PRP injection, wherein the greatest increase in hair density appeared to be obtained at 4 month follow up which was 55.46%, in comparison with 49.97% at 3 month follow up and 54.91% at 5 month follow up. This might be caused by PRP retention inside the hair follicles. Zhou et al. also reported the results from 4, 6, and 9 month follow up, wherein hair density only showed a statistically significant increase at 4 month, but not on 6 and 9 month follow up. The effect of PRP injection was reported to start decreasing after 9 month. These findings was consistent with the statements from Picard et al. regarding the appropriate protocol for PRP treatment, namely an initial treatment with 3 injection sessions at 1 month intervals, followed by maintenance treatments every 3 months. [26]

Dicle et al. found no significant changes in the mean hair density between baseline and after receiving treatment for 3 months, neither in the group receiving PRP injection (275.4 ± 54.5 vs 277.7 ± 47.5 hairs/cm^2, p = 0.703) nor the placebo control group (251.8 ± 39.0 vs 247.5 ± 45.4 hairs/cm^2, p = 0.999). In this study, patients with milder alopecia reported a greater increase in hair density. This finding might be caused by the fact that the hair loss in milder form of alopecia according to Norwood-Hamilton is usually limited to the frontal and temporal hairlines, in accordance with the study by Poonkiat et al. that reported highest hair density at the frontal and lowest hair density at the temporoparietal area. [27,28]

Gressenberger et al. reported that the median change in hair density at 4 weeks after the last treatment when compared to baseline value was -6.5 (-38.0 – 4.0) hair/cm^2 in the treatment group and -9.0 (-15.0 – 2.0) hair/cm^2 in the placebo group, while the second follow-up conducted at 6 months after the last treatment found a median change in hair density of -9.0 (-27.0 – 8.0) hair/cm^2 in the treatment group and -12.0 (-30.0 – 3.0) hairs/cm^2 in the placebo group. The difference in median hair density changes between treatment and placebo groups at 4 weeks and 6 months after the last treatment did not appear to be statistically significant (p=0.817 and p=0.366, respectively). This result might be influenced by the method used in PRP preparation, the number of PRP injection session (5 times with an interval of 4-6 weeks) and too long a delay before the clinical evaluation was carried, namely at 4 weeks and 6 months after the final treatment session. This is in line with the results from a previous study by Gkini et al., where hair density improvement was reported to follow an upward curve, reached their peak at 3 months, started to decrease at 6 months, and showed significant decrease after 1 year. Taking this into account, a booster session at 6 months is required to maintain and improve the results achieved. [29]

Meta-analysis of the mean change in hair density per cm^2 from 7 studies involving a total of 150 patients obtained a pooled SMD value of -0.889 with a 95% CI between -1.630 to -0.147 (p < 0.05). This overall results indicated that PRP injection treatment was significantly better than placebo in improving hair density in male patients with androgenetic alopecia. This result was consistent with the hypothesis that there was an increase in hair density after PRP injection was administered. The growth factors released by activated platelets in PRP was believed to promote proliferation, differentiation, angiogenesis, and cell chemotaxis required for new hair growth. IGF-1 has been shown to induce and prolong the anagen phase. Growth factors such as EGF and PDGF were reported to induce the KE pathway and upregulated the expression of B-cell lymphoma-2 (anti-apoptotic protein). Activated PRP was believed to influence the hair cycle by prolonging the anagen phase, preventing apoptosis, and delaying induction of the catagen phase. The telogen-anagen transition was also seen more rapidly than the control. Angiogenesis and increased follicular vascularization might play an important role in the initiation of the anagen phase. Normal hair follicles regeneration involved strong interactions between Wnt/β-catenin, Hedgehog, and Notch signaling transduction. In this pathway, Wnt/β-catenin signaling would determine the initiation of the hair growth cycle. The efficacy of this PRP treatment can be evaluated by assessing changes in hair density, a factor that has been sufficiently validated because numerous studies have been carried out to determine the normal value of hair density in various ethnic groups. [23,28,30,31]

The pooled analysis of all studies that were evaluated in the current meta-analysis has demonstrated that PRP injection treatment was more effective than placebo in improving hair density in male patients with AGA. However, this research has some limitations,

such as the limited number of RCT evaluating the administration of PRP injection in male patients with androgenetic alopecia, the influence of PRP quality on therapeutic outcome was relatively difficult to assess because of the diverse PRP preparation methods that was utilized in each study, and there was one study that only presented their results in the form of boxplot graph without specifying the numerical value of the mean hair density, thus disqualifying it from being included in the meta-analysis.

Conclusion

Platelet-rich plasma injection was found to be effective in improving hair density in male patients with androgenetic alopecia and PRP can be considered as an alternative treatment option to help improve treatment outcomes. However, the results of this meta-analysis should be interpreted with caution due to the small sample sizes and possible risks of bias from the pooled studies.

Abbreviations:

AGA: Androgenetic alopecia

GRADE: Grading of Recommendations, Assessment, Development and Evaluations

PRP: Platelet-rich plasma

RCT: Randomized Controlled Trial

PRISMA: Preferred Reporting Items for Systematic Review and Meta Analysis

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