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Treatment of Male-Pattern Alopecia with Platelet-Rich Plasma

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Keywords
Alopecia · Androgenetic alopecia · Baldness · Hair disorder · Platelet-rich plasma

Abstract
Background: Androgenetic alopecia (AGA) affects up to 80% of men and 50% of women throughout their lifetime, causing significant discomfort. Minoxidil, finasteride, and low-level laser light therapy are the only Food and Drug Administration-approved treatments for AGA, and they have shown positive results in randomized controlled trials and meta-analyses. However, their efficacy is limited, and new therapies are needed. Injection of platelet-rich plasma (PRP), a minimally invasive technique, has been described by several authors as a promising treatment for AGA. Although many studies report beneficial effects of PRP on AGA, there is no standardized practice for PRP preparation and administration or a standard method to evaluate results. ***Objective:*** The aim of this study was to evaluate the efficacy of manually prepared PRP in the treatment of male AGA. ***Materials and Methods:*** We treated 20 male patients with AGA with 3 monthly injections of PRP and analyzed results by Tricho-Scan®. ***Results:*** In this study, there was no statistically significant improvement in hair count or proportion of anagen hairs. ***Conclusions:*** This lack of response could be related to any of the variables during PRP preparation described above and also to the limited number of patients in the study.

Introduction

Androgenetic alopecia (AGA) affects up to 80% of men and 50% of women throughout their lifetime, causing significant discomfort related to appearance which may lead to anxiety symptoms, depression, and daily implications [1].

Minoxidil, finasteride, and low-level laser light therapy are the only Food and Drug Administration-approved treatments for AGA, and they have shown positive results in randomized controlled trials and meta-analyses. However, their efficacy is limited, and new therapies are needed [2].

Injection of platelet-rich plasma (PRP), a minimally invasive technique, has been described by several authors as a promising treatment for AGA [3–6]. PRP is prepared from autologous venous blood. Its platelets contain several growth factors (GF), chemokines, and cytokines that facilitate the healing process in hard and soft tissues [7–

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9]. Some of these GF stimulate hair regrowth: vascular endothelial growth factor helps promote microcirculation, transforming growth factor-B activates the dermal papilla and inhibits apoptosis during the cell cycle, platelet-derived growth factor stimulates stem cell mitosis, epidermal growth factor, fibroblast growth factor, connective tissue growth factor, and insulin-like growth factor-1 [10–12].

Although many studies report beneficial effects of PRP on AGA, there is no standardized practice for PRP preparation and administration or a standard method to evaluate results. The aim of this study was to evaluate the efficacy of manually prepared PRP in the treatment of male AGA.

Materials and Methods

Patients

From July 2018 to September 2018, 24 male patients were selected for the study. Of these, 20 healthy male patients between 18 and 45 years old with a clinical diagnosis of AGA were selected according to the following exclusion criteria. Patients should not have received any treatment for AGA for the last 6 months and should not take any continuous drugs for chronic diseases. Also, patients with blood disorders, such as anemia or blood clots, bleeding disorders, such as hemophilia, and blood cancers, such as leukemia, lymphoma, or myeloma, were excluded.

All patients provided written informed consent, and the study was approved by the ethics committee on human research of the University of Mogi das Cruzes.

Treatment Protocol

We selected the area most affected by AGA (vertex or frontotemporal) on each patient and treated the area with 3 monthly injections of PRP using a 3-mL syringe and a 30.5-gauge needle. Injections of 0.1 mL per cm^2 were given subdermally. We treated the rest of the scalp affected by AGA when there was more PRP left after treating selected areas.

We did not use any anesthesia prior to application. Patients were instructed not to wash their hair for 12 h.

PRP Preparation

We collected 24 mL of blood from each patient in 8 tubes of 3-mL sodium citrate solution (BD Vacutainer, BD Biosciences, San Jose, CA, USA). Tubes were centrifuged once for 5 min with 200 gravitational force (G) on a Thermo Electron LED centrifuge (Thermo Fisher, Germany).

We tested several protocols with different G forces and spin durations as well as single versus double centrifugation, comparing platelet concentration in peripheral blood and PRP collected. Platelet concentration was double or more than double at 200 G for 5 min.

After centrifugation, there was a yellow top layer (plasma); a thin middle layer, the buffy coat (platelets and white blood cells); and a red bottom layer (red blood cells [RBC]).

We collected the supernatant (plasma and buffy coat layer with a fraction of the RBCs) in a 10-mL syringe and added calcium gluconate in a 1:9 ratio (0.1 mL per 0.9 mL of PRP) in order to activate platelets and release GF based on the work of Gkini et al. [13].

A volume of 3–9 mL of PRP was obtained from patients after centrifugation. All materials used were sterile.

Outcomes

The primary goal was to achieve a change in hair density in the target area. Participants received a small tattoo before the start of treatment. A circular template of 2.2 cm^2 was centered over the tattoo. Hairs inside the template were clipped to approximately 0.5 mm in length. A 10-fold enlargement dermatoscopic digital image was performed 48 h after. This procedure was performed before treatment (D0), before the third injection (D1), and 30 days after the third injection (D2). Secondary outcomes measured were change in terminal hair density, proportion of anagen hairs, and terminal/vellus ratio. The hair counts were performed by the TrichoScan® software

Statistics

Statistical analyses used the ANOVA test to assess whether there were statistically significant differences between results measured at the 3 times D0, D1, and D2. Next, the Bonferroni multiple comparisons test (post hoc) was used to compare times by pairs (D0 to D1, D0 to D2, and D1 to D2).

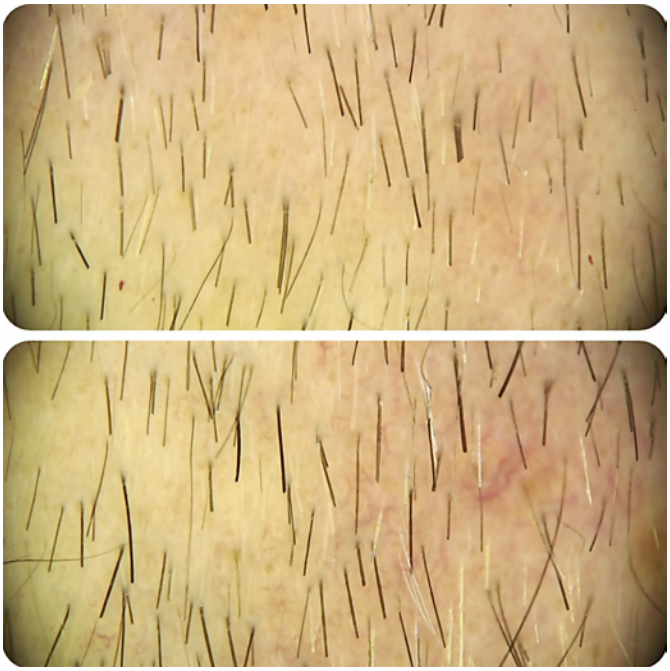
Results

One patient missed the last session and was excluded from the study. The main complaint was pain during injections. There were no other adverse effects, such as infection, telogen effluvium, scars, or others.

ANOVA tests were carried out for each parameter: total hair density, terminal hair density, anagen/telogen ratio, and terminal/vellus ratio, and no statistically significant differences were found. Nonetheless, the Bonferroni test was conducted, and it also indicated no statistically significant differences. Clinical comparative results are exemplified in Figure 1.

Discussion

PRP can be obtained in commercial kits or prepared manually by collecting blood samples and separating platelets by centrifugation. All systems of PRP preparation follow a similar method: blood collection involving use of an anticoagulant, such as sodium citrate or EDTA, to prevent blood clotting and consequent platelet activation, centrifugation to separate RBC and platelets, and activation with calcium chloride or calcium gluconate to release GF.



Color version available online

Fig. 1. Before and after 3 sessions of therapy with PRP.

However, the final composition of PRP may vary due to inherent differences in the concentration of platelets and GF between patients and variability during PRP preparation in factors such as blood volume collected, anticoagulant method used, centrifuge device used and its parameters, and whether a platelet activator was used. Also, clinical results may vary due to the method used to inject PRP, the number of sessions, and the interval between them [14].

The centrifuge device, spin rate or G force, duration of each spin cycle, and the number of cycles used for PRP preparation vary significantly between studies. Some authors do not even report this information. Cervantes et al. [15] reviewed 12 treatment protocols with different methods of PRP preparation. Ten of them had positive results and 2 had negative results. Objective end point measures also differed in some cases. Most analyzed hair density and hair count. One of the studies with negative results was done by Puig et al. [16]. They measured hair count and hair mass index in a double-blind, randomized, placebo-controlled trial with 1 PRP or placebo treatment that did not show statistically significant improvement. The second study with negative results was a half-head comparative pilot study with 2 monthly sessions. This study reported a mean decrease in the number of terminal and vellus hairs after 6 months of PRP treatment [17].

In the meta-analysis by Giordano et al. [18], all 16 studies used different centrifugation methods. Five reported a significant difference in the number of hairs per cm^2, favoring the PRP group.

Calcium gluconate and calcium chloride are the most frequently reported activation methods used [14]. However, this last step is controversial as platelets can be activated spontaneously after injection due to exposure to dermal collagen and thrombin even without prior activation [19]. Gentile et al. [20] evaluated GF concentrations in activated versus nonactivated PRP and did not find any significant difference with calcium activation.

In the review by Kramer and Keaney [14], only 32% of studies reported initial and final platelet concentrations. Among these, the average increase in platelet count was 3.8-fold. There is no consensus regarding which platelet concentration is best for PRP treatment. Some studies consider 1–3 times above baseline more therapeutically effective than 3–8 times above baseline. However, other studies suggest that platelet concentrations should be more than 1 million platelets per microliter [21–23]. Giusti et al. [24] consider 1.5×10^6 platelets per microliter the optimal concentration to induce angiogenesis in endothelial cells and suggest that higher concentrations would decrease this stimulus.

In our study, we assessed different spin cycle times and forces and single versus double cycle and found a higher platelet concentration at 200 G for 5 min with a 2-fold increase in platelet concentration. Although it is reasonable to think that the double cycle would result in a higher concentration, the second spin could impact the integrity and viability of the platelets. Also, higher forces or excessive spin duration could do the same.

We manually collected the supernatant plasma with a pipette, and the final product was orange-yellow because some RBCs were also collected. Kushida et al. [25] analyzed different commercial kits for PRP preparation and found significant differences in platelet recovery and the amounts of RBCs and granulocytes in the PRP. RBCs and white blood cells can induce inflammation locally, which could reduce the treatment effect for AGA and could also be related to burning pain at the injection site.

The volume of PRP obtained from patients varied from 3 to 9 mL. This can be explained by differences in patients' hydration status, lipemia, and hematocrit, which affect the final PRP characteristics [26].

PRP can be injected intra- or subdermally. In the review by Stevens and Khetarpal [27], subdermal bolus in-

jections were recommended, which are less painful and were the more efficient technique with 3 monthly sessions at least. On the other hand, Cervantes et al. [15] suggest intradermal injections of approximately 0.1 mL/cm^2 using the nappage technique based on analyzed studies with positive results. In our study, we subdermally injected 0.1 mL/cm^2 without anesthesia.

Lastly, there are many quantitative and qualitative measures defined in studies' objectives. Since there is no standard recommended method of assessment, it is difficult to assess which is the best. Most studies use hair count, hair density, and hair thickness; some use anagen/telogen ratio, histology and immunohistochemistry analysis, global photographs, and phototrichograms. We decided to compare only TrichoScan® hair counts as global photographs could confuse final analysis due to hair length.

In this study, there was no statistically significant improvement in hair count or proportion of anagen hairs. This lack of response could be related to any of the variables during PRP preparation described above and also to the limited number of patients in the study.

Conclusion

Although some patients experienced positive results after PRP treatment, statistical analyses did not show significance. This could be related to the small number of patients or PRP protocol variables. Further studies are needed to establish a standardized and effective PRP protocol.

Statement of Ethics

All patients provided written informed consent, and the study was approved by the ethics committee on human research of the University of Mogi das Cruzes.

Disclosure Statement

The authors declare no conflicts of interest.

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