



A Proposal of an Effective Platelet-rich Plasma Protocol for the Treatment of Androgenetic Alopecia

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ABSTRACT

Background: Platelet-rich plasma (PRP) has emerged as a promising treatment for androgenetic alopecia (AGA). In spite of the several studies previously reported, to date, a standardized protocol for PRP preparation and application, as well as a standard method for evaluating results has not been established. **Aims:** The aim of this study is to propose a standardized method for preparation and application of PRP for male AGA (MAGA) and female AGA (FAGA) and assess its safety and efficacy as a co-adjuvant therapy. **Materials and Methods:** Seventy-eight patients, 19 men and 59 women with AGA Grades II–IV in Ebling's scale, currently on treatment with topical minoxidil and/or oral finasteride for more than a year without improvement, were included in this study. PRP was prepared using a single spin method, and injected in affected areas for 3 monthly sessions, followed by 3 bimonthly sessions. A decrease of at least one grade in Ebling's scale was considered a successful result. **Results:** After the 6^o session, 71.4% of MAGA and 73.4% of FAGA patients reached a successful outcome while 21.4% and 16.3%, respectively, remained without changes. Only 7.1% of MAGA and 10.2% of FAGA presented worsening of their condition. **Conclusions:** PRP together with a periodical application protocol can be considered effective as a coadjuvant therapy in patients who no longer respond to pharmacological treatments. Ebling's scale was a practical and reliable parameter to allow a better evaluation in both MAGA and FAGA.

Key words: Androgenetic alopecia, hair loss, platelet-rich plasma

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INTRODUCTION

Androgenetic alopecia (AGA) is a nonscarring alopecia that may represent an important psychosocial issue.^[1] Despite being a very common condition drug therapies approved by the US Food and Drug Administration is limited to topical minoxidil and oral finasteride.^[2] Efficacy of these therapies is limited, and significant improvement is not always reached in all patients; therefore, there is an ongoing need towards finding new treatment options for this condition.^[3,4]

Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma, usually containing a higher concentration of platelets than the normal range (150,000–350,000 platelets/ μ L).^[5,6] When platelet alpha-granules become activated, they release numerous growth factors (GFs) which stimulate cell proliferation, differentiation, and angiogenesis.^[3,6,7] PRP has been used

in several fields of medicine for multiple purposes such as wound healing, tissue regeneration, rejuvenation, and orthopedic grafts.^[3,7-9] In the field of trichology, previous studies report that high concentrations of GFs in PRP achieve the following effects: new hair growth, thickening of preexisting hair shafts, proliferation of dermal papillae cells, and faster transition from telogen to anagen phase.^[10-12]

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With the exception of the recent proposed protocols of PRP application in different fields of dermatology by Alves *et al.* and Alves and Grimalt^[7,13] as far as we are aware, there is still no general consensus about the dose, frequency and ways of administration for PRP, as well as a reliable method for evaluating results. Therefore, clinical studies reported are difficult to compare due to high variability: small sample sizes, PRP treatment alone (not as a coadjuvant therapy), and short periods of patients follow-up. For all these reasons, our main objective is to propose a standardized method for preparation and application of pure PRP for male AGA (MAGA) and female AGA (FAGA) and evaluate its efficacy as a coadjuvant therapy in patients who present an intermediate degree of AGA. The method for evaluation of improvement was Ebling's scale, which is suitable to evaluate better both FAGA and MAGA.

MATERIALS AND METHODS

We conducted a prospective study that included 78 patients (19 men and 59 women), with an age range of 18–72 years. Exclusion criteria included coagulopathies, current treatment with anticoagulants, antiplatelet medications, or nonsteroidal anti-inflammatory drugs, and pregnancy. Grading of androgenic alopecia was assessed according to Ebling's scale, ranging from form I to V. We selected this method of evaluation since it is a reliable scale which can be easily assessed and can be applied in both men and women. Patients with Grades II–IV were selected for this study. All patients had been previously treated with topical 5% minoxidil and/or daily oral finasteride (1 mg in men and 5 mg in women) for at least 1 year without improvement. Intradermal injections of autologous pure PRP (0.1 cc/cm²) in affected areas were administered in 3 monthly sessions, followed by 3 bimonthly sessions [Figure 1]. Finally, maintenance was achieved with 2–3 annual follow-up sessions. Topical minoxidil and/or oral finasteride were not discontinued during PRP treatment. Follow-up assessment was documented every 2 months through a global clinical picture under standardized conditions and trichoscopy photographs of a previously determined area. A decrease in one grade of Ebling's scale was considered a successful result at any time of the treatment.

Platelet-rich plasma procedure

A peripheral blood sample of 18 cc was obtained from each patient in a 20 cc syringe previously loaded with 2 cc of 3.8% sodium citrate (anticoagulant). After obtaining a total of 20 cc of anticoagulated blood, the content was transferred to a dedicate CE mark PRP tube and

centrifuged using the equipment Omnigrafter® (Proteal, Barcelona, Spain): 1800 rpm, during 8 min, at 460-gauge. Separation of blood components into upper plasmatic portion with platelet-poor plasma, lower plasmatic portion with PRP, and lower globular portion rich in leukocytes and erythrocytes was obtained. Then, 3 ml of pure PRP were collected and platelets were activated adding 10% calcium chloride (CaCl₂) in a proportion 0.05:1 mL (CaCl₂:PRP) just before application. All steps were performed in a closed circuit which guaranteed a completely aseptic procedure.

RESULTS

From a total of 78 patients, 10 female and 5 male patients were lost to follow-up. Sixty-three patients concluded the study, 14 men and 49 women. After the 6^o session of treatment, 10/14 (71.4%) of MAGA and 36/49 (73.4%) of FAGA patients showed successful improvement characterized by a decrease in at least one grade in Ebling's scale [Figures 2-5] while 3/14 (21.4%) of MAGA and 8/49 (16.3%) of FAGA patients remained without changes. Worsening of the condition was only seen in 1/14 (7.1%) of MAGA and 5/49 (10.2%) of FAGA patients.

By the evaluation of global clinical and trichoscopy images, important changes such as the emergence of new vellus hairs, as well as improvement of hair quality, color, and width, were noted in the most cases [Figure 6].

DISCUSSION

In the field of trichology, previous studies report that high concentrations of GFs in PRP achieve the following effects: new hair growth, thickening of preexisting hair shafts, proliferation of dermal papillae cells, faster transition from telogen to anagen phase, and a reduction in the duration of the hair cycle.^[10] Other *in vivo* double-blind studies have confirmed that GFs in PRP may also stimulate cellular differentiation and proliferation of perifollicular collagen, fibroblasts, and blood vessels, generating a thicker epithelium that increases the hair cross-section.^[11]

Several signaling pathways have been proposed as the causal mechanisms for hair improvement after PRP treatment. Li *et al.* concluded that PRP increases proliferation of dermal papillae cells through stimulation of protein kinase B (Akt) and extracellular regulated kinase (ERK) signaling pathways, which contribute to apoptosis and cell growth regulation, respectively.^[5,6] Activated PRP also increases levels of Bcl-2, an anti-apoptotic protein, and upregulates

β -catenin, which promote a faster transition from telogen to anagen, and induce hair growth and follicular cell differentiation.^[5,12]

AGA is a disorder with relevant hormonal and genetic influences, and since the latter cannot be changed, treatment is mainly focused toward hormonal blockage. Increased levels of dihydrotestosterone (DHT), androgen receptors, and caspases generate cellular apoptosis and hair loss.^[14] DHT plays a relevant role in the inhibition of the hair follicle growth by inhibition of adenylyl cyclase (AMP-c),

which results in shortening of the anagen phase. All this mechanisms together result in the formation of vellus or miniaturized hairs, and if this stimulus persists, the hair may even disappear completely.^[5]

Treatment of AGA is essentially based on topical minoxidil and oral finasteride. Minoxidil prolongs the anagen phase through activation of potassium channels,^[15] increase of prostaglandin E2 channels^[8] and activation of the extracellular pathways of ERK and Akt.^[16] All these actions result in an increased proliferation of dermal papillae cells, as well as hair proliferation through an antiapoptotic effect by increasing the ratio of Bcl/Bax.^[9] Finasteride blocks caspases 1, 3, 8, and 9 and stimulates inhibitors of apoptosis (IAPs) such as X-linked IAP, generating a longer anagen phase and increasing follicular hair growth.^[17] GFs in PRP stimulate the growth of existing miniaturized hair follicles, without interfering in the hormonal pathways of AGA, thus, emphasizing the importance of using a combined therapy to achieve optimal results.



Figure 1: Infiltration of affected areas with the obtained pure platelet-rich plasma using five simultaneous 4 mm length mesotherapy needles and single needle

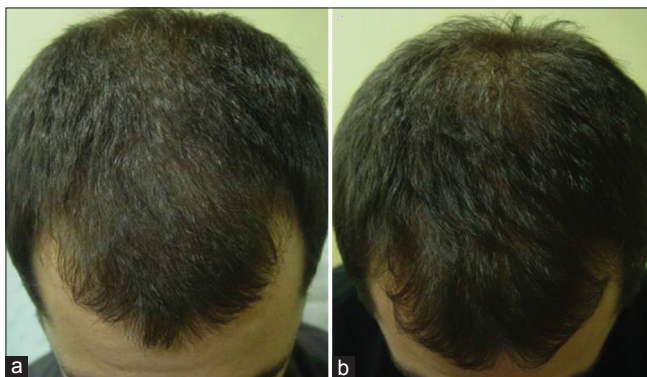


Figure 2: (a) A 33-year-old male with male androgenetic alopecia Ebling II, (b) showing improvement after 3 sessions of treatment

Successful results have been reported in previous studies. Cervelli *et al.* selected 10 patients with MAGA that were not currently on any other medication for the past 12 months. Evaluation was randomized, blinded, with a placebo half-head group, and evaluated by TrichoScan. After 3 months, a mean increase of 27.7 hairs/cm² was found in the treated areas, compared to 3 hairs/cm² in the control areas.^[15] Schiavone *et al.* studied 64 patients with MAGA and injected PRP enriched with leukocytes in two interventions separated by a 3-month interval. Evaluation was conducted by two evaluators using the Jaeschke scale and by clinical photographs, one of them stating improvement in all patients and the other in 62/64 patients.^[18] Kang *et al.* used a CD34+ cell-containing PRP preparation in 13 patients with AGA and placental extract preparation in a control group. They report an excellent improvement after 3 months of

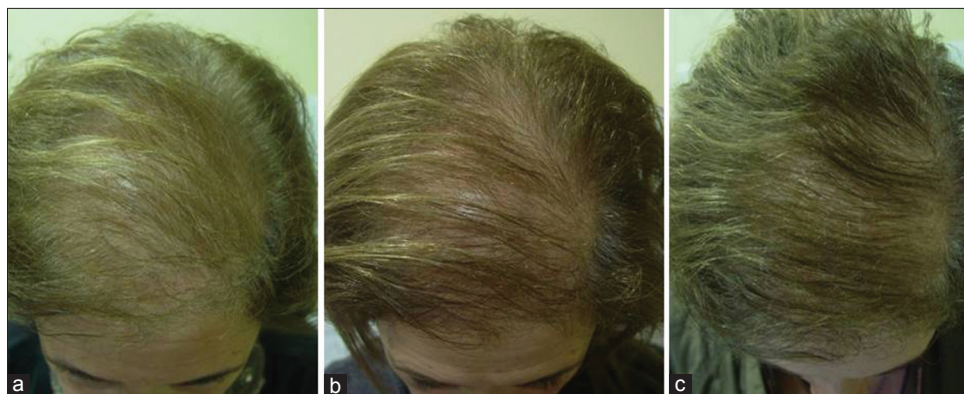


Figure 3: (a) A 51-year-old female with female androgenetic alopecia Ebling III, (b) excellent improvement at 3 months and (c) further improvement after 6 months, with a decrease of one grade in Ebling's scale (Ebling II)

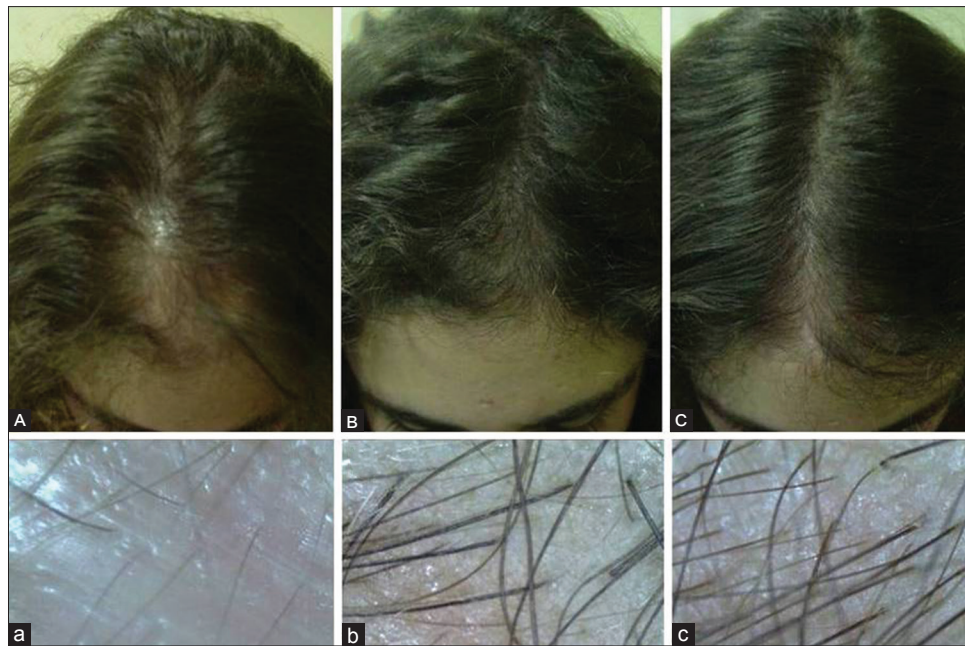


Figure 4: (A) An 18-year-old female with frontal female androgenetic alopecia Ebling III, (B) showing a decrease in one grade of Ebling's scale (Ebling II) after 3 sessions and (C) a decrease in two grades (Ebling I) after six sessions. Trichoscopy images (a-c) of the alopecic region taken with Fotofinder® showed improvement of hair density and width



Figure 5: (a) A 46-year-old female with female androgenetic alopecia Ebling IV, (b) notable improvement with decrease of one degree of Ebling at 3 months, and (c) further improvement at 6 months (decrease to Ebling II)



Figure 6: (a) Trichoscopy images were taken of specific, representative areas, using FotoFinder®. In these pictures improvement of the hair quality, width and density can be observed after 3 and 10 months (b and c)

treatment, with an increment in hair count of $20.5\% \pm 17\%$, and an increment in hair thickness of $31.3\% \pm 30.1\%$. They also evaluated a two-point score using a formula that included different evaluations of several points in the scalp, which also increased significantly after treatment.^[19] Singhal *et al.* treated ten patients with AGA (Ludwig score I and II, and Hamilton–Norwood score 1–4) and administered PRP alone for 3 months, using a control group which was treated

with medical treatment. Sessions of PRP were administered every 2–3 weeks for 3 months. Evaluation by pull test showed significant results, reporting an improvement of hair count, hair thickness, and alopecic degree.^[20] More recently, Alves and Grimalt^[13] reported a placebo-controlled, double-blind, half-head study in 25 patients with AGA with Stages II–V of Hamilton–Norwood and I–III of Ludwig, with no previous use of any medication. Evaluation was conducted by

global photography and phototrichogram, and they report significant differences 6 months after the first PRP treatment, with an increase in mean anagen hairs (67.6 ± 13.1), telogen hairs (32.4 ± 13.1), hair density (179.9 ± 62.7), and terminal hair density (165.8 ± 56.8) when compared with baseline; though when compared to control group, the only significant value was an increase hair density. They propose application of three initial treatments with 1 month apart, then wait 6 months, and perform another three cycles of PRP, with maintenance every 6 months or three cycles of PRP/year.^[7]

All of these studies demonstrate that improvement after PRP is evident, more commonly after the 3rd month. However, dose and administration protocols are not standardized, and neither are evaluation scales or methods, which in some cases were subjective and in some others complicated to assess in a daily practice basis.

CONCLUSIONS

After 6 years of personal experience of three of the authors (JF, LB and EN) with application of more than 600 sessions of pure PRP in more than 150 patients, we have found that our proposed method of preparation, application, and periodicity of PRP is effective since the third session. However, in this study we evaluated the results after the 6^o course of treatment to achieve solid results. We think that Ebling's scale is a better standardized and useful evaluation tool, which can be easily and accurately used for evaluation of AGA in both men and women. Our results state that more than 70% of patients (MAGA and FAGA) achieved at least a decrease of one grade in Ebling's scale after the 6^o session of treatment. We support the idea that PRP could be used as a coadjuvant treatment and patients should not discontinue previous topical and/or oral medication since PRP does not suppress hormonal response in AGA and may therefore not be enough when used alone. PRP appeared to be more effective in FAGA than in MAGA, and the best results were obtained in frontal and parietal areas. There were no side effects observed in our series of cases.

We strongly recommend the use of PRP as a coadjuvant treatment in patients with AGA who are no longer responding to the standard pharmacological treatments.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have

given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

There are no conflicts of interest.

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