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Research Article

Plasma Rich in Growth Factors Promotes Hair Growth on Female Androgenetic Alopecia

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- Anagen hair
- Telogen hair

Abstract

Androgenetic alopecia is a common hair disorder that affects around 45% of population at middle-advanced age. This condition has severe psychosocial impact especially among women, and few treatments have been proven to be effective against hair loss. The aim of this study was to evaluate the efficacy and safety of autologous plasma rich in growth factors (PRGF) injected intradermally for the treatment of female pattern hair loss. Two intra dermal injections of PRGF were administered every four weeks. Trichogramsat baseline and four months after the first treatment were performed in order to elucidate the anagen/telogen ratio. At the same time, macrophotographs were taken for overall improvement evaluation.150 PRGF treated cases of women with androgenetic alopecia were enrolled. Results showed a significant improvement among anagen hairs of 7.43 percentage points while a decrease of 6.38 percentage points was observed for telogen ones. Additionally, an overall improvement was noticed after PRGF treatment. Although additional randomized clinical studies are needed, our preliminary results suggest that plasma rich in growth factor therapy could be a safe and effective treatment for female pattern hair loss as the anagen/telogen ratio is significantly increased at four months follow up period.

INTRODUCTION

Female pattern hair loss, also known as female androgenetic alopecia, is a common hair disorder that affects over 21 million women only in the USA [1]. Given hair´s important role in determining self-image, social perception and psychosocial functioning, the psychological impact of an optimal hair appearance is essential. Recent studies have demonstrated the importance of hair to our self steemin some European countries [2]. While baldness is a commonly accepted condition among men population, 70% of women have proven to suffer from hair loss derived psychological impact. There are several pathologies in which hair loss has become a major concern within female population, being androgenetic alopecia the more prevalent one (68.8%), followed by telogen effluvium (11.3%) and alopecia areata (9.9%) [3]. Female androgenetic alopecia is a non scarring progressive miniaturization of the hair from terminal to vellous-like follicles that begins commonly at ages between 20 and 40. Women first notice hair thinning over the frontal area, and gradually the scalp becomes more visible. These miniaturized hairs result from the shortening of the anagen growing phases of the hair follicle cycle, and the prevalence of hairs in telogen resting phase. These changes are mediated through the interaction between androgens, their respective receptors and enzymes like 5-α-reductase and p450 aromatase [4].

In the last few years, several approaches have been tested with the aim of preventing female pattern hair loss including: topical or intradermal 5-α-reductase inhibitors such as finasteride [5] and dutasteride [6], prostaglandin activity related bimaprost [7] and minoxidil [8], Wnt signaling pathway activators like valproicacid [9] and follicular unit extraction (FUE) techniques as hair transplant treatment [10]. Moreover, some regenerative medicine approaches based on stem cells [11] and recombinant growth factors [12] are also being evaluated.

Another interesting alternative lies on the application of autologous plasma and platelet-derived proteins for the treatment of female pattern hair loss. In fact, some encouraging results have been achieved up to date [13]. The technology of plasma rich in growth factors (PRGF) is a new 100% autologous therapy whose benefits have been already demonstrated in dermatology [14] and other medical fields including orthopedic surgery [15], ophthalmology [16], neurobiology [17] and oral/maxillofacial surgery [18]. This technology is based on the recovery of a small volume of the patient´s own blood which is afterwards centrifuged and activated in order to obtain

autologous formulations enriched in proteins and growth factors free from pro-inflammatory leukocytes. PRGF is able to enhance the organism's self-renewal ability accelerating the transition between the different phases of tissue regeneration: hemostasis, inflammation, granulation tissue formation, epithelization, neovascularization and extracellular matrix deposition. No side effects have been demonstrated after the use of plasma rich in growth factors therapy in any of the aforementioned medical fields.

The objective of this study is to evaluate the effects of PRGF on women with female androgenetic alopecia, in order to elucidate the beneficial effects and safety of this autologous therapy on the recovery of hair loss.

MATERIAL AND METHODS

Patients

A retrospective study design was used and was performed following all the principles of the Declaration of Helsinki. This study includes patients who fulfilled the following selection criteria:

- Females patients
- diagnosed of androgenetic alopecia (AGA) degrees I-II in Ludwig scale (mild to moderate)
- Treated with PRGF.

All patients were evaluated before and three months after treatment with PRGF with macrophotographs and trichograms in order to evaluate the overall improvement and determine the anagen/telogen hair ratio respectively.

Plasma Rich in Growth Factors (PRGF-Endoret) preparation

For each patient, 18mL of peripheral blood was collected and was put into two 9 mL tubes containing 3.8% (wt/vol) sodium citrate. For PRGF preparation, the Endoret kit (BTI Biotechnology Institute) was used. Briefly the blood was centrifuged (BTI System IV, Vitoria, Spain) at 580g for 8 minutes and the plasma column was fractioned into fraction 1(F1) and fraction 2 (F2). F2 is defined as the 2 cm^3 platelet rich plasma just above the leukocyte buffy coat, and F1 is defined as the remaining plasma volume above the F2. Platelet poor F1 fraction was discarded and only the platelet rich F2 fraction was used in the study. PRGF activator (BTI Biotechnology Institute, SL, Miñano, Spain) was added to the collected F2 fraction to provoke the release of a pool of bioactive growth factors and cytokines from the alpha granules of the platelets. Then, PRGF was ready to be injected into the patient´s scalp.

Treatment protocol

All patients provided written informed consent prior to the beginning of the treatment. A global blood analytic was obtained from each one with the aim of confirming the suitability for the treatment with PRGF. Patients with infectious processes or platelet derived blood disorders were considered non-suitable for plasma rich in growth factor therapy. Patients with skin diseases or scalp wheals were not included in the study. Additionally, all patients were previously examined to exclude any signs on hair of inflammation, scarring or erythema.

A 10mL syringe was loaded with 2cm^3 of PRGF and briefly, intra dermal injections were carried out into hair depleted areas of the patient´s scalp, using 30G needles. For the homogenization of the PRGF injections, an electronic DHN1 mesotherapy gun (Dismedical, Madrid, Spain) was used. After each treatment, a fingertip pressure based craniosacral massage was provided by a trained clinician during 10 minutes in order to relieve the pain around the injected area and with the aim of spreading the PRGF all over the scalp. Subjects were treated every 4 weeks for two treatment sessions.

Assessment

All patients were evaluated at two stages: at the beginning of the study and four months after the first PRGF treatment. The effects of plasma rich in growth factors on hair growth and androgenetic alopecia recovery were assessed with the help of global photography using a digital camera (Nikon, Madrid, Spain) and standardized trichograms.

Trichograms were performed by a trained and blinded evaluator. 20 single hairs were carefully removed of the parietal area of the patient´s scalp using Kocher forceps (Figure 1). The forceps were placed at a distance of approximately 2cm of the scalp and hair was firmly pulled out in the hair growth direction. Once the hairs were extracted, they were placed in a slide and mounted with a cover glass. Finally, number of anagen (active growth) and telogen (resting phase) hairs were counted under an optical microscope at 2.5x and 5x objectives. Representative images of both anagen and telogen hairs were obtained (Leica DM IRB, Leica Microsystems, Wetzlar, Germany) (Figure 2).

Statistical analysis

A complete descriptive statistic was performed. For this

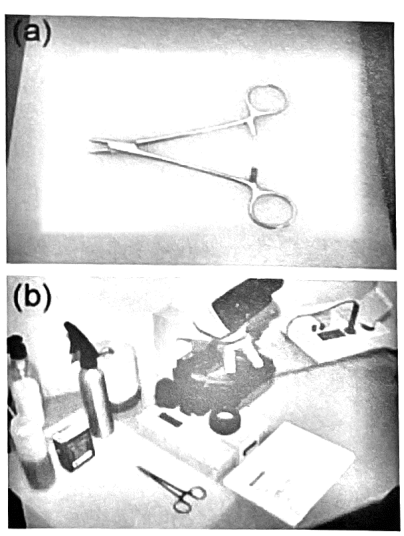

Figure 1 a) Kocher forceps and slide used to permorm the trichograms. b) Hairs where mounted on a slide and viewed under 2.5x and 5x objectives using an optic microscope.

purpose, absolute and relative frequency distributions were calculated for qualitative variables, while mean values and standard deviations were assessed for quantitative variables. After checking the normal distribution (Shapiro Wilk test) of the variables, differences in percentage of anagen-telogen hairs between pre and post-PRGF treatment, was analyzed using the Wilcoxon non-paramentric test. In all cases, the applied statistical significance level was 5% ($p< 0.05$). SPSS v15.0 for Windows statistical software package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

One hundred and fifty women patients with AGA were finally included and evaluated. All cases were treated with PRGF between January 2012 and October 2013. Donor age ranged from 25 to 70 years old caucasian women being a medium-advanced 57 age year woman with type II Ludwig alopecia, the median patient.

The comparison between the trichograms performed at time point 0 and four months after the PRGF treatment, revealed a significant increase in the number of anagen hairs while a significant decrease among telogen ones was observed. Before any treatment was done, anagen hairs reached the 56.33%±6% of the total, and after PRGF injections, they increased significantly up to 63.76%±3% ($p<0.05$). On the other hand, telogen hair count was significantly reduced from 25.95%±5% to 19.57%±2% after PRGF treatment (Figure 3). These results demonstrate that an improvement of 7.43 percentage points was achieved among the number of anagen hairs, while for telogen hairs; a decrease of 6.38 percentage points was obtained.

All of the patients were satisfied at the end of the treatment period. As Figure 4 illustrates, a significant improvement of hair volume and quality was achieved after two PRGF intra dermal injections and four months of follow up period. Subjectively hair density was noticeably increased in those areas where the androgenetic alopecia derived hair loss was most severe.

A total of 150 cases of women suffering from female androgenetic alopecia were enrolled in this study. All of them finished de study with no adverse effects apart from transient post-treatment erythema and edema that disappeared after 24 hours of the PRGF injections. Other possible side effects such as secondary bacterial or viral infection, post-therapy blister formation and hypopigmentation were not observed.

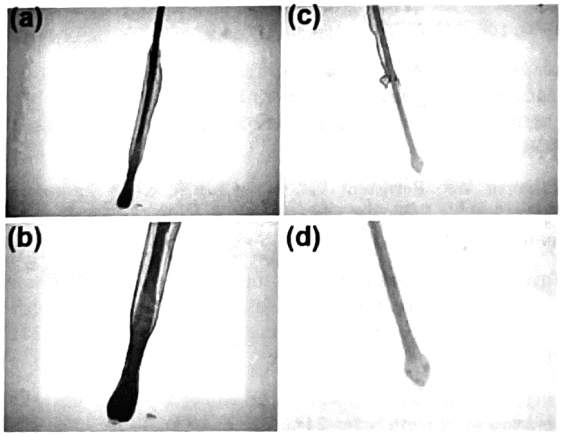

Figure 2 Representative microphotographs taken after trichogram analysis of anagen hairs under a) 2.5x and b) 5x objectives and telogen hairs c) 2.5x d) 5x.

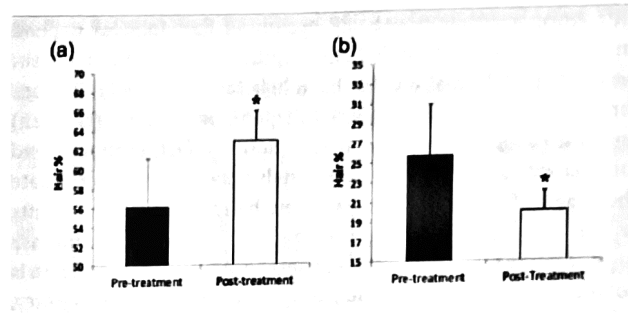

Figure 3 a) Percentage of angen follicles increased significantly after PRGF treatment, while b) a decrease among telogen ones was observed. *p<0.05.

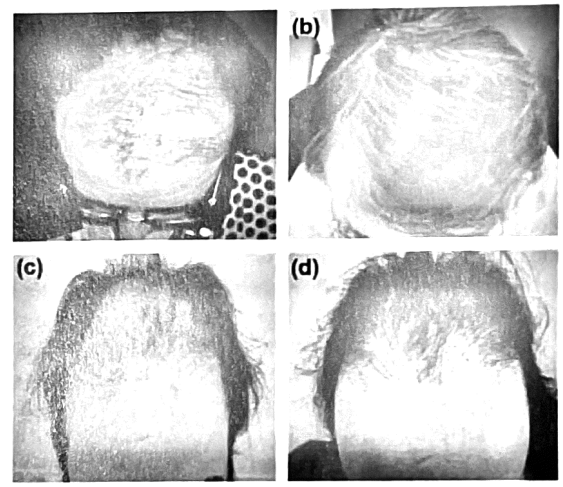

Figure 4 Representative photographs of two patients taken a), c) at time point 0 and b), d) after 4 months of PRGF treatment.

DISCUSSION

Each platelet from PRGF contains around 40 α-granules that upon activation release a wide variety of cytokines, growth factors and other bioactive biomolecules. Some of these proteins include platelet derived growth factor (PDGF), fibroblast growth factor (FGF), transforming growth factor β1 (TGFβ1), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), insulin-like growth factor (IGF), and epidermal growth factor (EGF).The pool of proteins present in PRGF, promoteskin repair by inducing proliferation, migration, angiogenesis, biosynthesis and extracellular matrix remodeling [19]. As growth factors have proven to significantly induce tissue regeneration, we wonder whether they could be helpful in the stimulation of dermal papilla and germinal matrix cells involved in folliculogenesis. In fact, some of these growth factors separately have proven to induce hair follicle neogenesis and hair growth

[20]. Normal development and cycling of hair follicles depend on the interaction of the follicular epithelium with the adjacent mesenchymal dermal papilla. Each hair follicle perpetually goes through three stages: growth (anagen), involution (catagen) and rest (telogen) [21]. The trichogram is a fast, economic and minimal invasive method that is widely validated to differentiate the stage of a hair follicle. Anagen hairs exhibit long shafts with uniform diameter and rectangular shape,while an intense pigmentation within the bulb is observed. Additionally, a sheath is surrounding the proximal end of the hair follicle. On the contrary, telogen hairs shafts are shortened and the bulb is thickened, club shaped and absent of any pigmentation or surrounding sheath. The decrease of anagen hairs and the increase of telogen ones is one of the hallmarks of androgenetic alopecia [22].

Interestingly, our results show that PRGF increased significantly the percentage of anagen hairs after 4 months of treatment. Two sessions of PRGF intra dermal injections were able to induce an improvement of 7.43% among the total number of anagen hair. Moreover, our results demonstrate a significant decrease in the number of telogen hairs after PRGF treatment. The percentage of telogen hairs decreased more than 6 percentage points comparing with the basal visit. These results are consistent with others in which a clinical improvement in the mean number of hairs, along with hair thickness and several phototrichogram results where improved after patient's own proteins were used [23]. In fact, some studies demonstrate that this autologous therapy improves dermal papilla cell proliferation and hair re-growth while decreases hair dystrophy and burning/itching sensation [24]. Our results showing an increase in anagenand a decrease in telogen ones, are consistent with patient's satisfaction and overall improvement seen in the photographs after PRGF treatment.

Research around biomolecular mechanisms by which androgenetic alopecia develops is still ongoing. It seems that hair follicles of patients with this pathology have increased levels of dihydrotestosterone (DHT) as a result of the up regulation of 5-α-reductase activity, which converts the peripheral testosterone into DHT. Ultimately, DHT binds to the androgen receptor of the hormone receptor complex and activates the genes responsible for gradual transformation of terminal follicles to miniaturized ones by means of down regulation of dermal papilla cells 4. However, recent studies have demonstrated that the use of PRGF may induce the proliferation of dermal papilla cells up regulating ERK and Akt pathways involved in mitogenesis and cell growth while increasing the expression of Cyclin D1 and Cdk4, involved in cell cycle progression [25]. The pool of bioactive molecules present in PRGF could influence the maintenance of growing follicles. In fact it has been proved that VEGF, present in PRGF, is a potent inductor of perifollicular neovascularization that may help in hair regrowth processes [26]. Additionally, platelet derived growth factor (PDGF) acts on follicular receptors, triggering a signaling cascade between dermal papilla cells and follicular keratinocytes ending up in hair canal development [27]. Other potential role of PRGF may be the antiapoptotic effect shown among dermal papilla cells by the up regulation of antiapoptotic Bcl-2 regulator. This mechanism has been shown to be related with the induction of stem cell derived anagen phase prolongation via FGF-7/b-catenin signaling pathway activation [28].

Although additional studies are needed to elucidate the underlying mechanism by which this autologous technology regulates hair follicle activity, our findings suggest that PRGF offers a safe and effective therapy that enhances hair growth improving the anagen/telogen ratio.

CONFLICT OF INTEREST

Eduardo Anitua is the Scientific Director and Ander Pino is a scientist at BTI Biotechnology Institute, a dental implant company, which investigates in the fields of oral implantology and PRGF-Endoret technology.

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