Microscopic and Histologic Evaluation of the Regenera® Method for the Treatment of Androgenetic Alopecia in a Small Number of Cases

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ABSTRACT

Alopecia is a generalized problem concerning a large sector of the population, both men and women. Some of the most common treatment options for androgenic alopecia are a useful, though the results obtained are not always successful. Apart from known medical treatments, there are other possibilities, like regenerative medicine procedures. The goal of this paper is to assess changes occurring on the scalp, hair bulbs and hairs after applying an autologous suspension obtained using the Rigenera® system.

Keywords: *Alopecia, AGA, regenerative medicine, Rigenera.*

INTRODUCTION

Alopecia is defined as any type of pathological hair loss anywhere on the skin surface.[1] It is a generalized problem concerning a large sector of the population, both men and women, especially when located on the scalp. The wide range of products and treatments against hair issues reflects their high demand and the incidence of this problem.[1] Alopecia can be classified in two main groups: scarring alopecia and non-scarring alopecia (Table I). The main difference is that in non-scarring alopecia, hair follicles are not destroyed, whereas scarring alopecia[2] involves their destruction. The most frequent type is androgenetic alopecia (AGA), also known as "common baldness". It occurs when the hormone 5-α-dihydrotestosterone (5-α-DHT) acts on predisposed hair follicles of the scalp, gradually miniaturizing them until they disappear.[3]

Table1. *Classification of Alopecia.*

Scarring	Non-Scarring
Infectious	Androgenetic (AGA)
Physical-chemical agents	Areata
Neoplasias	Traumatic
Dermatosis	Drugs
Hereditary diseases	Systemic diseases
	Hereditary syndromes

The most common treatment options for AGA are described in Table II. Topical 2% and 5% Minoxidil is a useful treatment both for men and women.[4] However, results obtained are not always successful. This is due to: i) inefficacy of the drug; ii) occurrence of side effects, like pruritus, desquamation or headache; iii) failure to continue performing the treatment; and iv) withdrawal from the treatment due to patients discomfort, who complain about their hair looking greasy or dirty after application.

Finasteride and, more recently, Dutasteride have been approved to treat male pattern AGA.[2] Orally administered at 1 mg per day, these drugs act by inhibiting the 5-α-reductase enzyme type 2 in the case of Finasteride, or as inhibitors of 5-α-reductase enzymes types 1 and 2 for Dutasteride. Thus, they block the conversion of free testosterone to 5-α-DHT, reducing its direct action on hair follicles.[3] The need of a daily schedule for a long time causes high rejection from the patients, who often withdraw from the treatment. Women have an alternative, which is also orally administered: antiandrogens, like spironolactone and cyproterone acetate.[3] However, due to their interrelation with other

hormones and changes in hormonal balance that women suffer throughout their lives, it is not possible to prescribe standardized doses or a simple action protocol.

On the other hand, there are other different treatments. Platelet rich plasma (PRP), for instance, involves a high economic cost for the patient, provides different results and lacks standardized doses.[4] With capillary surgery, hair follicle transplants are performed using the FUE (Follicular Unit Extraction) or FUSS (Follicular Unit Strip Surgery) techniques, and as long as they are properly indicated and carried out, they yield excellent results in male patients. In female pattern AGA (FAGA), hair transplant is not usually a good therapeutic option.

Table2. *Classification of medical treatments for alopecia.*

Topical	Oral Drugs	Injectable Drugs	Fototherapy	Surgery
Minoxidil	Finasteride	Cortisone	Laser	FUE
Antimycotics	Dutasteride	PRP	LED	FUSS
	Corticosteroids			
	Antiandrogens			

Apart from known medical treatments, there are other possibilities with less solid scientific foundation, but that nonetheless are highly accepted by the population: oral or injectable multivitamin supplements, shampoos, lotions, masks, homeopathy and phytotherapy, among others. Very few of these help to stop hair loss or are really effective to treat alopecia.[3]

Results obtained with regenerative medicine procedures for the treatment of complex injuries,[5] regeneration of soft tissues[6] and bone regeneration,[7] have attracted a lot of interest in their application for several pathologies or hair conditions.

The goal of this paper is to objectively evaluate a very small number of patients with AGA to assess changes occurring on the scalp, hair bulbs and hairs after applying an autologous suspension obtained using the Rigenera® system.

METHODS

The treatment consists of the mechanical disintegration of a sample of tissue obtained by a skin punch and subsequent filtration (50 microns) to be intradermally administered in the affected area according to technical specifications (Regenera® Protocol, Rigenera® System, Human Brain Wave SRL, Turin).

For this descriptive study, 3 patient volunteers were consecutively and randomly recruited. The defined inclusion criteria were: males over 18 years old diagnosed with male pattern AGA. Exclusion criteria included: allergy to lidocaine, healing issues, scarring alopecia, chronic drug treatment, oncologic processes and having performed any hair loss treatment —topical, oral or injectable— between 3 months prior and 3 months after the date of applying treatment — except taking vitamin supplements and applying topical lotions or shampoos.

The suspension was applied on the scalp using mesotherapy, and the variables assessed were:

- Hair thickness: Measured with a micrometer (MDC-1"MX Mitutoyo® model, Mitutoyo Corporation™)

- Hair Loss Test: At control visits, 30 days after treatment, involving counting hairs over a white towel after combing it forward for 60 seconds

- Biopsy[9-13] for immunohistochemical stains: Ki-67 for the identification of cells found in the proliferative phase of the cell cycle, CD34 for the location of the vascular endothelium and Vimentin[11] for the location of an intermediate filament indicating an increase in fibroblasts and perifollicular collagen.

Follicular units and biopsies were taken from the upper parietal region of the scalp.

RESULTS

Three male patients were treated with the Rigenera® system. According to the scale of Hamilton, two had alopecia type III, and one had alopecia type IV. The results of the micrometer measurements are summarized in Table 3.

Table3. *Measurements with micrometer.*

Patient 1	Hair 1 (Mm) Total (Proximal - Intermediate – Distal)	Hair 2 (Mm) Total (Proximal - Intermediate – Distal)	Hair 3 (Mm) Total (Proximal - Intermediate – Distal)
Baseline	0.186 (0.058 - 0.074 - 0.054)	0.152 (0.054 - 0.050 - 0.048)	0.15 (0.052 - 0.051 - 0.047)
1 Month	0.185 (0.065 - 0.065 - 0.055)	0.187 (0.064 - 0.072 - 0.051)	0.179 (0.063 - 0.062 - 0.054)

Patient2	Hair 1 (Mm) Total (Proximal - Intermediate – Distal)	Hair 2 (Mm) Total (Proximal - Intermediate – Distal)	Hair 3 (Mm) Total (Proximal - Intermediate – Distal)
Baseline	0.183 (0.062 - 0.062 - 0.059)	0.181 (0.060 - 0.060 - 0.061)	0.184 (0.062 - 0.060 - 0.062)
1 Month	0.206 (0.072 - 0.068 - 0.066)	0.161 (0.056 - 0.053 - 0.052)	0.218 (0.075 - 0.072 - 0.071)

Patient 3	Hair 1 (Mm) Total (Proximal - Intermediate – Distal)	Hair 2 (Mm) Total (Proximal - Intermediate – Distal)	Hair 3 (Mm) Total (Proximal - Intermediate – Distal)
Baseline	0.176 (0.056 - 0.064 - 0.056)	0.152 (0.056 - 0.050 - 0.046)	0.165 (0.058 - 0.056 - 0.051)
1 Month	0.157 (0.051 - 0.056 - 0.050)	0.169 (0.057 - 0.057 - 0.055)	0.197 (0.070 - 0.067 - 0.060)

In the first patient, measurements show a difference of +0.063 mm, which means a 12.90% increase in hair thickness. In the second patient, the difference is +0.037 mm, which means a 6.75% increase in hair thickness. And in the third patient, measurements show a difference of +0.03 mm, which means a 6.08% increase in hair thickness.

Hair Loss Test results are summarized in Table 4.

Table4. *Hair Loss Test.*

Patient	Hair Loss Before Treatment (No. of Hairs)	Hair Loss After Treatment (No. of Hairs)	Difference (%)
1	23	14	39.13
2	19	13	31.57
3	17	5	70.58

The histological study with hematoxylin-eosin showed a slight increase in the epidermis thickness, an increase in the amount of perifollicular collagen fibers and an increase in the amount of existing vessels in the follicle bulb. Using immunohistochemical techniques, [11] the following differences between both groups were observed: an increase in cell mitotic activity with Ki-67 staining, an increase in the amount of perifollicular vascular structures (CD34 staining), an increase in the amount of collagen fibers and fibroblasts in the dermis (vimentin).

DISCUSSION

An increase in the mean of hair thickness, together with reduction of its loss, have been objectified; on the other hand, immunohistological findings are encouraging. Usually, a reduced amount of Ki-67 immunoreactive cells is observed during involution of hair follicles. This denotes a suppression of hair growth and reflects the atrophy of the hair follicle. Cases treated with the Rigenera® system, however, showed an increase in mitotic activity, suggesting higher metabolic activity consistent with the hair growth phase. Likewise, CD34 and Vimentin stains were also quantitatively greater during post treatment, reflecting an increase in perifollicular vascularization, as well as an increase in fibroblastic activity in the adjacent area to the hair bulb.

Methodologically speaking, this small number of cases provides conclusions that are extremely

interesting, but with a very limited scope. The sample size, lack of a control group, and short duration of the observation period determine how to interpret the results obtained.

In conclusion, given the results, using the Rigenera® system seems to be a promising option to treat and slow down the evolution of AGA. However, controlled, randomized, longer clinical trials, with a larger sample, control and placebo groups and quantifiable methods are necessary to irrefutably corroborate these findings.

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