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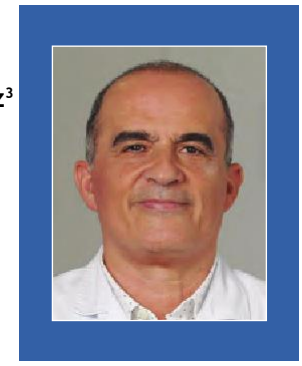

Dr. Enrique Lorente Prieto.

Treatment of androgenetic alopecia (AGA) with *Sabal serrulata* and biomimetic peptides delivered with microneedling

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SUMMARY

Introduction. *Microneedling* is a minimally invasive technique that allows the opening of microchannels in the skin, favouring the topical absorption of sterile medical and/or cosmetic preparations. Its use is widespread in the treatment of different pathologies, including androgenetic alopecia (AGA).

The aim of the study is to assess the efficacy of *microneedling treatment* of AGA in serial microneedling with commercially available sterile cosmetic preparations.

Method. Ten male voluntary patients diagnosed with AGA, accentuated by the stress of the coronavirus pandemic (SARS-CoV-2), were included. They underwent 10 treatment sessions, performed weekly, using the *microneedling* technique followed by the application of 2 sterile topical cosmetic products containing *Sabal serrulata* and biomimetic growth factors. Daily home treatment with topical lotion and intake of a nutritional supplement was also prescribed.

Results. At the 5th session, a reduction in hair loss was observed in all patients. A decrease in AGA on the Sinclair scale was observed in 60% of patients, compared to the start of treatment, as measured by trichogram. At the 10th session, 70% of treated patients showed an improvement in cumulative hair thickness (mm/cm).[2]

Conclusions. Despite the limited number of patients, transcutaneous administration of sterile topical cosmetics by *microneedling* together with home treatment may be a successful approach to androgenetic alopecia.

Key words. Androgenetic alopecia, *microneedling*, sterile cosmetics, *Sabal serrulata*, biomimetic peptides, nutritional supplements.

ABSTRACT

Introduction. Microneedling is a minimally invasive technique, which allows the opening of microchannels in the skin, favouring the topical absorption of sterile medical and/or cosmetic preparations. Its use is widespread in the treatment of different pathologies, including androgenetic alopecia (AGA).

The aim of the study is to assess the efficacy of microneedling in the treatment of AGA, by serial micropuncture, with commercial sterile cosmetic preparations.

Material and Method. Ten male volunteer patients diagnosed with AGA, accentuated by the stress of the coronavirus pandemic (SARS-CoV-2), were included in the study. They underwent 10 treatment sessions, performed weekly, using the microneedling technique followed by the application of 2 sterile topical cosmetic products containing *Sabal serrulata* and biomimetic growth factors. Daily home treatment with topical lotion and intake of a nutritional supplement was also prescribed.

Results. At the 5th session, a reduction in hair loss was observed in all patients. The 60% presented a decrease in the AGA on the Sinclair scale, compared to the begin- ning of the treatment, which was objectified by tricho- gram. At the 10th session, 70% of the patients treated presented an improvement in cumulative hair thickness (mm/cm).[2]

Conclusions. Despite the limited number of patients, the transcutaneous administration of sterile topical cosmetics by microneedling together with home treatment may be a successful approach to androgenetic alopecia.

Keywords. Androgenetic alopecia, androgenetic alopecia , microneedling , sterile cosmetics , *Sabal serrulata, biomimetic peptides, nutritional supplements.*

INTRODUCTION

Alopecia is the reversible or irreversible loss of previously existing hair follicles resulting in a diffuse or localised decrease in hair density [1]. Alopecia can be classified in various ways, depending on its origin and manifestations, the most common being androgenetic alopecia (AGA), commonly known as common baldness. This type of alopecia accounts for 95% of the diagnoses made in the clinic and is predominant in the Caucasian race. It affects up to 80% of the general male population and 50% of the female population at some point in their lives, and its prevalence increases with age [2]. Hair loss can affect self-esteem and lead to depression. The impact on quality of life and the high incidence of AGA make it the most frequent cause of consultation in trichology [3].

AGA is a pathology with a genetic basis, variable penis and hormonal pathophysiology, which causes a progressive miniaturisation of the follicles of the scalp, mainly in the frontal, parietal and temporal areas [1]. Genetic predisposition to alopecia accounts for 80% of existing cases [4]; genetic polymorphism explains the different ages of onset and degrees of presentation of AGA [5]. Follicular miniaturisation is a response of the hair follicle cells to the rate of circulating androgens in the blood in genetically predisposed individuals, even if this rate is normal [6].

Testosterone is metabolised into dihydrotestosterone (DHT), by the enzyme 5-alpha-reductase (5-αR), in its isoenzyme 1 and isoenzyme 2 forms. DHT has a high affinity for androgen receptors in the hair bulb, and is considered the main responsible for androgen-mediated effects on the scalp of AGA patients [7]. Under the influence of androgens, the dermal papilla of hair follicles secretes a significant amount of cytokines, such as TGF β-1, IL-1 α and TNFα that may influence the shortening of the anagen phase [7].

Follicular miniaturisation is a theory that explains the decrease in scalp density affected by AGA, which involves the shortening of the anagen phase in the hair cycle, and an ascension of the follicle from the reticular dermis to the papillary dermis that occurs progressively with each new hair cycle. The previously larger follicular units with terminal hairs become smaller and exhibit a downy hair pattern [8]. It also explains the decrease in scalp density affected by AGA.

A large number of molecular signals are involu- ted in the phases of the normal hair cycle. The tran-

The telogen to anagen follicle differentiation is associated with activation of the Wnt/β-catenin / LEF-1 (Lymphoid enhancer-binding factor 1) signalling pathways, exogenous Sonic Hedgehog (Shh) proteins and STAT3 mediator proteins that mediate gene expression in response to specific cellular stimuli, in addition to down-regulation of bone morphogenetic protein (BMP) signalling. The growth factor BMP-4 also appears to play an important role in suppressing follicular growth and differentiation during the telogen phase. In AGA, circulating androgens enter the follicle through the dermal papilla (DP) capillaries, bind to the androgen receptor within the DP cells and then activate or repress the molecular signalling pathways responsible for premature anagen to catagen trans- sion and follicular miniaturisation. This includes suppression of Wnt, STAT 3 and Shh stimulatory pathways, and positive regulation of suppressor pathways such as BMP [8 - 10].

Another explanation for the decrease in hair density is the increased duration of the kinogenic phase, which may occur in conjunction with the minia-turisation process. This is the so-called empty follicle phase that follows an exogenous event that causes hair loss. This kenogenic phase can last from 3 months to a year, but in cases of AGA it can be longer [10]. An increase in follicles in this phase may be more important for the decrease in hair count than miniaturised hair follicles. Rushton *et al* concluded that, after a 12-month treatment of AGA, hair regrows more from the conversion of follicles from kenogene to anagen than from increased activity in miniaturised hair follicles [11].

Although the pathophysiology of AGA is closely related to androgen metabolism, scientific evidence suggests that it is also associated with dysregulation in the expression of inflammatory cytokines, with the consequent chronic microinflammation that acts as an aggravating factor in alopecia [12]. This inflammatory scenario can be caused by both endogenous and exogenous factors: endogenous bacterial flora in cases associated with seborrhoea, oxidative stress, ageing, smoking, ultraviolet radiation and pollutants [8]. With regard to exogenous factors, lifestyle modification can potentially reduce the extent of clinical manifestations [7].

The use of the *microneedling* technique has undergone a significant evolution in recent years, allowing it to be introduced into the therapeutic arsenal as a low-cost tool, with a low learning curve, comfortable for the patient and minimally invasive [9, 13 - 16].

The device uses a single-use head with a number of fine solid needles, connected to a motor that rotates the head and thus facilitates skin micropunctures that will generate microchannels through the outer layer of the skin, allowing the passage of small molecules of active substances. These micropunctures in turn stimulate neocollagenesis, neovascularisation, expression of Wnt proteins and release of growth factors [17 - 19]. *Microneedling* can be used as an adjuvant treatment for AGA, associated with other light, physical or cosmetic techniques with an excellent safety profile [20].

Currently, the first-line treatments for AGA in men are oral anti-androgen drugs, 5-α reductase inhibitors: dutasteride and finasteride; dutasteride is the drug of choice as it inhibits both 5-α reductase isoenzymes, while finasteride inhibits only the type 2 isoenzyme. In various clinical trials, dutasteride has been shown to have a similar safety profile to finasteride, with greater clinical efficacy [21]. *Sabal serrulata* is also able to inhibit both 5-α reductase isoenzymes in human prostate tissue [22], although other studies have shown that oral intake of *Sabal serrulata* and certain nutritional supplements could increase the number of hairs in patients suffering from AGA [23, 24].

The aim of this study is to test the efficacy and safety of transdermal treatment of sterile cosmetic preparations already marketed and delivered via *microneedling*.

MATERIAL AND METHOD

The study was conducted in accordance with the principles set out in the Declaration of Helsinki, updated in 2013. It is a prospective study on the efficacy and safety of the combined use of microneedling and the subsequent application of topical preparations. Ten male volunteer patients, previously diagnosed with AGA, with a mean age of 46 years (range: 34 - 57), with Sinclair scale score 3.9 to 2.06, were enrolled. The study period was from September 2020 to December 2020 and was conducted at the Toska- niMed Medical Unit, Barcelona.

The following inclusion criteria were considered:

- Diagnosis of AGA not less than 3 years old.
- Increased hair loss in the months of confinement, from March to May 2020.
- No treatment for AGA in the 6 months prior to the study.
- Absence of known allergies.
- PCR and antigen test negative during the study and its development.
- Accept, understand and sign the specific informed consent.
- Explicit commitment to carry out the recommended home treatment.

Material used

A ***microneedling*** device (Neopen® Toskanimed, Equipmed USA LLC, Newport Beach, USA) with a head of 16 solid 33G-gauge steel needles was used. Trichoscopy, for scalp and hair imaging, was performed using the Handyscope® dermatoscopic device (FotoFinder Systems GmbH, Bad Birnbach, Germany).

The **trichoscopy** images obtained were analysed by the independent company TrichoLAB®, specialised in the analysis of trichological images, following the measurement protocol designed by themselves, which uses the Sinclair scale for the evolution of AGA as a reference. In this way, the subjective perception of the researchers was avoided.

The **photographs** were taken with high-resolution imaging equipment (VisioFace® RD, Courage + Khazaka electronic GmbH, Cologne, Germany) before the start of treatment and after the 5th and 10th session. To ensure the reproducibility of the images obtained from the study area, the equipment offers the option of superimposing the images taken in the last session with those of the current session. In addition, it has a homogeneous lighting system that allows the photographic shots to have the same lighting conditions in all the captures.

The sterile cosmetic products used were (Hair Cocktail Plus®, Toskani, SL, Barcelona, Spain) and their composition is detailed below:

- **Vial nº 1.** (Hair Cocktail Plus® of 10 ml, Toskani, SL, Barcelona, Spain) composed of:
  - *Sabal serrulata*, inhibitor of both 5αR isoenzymes.

    Coumarin and troxerutin stimulate microcirculation by promoting the arrival of micronu- trients to the follicle and hair bulb.
  - Vitamins B6 and B8, with anti-seborrhoeic action.
  - Glutathione, as an enzyme activator.

    Panthenol, with anti-inflammatory action.
- (HCPR® of 5 ml, Toskani, SL, Barcelona, Spain), consisting of:
  - Biomimetic peptides, prolong the anagen phase and shorten the telogen phase.

- bFGF (basic fibroblast growth factor), promotes blood microcirculation in the scalp.
- IGF-1 (insulin-like growth factor-1), strengthens hair by stimulating hair follicles.
- VEGF (vascular endothelial growth factor), promotes local angiogenesis.

Minerals (sodium, potassium, magnesium and iron).

- Amino acids and vitamins A, B, C, E and K as micronutrients that stimulate regeneration and strengthen hair.
- The pre-treatment preparation did not require topical local anaesthetics, disinfection was performed with chlorhexidine digluconate (Cristalmina® 1%, Laboratorios Salvat, Esplugues de Llobregat, Barcelona).

The treatment **protocol** for each weekly session was performed by separating the hair in longitudinal lines, unwinding the head of the device 2 times, first in the occipital-frontal direction and then in the fronto-occipital direction. The needles of the head were adjusted to a depth of 1 mm and the rotation speed was set at 7200 rpm. No bleeding was provoked, only erythema. Vial 1 was used for the first 6 treatment sessions, and vial 2 was used for the 7th to 10th sessions. After each procedure, no aftercare was required, except for not showering for the next 8-10 hours or bathing at the beach or swimming pool. All patients were able to return to their daily activities.

Three high-definition **trichoscopies** were performed, before the start of treatment (T0), after the 5th session (T5), and after the 10th session (T10), in each of the following areas: middle frontal area, 2 cm behind the hairline; left temporal area, 3 cm above the pinna, and in the occipital protuberance. The objective variables provided by the trichocopies were evaluated by the independent TrichoLAB® laboratory. The following variables were considered:

- Average number of hairs per control area [N/cm].[2]
- Average hair shaft thickness [µm].
- Cumulative hair thickness per surface area [mm/cm].[2]
- Sinclair scale.

The **home treatment** consisted of:

- Topical lotion (Anti Hair-loss lotion®, Toskani, SL, Barcelona, Spain). One application every night consisting of 7 sprays. Its composition includes:
  - *Sabal serrulata*.
  - Aminexil, with vasodilator effect similar to minoxidil and collagen regenerator.
  - Zinc, hair growth stimulator.
- Oral treatment (Densihair Boost Capsules®, Toskani, SL, Barcelona, Spain). It was recommended to take 2 capsules a day, with the following composition: *Sabal serrata*, zinc, selenium, biotin, lysine, cystine and arginine.

At the end of the treatment, all volunteers were surveyed to find out their level of satisfaction.

Statistical analysis. Data were expressed as mean ± standard deviation. An analysis of the norma- lity of the sample was performed using the Shapiro-Wilk test and the Wilcoxon non-parametric test was applied to compare the results between all study areas and with the baseline state. Statistical analyses were performed using SPSS v.20 (IBM, Madrid, Spain). A p value < 0.05 was considered to indicate statistical significance.

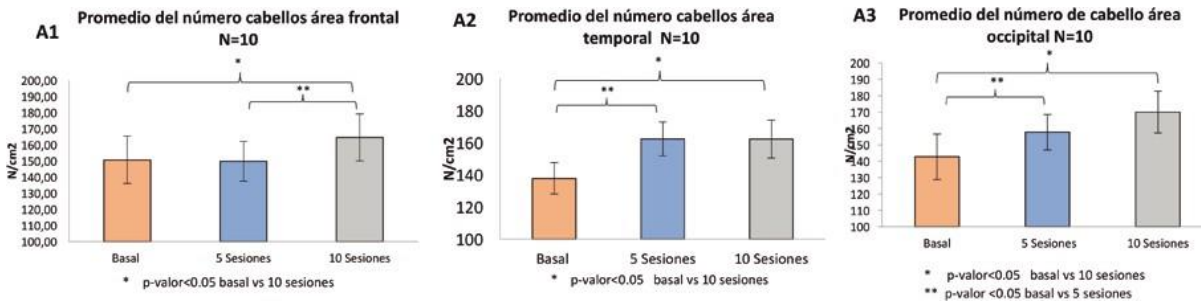
Participants' satisfaction data were obtained through individual surveys and expressed as a percentage.

The statistical study of the trichological analysis was performed by Tricholab System® based on the Trichoscopy Derived Sinclair Scale.

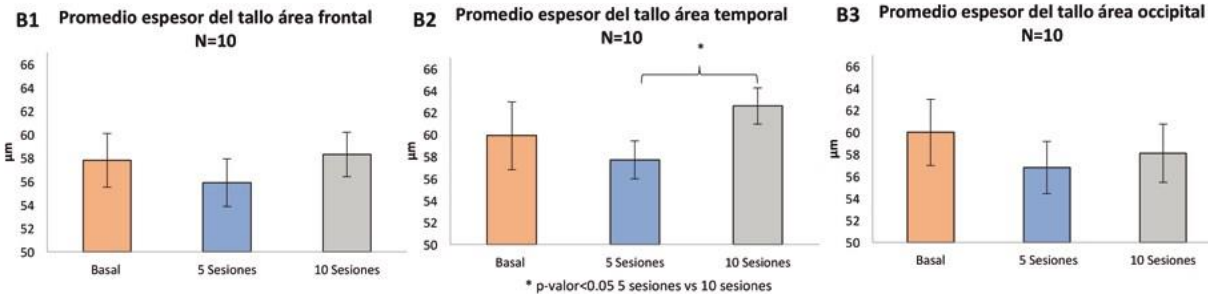
RESULTS

All enrolled patients completed the study. No volunteers showed any adverse effects during treatment or post-treatment. Secondary effects were those intrinsic to the micropuncture technique, being labelled as mild and transient: erythema and mild discomfort during the procedure.

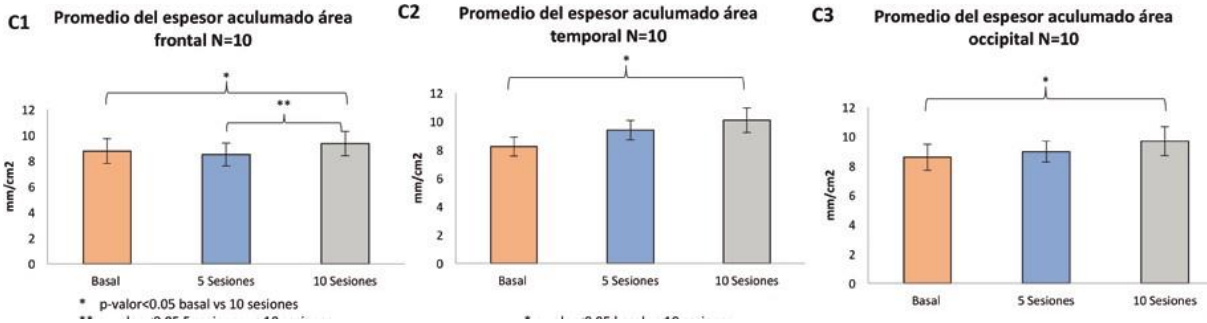
1) The **number of hairs** per area (N/cm2) was recorded in the frontal, temporal and occipital areas. The best result was obtained in the occipital area with an average of 16.9% ($p = 0.005$) compared to the baseline measurement. In the frontal and temporal areas, less significant improvement averages were obtained, 11.7% ($p = 0.036$) and 8.67% ($p = 0.047$) respectively (Figure 1). The overall improvement rate for all patients was 40%.
2) The result of the measurement of hair **shaft thickness**, expressed in µm, in the same areas showed a thinning in all areas from the initial baseline measurement to that taken after the 5th session, being greater in the occipital area (3.4%). Stem thickness recovered in all areas after measurement after the 10th session: increasing 0.7% in the frontal area, 4.4% in the temporal area and 0.5% in the occipital area (Figure 2).



Average results of the trichoscopic analysis of the number of hairs / cm² of the patients, in the frontal (A1), temporal (A2) and occipital (A3) areas, according to baseline count and after sessions 5 and 10.
The best result corresponds to the occipital area.



Results of the trichoscopic analysis of the average thickness of the hair shaft in the frontal (B1), temporal (B2) and occipital (B3) areas according to baseline count and after sessions 5 and 10.
A thinning of the hair thickness from the baseline result is observed in all of them until after the 5th session, with subsequent recovery, except in the occipital area.



Results of the trichoscopic analysis of the average cumulative thickness of the hair shaft in the frontal (C1), temporal (C2) and occipital (C3) areas. A progressive increase can be observed, from the baseline measurement to that taken after the 5th and 10th sessions, in all areas, especially in the temporal area.

The overall percentage of improvement of the patients, from the baseline measurement to that obtained after the 10th session, was: 60% in the frontal area, 70% in the temporal area and 40% in the occipital area.

3) The cumulative **hair thickness** (mm/cm²) showed an improvement at the end of treatment of 6.8% (p = 0.048) in 40% of patients in the temporal region, and 10.5% (p=0.046) in 69% in the occipital region (Figure 3).

4) The evolution of AGA according to the **Sinclair scale**, measured with the TrichoLAB® technology comparing baseline and after the end of the 10 sessions, was as follows:

a. In the frontal area, 30% of the patients achieved a significant improvement, whereas

Figure 4. Results obtained with treatment in the occipital area of the 43-year-old patient (no. 3) with initial AGA of 2.3 on the Sinclair scale. A, baseline condition. B, after 5 sessions. C, final result after 10 sessions.

Figure 5. Results of the 57-year-old patient (No. 5), with baseline AGA on the Sinclair scale of 3 and 2.8 for the temporal and frontal areas, respectively.
A, before treatment. B, at the end of the first 5 sessions. C, results at the end of 10 sessions.

40% were unchanged and the remaining 30% worsened slightly (estimated 1.23%). Overall, the average improvement went from 2.7 ± 0.2 from baseline to 2.8 ± 0.1 at the end of treatment.

b. In the temporal area, only 40% of patients showed improvement while 60% suffered a worsening compared to baseline. The average baseline status went from 2.7 ± 0.1 to 2.6 ± 0.1 in the final state.

c. In the occipital area, 60% of those treated experienced a degree of improvement and 40% experienced a slight worsening (estimated at 1.1%). The mean of the baseline condition went from 2.7 ± 0.2 to 2.7 ± 0.3 in the final condition.

Figures 4, 5 and 6 show details of the results achieved, before starting treatment and after completing 5 and 10 sessions, in 3 of the 10 patients treated.

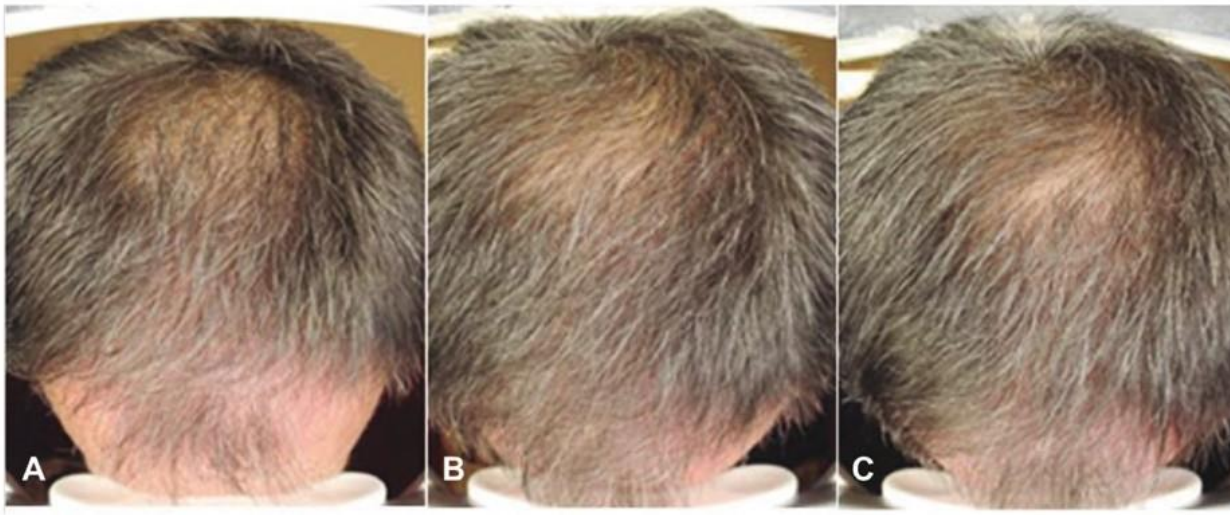


Figure 6. 57-year-old patient (No. 8) with baseline AGA on the Sinclair scale of 3.2 and 2.8 for the temporal and frontal areas. A, baseline condition. B, results after 5 sessions. C, results at the end of 10 sessions.

The patients' assessment of the results achieved were as follows:

a. All patients were satisfied with the results obtained.
b. Regarding the perception of the anti-hair loss effect of the treatment, 50% of the volunteers reported a strong improvement, 40% of the participants reported a moderate improvement and the remaining 10% reported a slight improvement.
c. In terms of hair density, 20% of respondents reported a strong improvement, 50% reported a moderate improvement and the remaining 30% reported a slight improvement.
d. Finally, regarding the appearance of new hair, 60% of participants noted a moderate improvement, 30% noted a slight improvement and the remaining 10% did not identify any improvement.

DISCUSSION

AGA is characterised by a progressive reduction in hair diameter, length and pigmentation in both men and women, causing not only aesthetic but also emotional problems [3]. Despite the existing social demand, there are currently only two drugs approved by the FDA for the treatment of AGA: topical minoxidil and finasteride [25]. However, some studies on the combination of the *microneedling* technique with minoxidil [16] have shown more effectiveness than the isolated use of minoxidil, reinforcing the hypothesis that *microneedling* favours the penetration of active ingredients while at the same time potentiating the neovascularisation [26]. On the other hand, treatments based on the inhibition of 5-α reductase action, finasteride and dutasteride, are effective in the treatment of AGA, although they are not free of adverse effects that limit or discourage their use [27].

For this reason, studies are being conducted on the basis of natural extracts that exhibit 5-α reductase inhibitory properties, such as *Sabal serrulata*, which have the potential to be effective in the treatment of AGA. Among their advantages are that they are well tolerated in humans and can be administered orally and topically; in the latter case, with the aid of *microneedling* for more effective absorption and therapeutic response, thus opening up a wide range of possibilities for the treatment of AGA. It should not be forgotten that not only hormonal factors contribute to the aetiopathogenesis of AGA, but also inflammatory and oxidative factors [27].

For the present study, a treatment protocol was proposed to address these three pathways involved in the aetiopathogenesis of AGA; firstly, using the *microneedling* technique to deliver the active ingredients contained in sterile cosmetic vials already on the market (Vials 1 and 2), complemented by a topical lotion and a food supplement, which were prescribed to all patients, forming part of the standardised protocol for all volunteers. Taking into account the typical male pattern of hair loss, with greater loss in certain areas, especially the frontal and occipital areas, the results have shown a particularly striking effectiveness in the occipital area, being

moderate-light hair loss in the frontal area, congruent with the hypothesis that the combination of treatments based on *Sabal serrulata* and biomimetic peptides combined with the action of serial micropuncture, which favours the penetration of these molecules while improving microcirculation in the area [8]. The results analysed show that hair loss has been slowed, with an increase in the number of new hairs and an increase in accumulated thickness [28].

A significant increase in the thickness of the hair shaft has also been observed, both in the frontal and temporal areas, being less in the occipital area. The decrease in the thickness of the hair shaft in this area has been interpreted in relation to the appearance of new hairs in this area.

The non-uniform response in the different areas may be conditioned by the short follow-up period, which we consider to be one of the limitations of the present study. It is therefore advisable to undertake further studies with longer follow-up periods in order to observe late responses to treatment and to verify the duration of the results obtained. Longer-term follow-up controls are needed, which will ultimately allow better adjustment of the frequency of future sessions.

Also, although the number of cases included in the Although the results achieved and the degree of satisfaction expressed by the patients showed that the treatment had met the volunteers' expectations in all cases. The number of sessions performed is consistent with the efficacy observed in terms of hair loss reduction, the safety of the active ingredients and the *micro-needling* technique used.

CONCLUSIONS

Transcutaneous administration of sterile cosmetics for topical use, vehicleised with *microneedling*, is a promising technique that shows evidence of decreased hair loss and new hair growth.

Side effects observed during treatment were limited to the appearance of erythema, accompanied by short-lasting itching in the treated area. The treatment is therefore considered effective, safe and accompanied by a high degree of patient satisfaction.

Conflict of interest

The ToskaniMed laboratory has provided the products and the use of its facilities to carry out the treatments used for this study.

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