

Vacuum Therapy in Association with Plant Active Compounds, Prebiotics and Probiotics for Treatment of Androgenetic Alopecia, Female Pattern Hair Loss and Telogen Effluvium, Part II: Follow-up Study

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Abstract

Aims: To present follow-up data regarding previously published study on vacuum therapy alongside the use of cosmetic emulsions containing plant bioactive compounds used for treatment of androgenetic alopecia (AAG), female pattern hair loss (APF) and telogen effluvium (TE) and associated symptoms.

Methods: The 07 participants from the initial study (n=25) who suffered from more severe cases of hormonal androgenic/estrogenic alopecia accompanied or not by effluvia were selected by a multidisciplinary team for follow up. These participants were followed up for 10 months after the initial study had concluded (D=57 days) by means of clinical and dermatological evaluations utilizing a digital microscope USB 1600; questionnaires were also used to assess subjective perception of the participants. 07 participants from the initial study (n=25) who suffered from more severe cases of hormonal androgenic/estrogenic alopecia accompanied or not by effluvia were selected by a multidisciplinary team for follow up. These participants were followed up for 10 months after the initial study had concluded (D=57 days) by means of clinical and dermatological evaluations utilizing a digital microscope USB 1600; questionnaires were also used to assess subjective perception of the participants.

Results: The benefits of activating capillaries utilizing vasodilating negative pressure at short, medium and long term were evident, making it clear this technology has applications. In part I of the study the efficacy of vacuum therapy alongside other dermatological procedures was assessed by both a dermatologist and a trichologist at the start and at the end of the study after 57 days. In part II of the study, the same team of researchers observed that 29% of the participants showed intense hair growth (n=02), 42% showed moderate hair growth (n=03) and 29% showed light hair growth (n=03) after therapeutic interventions had concluded.

Conclusion: Participants demonstrated increased hair growth 10 months after therapeutic interventions had ceased, with no further need of any home care hair products. These results evidence the effectiveness of the therapies previously employed for treatment and symptom remission of AAG, AGA and TE.

Keywords: Hair vacuum therapy • Androgenetic alopecia • Microbiome • Telogen effluvium • Female pattern hair loss

Introduction

Telogen Effluvium (TE) is a form of alopecia characterized by lack of scarification, afflicting individuals which already suffer from hair loss, and in its more intense occurrences adds up to losses of more than 100 hair strands per day; this is linked to a sudden change from “anagen” growth to “telogen” rest. The heterogeneity of TE is linked to several triggers associated with nutritional deficiencies, metabolism alterations, and use of medications, fever and other stress-inducing conditions [1-3]. TE can also be developed under other conditions, such as after surgery, pregnancy, infectious diseases, chemotherapy and even after strong virus-associated inflammatory reactions, such as seen in COVID-19 [1,4].

Under specific circumstances, Chronic Telogen Effluvium (ETC) can develop into other forms of alopecia, among them female pattern hair loss (APF) and Androgenetic Alopecia (AAG). Diagnosis of alopecia most often require recognition of histological, clinical and dermatoscope patterns, and

even then identifying the condition can be difficult; despite such difficulties, diagnosis of alopecia is reasonably frequent [2,3,5].

The homeostasis of skin microbiome is responsible for preventing proliferation of pathogen microorganisms and ensuring hair scalp physiology does not change drastically or abruptly. The skin microbiome is littered with a plethora of different fungi, bacteria and viruses, and their distribution varies among different skin areas among different people due to different skin pH, fat content, humidity and temperature. Other factors can also contribute to the establishment of microbiological populations, such as use of cosmetics, either normal birth or cesarean section, sun exposure, among others [6,7]. Hair scalp produces plenty of sebum due to the high amount of fat glands found in it, which may also possess antimicrobial properties. However, even if sebum prevents colonization of hair scalp by pathogens, some still manage to do so, such as *Cutibacterium spp.*, *Malassezia spp.* and *Corynebacterium spp.*, which even benefit from the presence of human physiological sebum. In research, hair scalp smears are often used for analysis of hair microbiome [7,8].

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Received: July 05, 2021; **Accepted:** July 21, 2021 ; **Published:** July 28, 2021

Negative-pressure generating vacuum therapy is a technique derived from therapeutical practices employed on the treatment of Fibroedema Gelloid (FEG), which acts upon subcutaneous tissue increasing local blood flow and eliminating subcutaneous infiltrate from interstitial liquids allowing metabolic exchanges to take place. This technique is non-invasive, and combines skin suction and negative pressure benefiting hair physiology greatly. Some studies evidence that mechanical stimulation of subcutaneous tissue via negative pressure under vacuum therapy leads to renewed vasoconstriction/vasodilation events, renewing and stimulating natural production of collagen and elastin [9]. The well-known use of platelet-rich plasma for treatment of hair loss due to AAG, APF or TE also makes use of vacuum therapy for inducing cell renovation and stimulating collagen production [10].

Toffanello et al. have carried out a clinical study demonstrating vacuum therapy in association with plant bioactive compounds can be an efficient therapeutic alternative for treatment of hair loss seen in AAG, APF and TE by reducing their overall symptoms [11].

The goal of this study was to compile the follow-up results on a previous work carried out on hair therapy utilizing vacuum therapy alongside the use of cosmetic emulsions associated with plant bioactive compounds and growth factors which might induce remission of symptoms caused by AAG, APF and TE. This study is the second part of the previously published work carried out by Toffanello and collaborators [11].

Methods

This work was carried out in accordance with regulations determined by the Brazilian Committee on Research Ethics (CEP/CONEP) under final approval number CAAE 07857119.9.0000.8227, as determined in 2019, February 29th. Experimental designs were conceived at the Ecolyzer Laboratory in São Paulo, Brazil.

The first step of the clinical study was carried in 2019 in the period between March 11th and May 7th, including 25 participants of both sexes aged between 18 and 65 years. All participants were assessed by a dermatologist physician, and the ones presenting unspecific hair loss, baldness or TE were further assessed by a trichologist. The study was carried out in a single center, and evaluation of the effects of 3 products available in the cosmetic market alongside vacuum therapy was carried out in a simple-blind manner throughout a 57-day period (D57) [11].

Participants were subjected to 2 hair washes utilizing Antiqueda shampooing. At the second wash, the shampooing remained on the hair of the participant for 3 minutes before being removed and the hair dried; next, the Epigem lotion was applied to the hair scalp on baldness areas, which remained on for further 15 minutes. Then, suction cups (multiplatform equipment Stim Hair, series number 150023 – Brazil) were applied to the hair scalp generating 520 mmHg of negative pressure, which was massaged in circular motions in order for local hyperemia and tissue displacement to be carried out for a total time of 15 minutes. At the end of the procedure, epicranial massage utilizing the thumbs was carried out aiming to stretch skin tissue; sliding movements were directed to parietal and occipital lymph nodes. Next, the hair scalp was dried with a towel and 10 drops of Nanofactor solution were applied to hair loss areas at days D0, D7, D14, D21, D28, D35, D42 and D49. Each time a participant went through this procedure in the laboratory, he or she were also instructed to wash their hair twice per week with the Antiqueda shampooing and utilize the Epigem lotion before sleeping, and repeat this whole procedure in the next day [11].

Evaluation of the efficacy of the therapeutic intervention relied on three different methods: assessment by dermatologist/trichologist either via direct visual observation or via digital phototrichogram; sensory evaluation by a trained technician; having the participants answer a questionnaire on their own perception regarding efficacy of the therapeutic interventions [11].

The second part of the study consisted of following up for 10 months the

individuals enrolled in the first part of the study after it had concluded (D=57 days), in the period of May, 2019 to March, 2020. Follow-up data were obtained at March 26th, 2020 via clinical and dermatological evaluations alongside questionnaires answered by the participants. From the initial study, 7 individuals (out of 25) who completed the study (and no longer made use of vacuum therapy and cosmetic products after 57 days) were selected and chosen for further assessment. Participants were selected by the multidisciplinary research team involved with the study (comprising a dermatologist, a biologist, a trichologist and other researchers), which had taken into account the most severe cases of androgenic and estrogenic alopecia, which could have been accompanied or not by visible effluvia.

At clinical and visual assessment, participants were screened for type of hair loss, hair color, and amount/caliber of hair strands, density of hair strands, oiliness and presence of erythema /itching in hair scalp. At March 26th, 2020 data acquisition utilizing a dermatoscope was standardized by small punctures done to a plastic cap shaped to best fit the scalp of each participant. This ensured measuring the effects of vacuum therapy and of the cosmetic products in a specific area of hair scalp was precise, as shown in Figure 1.



Figure 1. Photo registries showing the punctures made to the plastic cap shaped to best fit the hair scalp of each participant in order to ensure precise measures at 26th March, 2020.

Results and Discussion

At this second stage of the study, all 7 participants, 3 males (aged between 28 and 49) and 3 females (aged between 19 and 65), were followed up throughout the whole 10 months period after the end of the initial study. All returning participants concluded the study at its second stage. Clinical evaluations were carried out by the multidisciplinary team (dermatologist, biologist, trichologist and other researchers) present at the moment the participants returned for their assessment.

Clinical evaluation

Dermatological clinical evaluation was of large importance for assessment of the effects of vacuum therapy. Assessment by a physician not only provided insight on everyday clinical aspects of hair loss, but it also allowed for a better following of any changes or remission which have been observed throughout the course of the therapy employed in this study.

All participants had their impressions on overall hair scalp health, hair growth and reduction of areas afflicted by hair loss assessed via a questionnaire. These data aid clinical evaluations and better guide decision-making and the coming to conclusions.

Figure 2 shows that, after ceasing therapeutical interventions, 29% of the participants had intense hair growth (n=02), 42% had moderate hair growth (n=03) and 29% had light hair growth (n=02). It was also demonstrated that 57% of the participants showed increased strand thickness (n=04) and 43% showed slight increase of strand thickness (n=03).

After therapeutical interventions had ceased, it was observed that 43% of the participants showed moderate hair loss (n=03), 14% showed light hair loss (n=1) and 43% showed no hair loss at all (n=3). These results are shown in Figure 3.

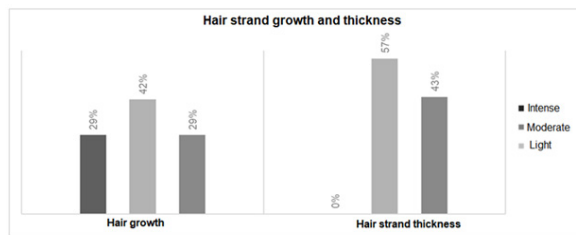


Figure 2. Clinical dermatological evaluation on hair growth (n=07) and on hair strand thickness 10 months after therapeutical interventions had ceased (n=07).

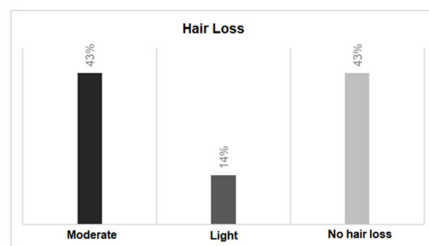


Figure 3. Clinical dermatological evaluation on hair loss (n=07) 10 months after therapeutical interventions had ceased (n=07).

Evaluation of subjective efficacy

A total of 14% of the participants noticed there was no increase in hair strand thickness (n=01), 14% noticed a light increase in hair strand thickness (n=01), 29% noticed an increase in hair strand thickness (n=02) and 43% of the participants showed actual increase in hair strand thickness (n=03) after ceasing of the therapeutic interventions.

As for noticing the growth of new hair strands, 13% of the participants did not notice any new strands (n=01), 29% noticed a slight number of new strands (n=02), 29% noticed a moderate number of new strands (n=02) and 29% noticed a considerable amount of new hair strands (n=02).

Clinical evaluation of hair strand thickness showed there was a moderate increase for 57% of the participants (n=04) while subjective assessment showed only 29% of the participants (n=03) noticed the same. However, subjective assessment also showed 43% of the participants noticed intense increase of hair thickness (n=03), evidencing the results between clinical evaluation and individual perception are comparable. Increased thickness of hair strands at their root indicates healthy hair physiology and likely anagen growth, which in turn indicate increased absorption of nutrients and thus a healthy distribution of anchoring proteins [12].

Subjective assessment of growth of new hair strands was not skewed towards increased perception for either light, moderate nor intense growth of new strands (29%, n=06). Such perception might be linked to noticing an increase in thickness rather than an increase of number of strands, as increased thickness of hair strand roots leads to a larger coverage of bald areas. However, clinical evaluation showed 29% (n=02) of participants had light increase of number of hair strands, 42% had moderate increase of number of hair strands (n=03) and 29% had moderate increase of hair strands (n=02).

The results make evident that, after 10 months of not being subjected to any sort of therapeutical intervention or not using any cosmetic formulations, vacuum therapy led to beneficial effects on hair growth. Hair loss was decreased and common symptoms associated with AAG, AGA and TE was reduced; benefits associated with increased local blood flow were also verified clinically and subjectively. Results between clinical and subjective evaluations were comparable, making clinical evaluations much more credible.

Thus, it is feasible to assume vacuum therapy can be viable in treating decreased blood flow in hair scalp capillaries due to its vasodilation actions

associated with negative pressure at both short and long term. At the first stage of this study, results showed there was significant difference between the number of hair strands in anagen growth phase at D1 (68.5) and at D57 (78.9) ($p=0.038$) and between strand density at D1 (9.95) and at D57 (12.34), $p=0.029$, meaning hair fibers became stronger after therapeutical interventions [11].

As a follow-up, during the second stage of the study, participants were monitored for 10 months for intermediate and long-term assessment of the effects of the vacuum therapy; no therapeutical interventions nor the use of any home care cosmetic products were used during this period. It was observed that, even after ceasing therapeutical interventions, vasodilating negative pressure in association with cosmetic lotions containing plant-based bioactive compounds and growth factors had prolonged beneficial effects. Throughout the 57 days at the beginning of the study (part I), the circular massage was essential for normalizing local hair scalp physiology and increasing activity of capillary vessels, facilitating permeation of growth factors and also of bioactive factors responsible for strengthening tegument microbiota. Such hypothesis takes basis on the data shown in the graphs (part II) (1) index of hair growth (2) and index of hair strand thickness, which are also reinforced by the comparable subjective analysis of the participants, making the potential use of vacuum therapy a credible option for treatment of hair loss.

The fluid cosmetic formulations and their bioactive compounds found in the home care products used during the first stage of the study were [11]:

(a) anionic-amphoteric micelle system resulting in a soft tensioactive of pH 5.5 (Shampooing Antiqueda) [13,14];

(b) anionic/polymeric emulsifying system (Nanofactor solution) containing Cooper tripeptide-1, Sh-polypeptide-9 and Sh-oligopeptide-2; these in association can increase the number of hair follicles and their growth due to the formation of compounds bioidentical to skin such as glycosaminoglycans of antioxidant and anti-inflammatory properties [13-15];

(c) maltodextrin-encapsulated system (Epigem lotion) containing association of *Serenoa serrulata*, *Pilocarpus microphyllus*, *Persea gratissima* and *Prunus cerasus* extracts alongside *Malpigia glabra*, *Saccharomyces cerevisiae* and yeast extracts. These compounds work as both prebiotics and postbiotics and prevent formation of edemas and sebum and also inhibit the 5-alpha reductase enzyme [8,16].

Antiqueda shampooing plus the Nanofactor solution temporarily acted upon the stratum corneum lowering its impedance and favoring the permeating of bioactive compounds to deeper epidermis layers. The Epigem lotion containing several extracts interacted with the lipid barrier of the hair scalp skin, preserving its healthy physiological state. Applying of these cosmetics was done in a manner that would not lead to any irritation or local inflammation which could have interfered with their efficacy or the efficacy of the associated vacuum therapy [17,18].

Under this context of therapeutical alternatives, it is reasonable to expand the present work into further studies assessing the efficacy of vacuum therapy for treatment of post-COVID-19 development of TE.

TE is the most common form of hair loss caused by nonscarring alopecia, especially among females. This is due to several risk factors, such as severe stress, non-supervised use of medication, poor nourishment, extreme diets, diabetes, among others; it is common for large chunks of hair to fall off when combing or untangling hair [1,2,3]. Other factors also might predispose the development of TE, such as surgeries, pregnancy, infectious diseases, chemotherapy and conditions associated with severe inflammatory processes, such as those seen in COVID-19 [1,4].

Stress-inducing factors are directly linked to hair loss of diffuse rarefaction, especially among women; TE usually develops throughout 60 to 90 days after stressful episodes. Stress levels associated with COVID-19 are undeniably high at both physiological and psychosocial levels, and

several studies link viral infection to development of TE. Also, use of drugs for COVID-19 treatment such as hydroxychloroquine, azithromycin, among others, can also be predisposing factors for development of TE. Global epidemiological factors including psychosocial stressors must also be considered when assessing the relationship between COVID-19 and the onset of TE. Further studies are still necessary to better elucidate how the viral infection in COVID-19 and concomitant inflammation disrupt the homeostatic pattern of anagen/telogen growth of hair strands, which lead to development of TE [19].

A balanced skin microbiome leads to skin physiology remaining under homeostasis, ensuring it exerts its functions as a physical barrier to environmental factors, preventing aggressive infections caused by any bacteria or viruses and aiding skin regeneration should any damage occur [18].

Almeida and collaborators [20], all dermatologists, studied 45 patients at 3 different clinical centers at both North and South America who had tested positive for COVID-19. The following parameters were evaluated: laboratory tests, medical history, clinical photographs, dermatoscope assessments; also, major skin manifestations which can arise due to COVID-19 infection were described alongside recommended treatments. Table 1 compiles such skin conditions and/or their aftermaths as seen in COVID-19 patients.

Table 1. Skin manifestations and/or their aftermaths as seen in COVID-19 patients [20].

Patients (n=) and (%)	COVID-19 (serology or PCR) (+) or (-)	Skin manifestations
11% (n=05)	(+)	Morbilliform rash
20% (n=09)	(+)	Itching/Erythema
51% (n=23)	(+)	Papule/Pustules
13% (n=06)	(+)	Petechia/Vasculitis
04% (n=02)	(+)	Livedo reticularis
15.6% (n=07)	(+)	Chilblain
22.2% (n=10)	(+)	Telogen effluvium

In this study, the etiological relationship between the inflammatory process seen in COVID-19 with itching, rash and papule/pustule lesions is not elucidated. The same is true for TE, where no causal relationship is described, even though it was the skin manifestation most frequently observed (22%); in these patients, it was observed their hair scalp had a very low oxygen concentration alongside considerable vasoconstriction, as shown in Figure 4. Diagnoses were carried out via traction tests, assessment of hair scalp biopsies and trichoscopy [20].

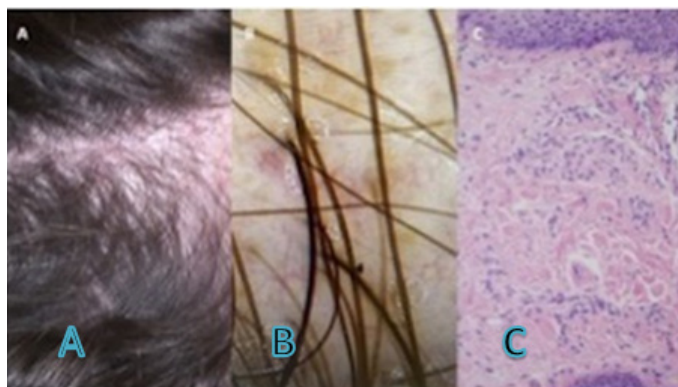


Figure 4. A: Telogen effluvium; B: hair scalp assays; C: histological analysis evidencing edema and dilated surface vascular plexus not accompanied by inflammation [20].

The histological analysis shown in Figure 4C evidences local edema and dilated plexus located in the surface area of the reticular dermis. In this context, small arterioles and capillary loops ascending to the surface of

the dermal papilla return to venous capillaries; however, the dilating plexus reduce tissue oxygenation, nutrition and hormone signaling, consequently leading to vasoconstriction followed by follicular contraction. Local inflammation is often observed alongside hair loss, but it was not described in this study [20].

Toffanello and collaborators (2020) carried out a clinical study investigating the efficacy of vacuum therapy alongside the use of formulations containing plant bioactive compounds for treatment of AAG, APF and TE and their symptoms, and it was shown such therapeutical interventions were indeed successful. Symptoms associated with these conditions are usually manifested as excessive oiliness, local erythema, itching, follicular inflammation, hair loss and reduced hair strand thickness [11].

The present work evidenced negative pressure can improve vasodilation of blood capillaries consequently increasing oxygenation of subcutaneous tissue, improving metabolic exchanges. Balance between pathogen and non-pathogen microorganisms prevent dysbiosis and the onset of several skin manifestations, which can further develop into more serious conditions, culminating in hair loss. The use of cosmetic formulations containing prebiotic and postbiotic compounds can improve such microorganism balance, greatly contributing to hair scalp physiology [11,21]. Figure 5A shows a poorly oxygenated hair scalp, lacking vascularization and with a deficit of hair strands. Figure 5B shows a hair scalp after 10 months of the last therapeutic intervention; a pinker color indicating tissue mobility and better oxygenation can be observed, as well as increased hair strand thickness.

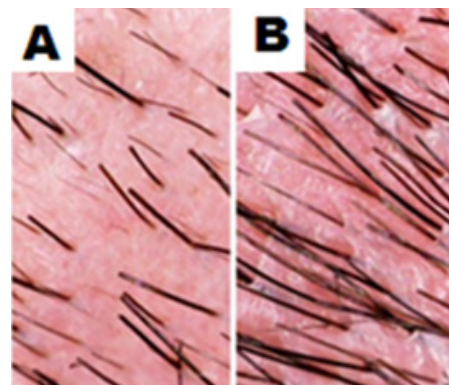


Figure 5. Follicular projections showing: A – Decreased hair strand thickness; B – Increased hair strand thickness.

Hair growth percentages, regardless whether for light, moderate or intense growth were all increased after vacuum therapy, showing how beneficial such therapeutical intervention can be by increasing tissue nourishment and hormonal signaling via activation of capillaries due to vasodilation. Activating hair scalp microcirculation led to benefits even 10 months after therapeutic interventions had ceased: hypervascularization, hyperoxygenation and draining of interstitial liquid [11,12,21].

In the study by Almeida and collaborators [20], histological analysis of hair scalp of COVID-19 patients showed dilation of surface vascular plexus, effect being likely associated with impaired tissue nourishment and lacking oxygenation, leading to physiological imbalances compromising anagen/telogen growth cycles, culminating in abrupt hair loss. These patients showed the development of telogen effluvium as the second most common post-COVID-19 skin manifestation, which might even have arisen due to side effects of misused medications. The histological analyses also showed a low level of local inflammation in all 22.2% patients which had developed post-COVID-19 TE, which is very unusual in individuals suffering from any sort of alopecia, as inflammation is commonly observed in under such conditions [20].

Conclusion

Vacuum therapy (negative pressure alongside hair scalp massaging) in association with the use of cosmetic formulations containing prebiotic and postbiotic plant-based compounds was effective in significantly attenuating the symptoms of AAG, AGA and TE. Hyperemia, erythema and any descaling processes were attenuated by vacuum therapy associated with the use of cosmetic formulations, improving oxygenation, balancing vasoconstriction/vasodilation and ensuring skin microbiome would not be altered, as shown in the First Stage of the Study.

Participants, after 10 months of the last therapeutic intervention and also not using any home care cosmetic products, showed symptoms associated with AAG, AGA and TE were clearly reduced, evidencing the lasting effects of vacuum therapy as shown in the Second stage of the Study. Such could be verified due to rigorous dermatological evaluation carried out by the multidisciplinary team of researchers in accordance with subjective evaluations by the participants themselves, clearly showing reduced hair loss, increased hair strand number and increased hair strand thickness.

Vacuum therapy can thus be useful for reestablishing normal hair scalp physiology due to its vasodilating properties. Also, the therapy of post-COVID-19 TE relies mostly on oral medication and topical cosmetics; the present work shows a potential treatment alternative for TE developed after COVID-19.

Acknowledgements/Funding

The authors acknowledge Self Cosmeceutics® for donating the cosmetic products utilized throughout the study, Ecolyzer Laboratory for carrying out treatment efficacy evaluations on study participants and also HTM Electronics® for donating the necessary equipment for vacuum therapy, which was used throughout the whole study.

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How to cite this article: Toffanello, Airton, Gomes Joao Paulo Correia and Moraes Carla Aparecida Pedriali. "Vacuum Therapy in Association with Plant Active Compounds, Prebiotics and Probiotics for Treatment of Androgenetic Alopecia, Female Pattern Hair Loss and Telogen Effluvium, Part II: Follow-up Study" *J Cosmo Trichol* 7(2021):167