Innovative techniques and protocoles for preventing and treating alopecia

S. BEILIN et S.BOISNIC
Paris
Benefits of combining mesotherapy (NCTF® 135 HA) and LEDs in the treatment of alopecia

Patient No.1. Stage II as per Ludwig classification.
1st mesotherapy and LED session on 09/11/2010

Photo No. 5: 5th treatment session 03/01/2011

Photo No. 6: 7th treatment session 19/01/2011
Regrowth clearly visible, marked improvement in the quality of hair and repigmentation (observed from the 6th session).

Photo No. 7: 8th treatment session 01/02/2011 Regrowth clearly visible from the front

Photo No. 8: 11th treatment session 03/06/2011
Continuation of the regrowth and improvement in the quality of hair (shiny and re-pigmented).

Photo No. 9: 20th treatment session 10/04/2012
Standard regrowth on the frontal and particularly temple hair line.

Photo No. 10: 21st treatment session 16/07/2012
Continued improvement.
CONCLUSION AND DISCUSSION

1 - DISCUSSION

The objective of this pilot study was to confirm the benefit of treating the tissue ageing element in androgenic alopecia. The NCTF® 135HA injections conventionally used for ageing skin in the mesotherapy technique «Mesolift» proved to be effective for ageing of the scalp and secondarily for the regrowth and quality of scalp hair (appearance, density, colour). The intake of antioxidants (vitamins C, E, glutathione), tissue nutrients in the form of vitamins (C, E, B group), coenzymes, nucleic acids, amino acids and non-cross-linked hyaluronic acid has boosted cellular metabolism with the synthesis of dermal proteins and angiogenesis; it has restored the extracellular matrix and helped fight against inflammation and tissue fibrosis. By increasing mitochondrial ATP production, the LEDs have, in synergy with the NCTF® 135HA injections, helped revive a normal cellular metabolism. The cell proliferation at the dermal papilla and the lengthening of the anagen phase and resulted, firstly in the immediate stoppage of hair loss (at the end of 1st month of treatment) and secondly, in hair regrowth, improvement in the quality and density of hair (from the end of 2nd month of treatment). This also explains better control of hair grats.

By allowing the scalp and hair bulb to fight effectively against oxidative stress, the treatment prevents cellular and particularly melanocyte apoptosis, inflammation and fibrosis of the hair follicle. The hair grows back with its original pigmentation that is to say, it is blacker in the case of our patients. The scalp regains its flexibility and its vascularisation leading to normal oxygenation, which makes it easier to inject on an appropriately vascularised scalp with proper bleeding.

The rapid stoppage of hair loss is the result of synergistic effects of LEDs and NCTF® 135HA injections. It is likely that the systematic loco-regional passing of injected metabolites for anti-radical and vasodilator effects (vitamin E and vitamin PP) explains the observed improvements (in women) in the quality of the skin (botox-like effect on forehead lines and wrinkles and crown’s feet), vision, and regrowth of patient’s eyebrows. These side effects are undoubtedly related to injections given to women in the lower temple areas compared to the men who are primarily treated on the top of the skull and the temple hair line. The wave lengths used during the irradiation by LEDs can vary during the protocol. The red light seems more appropriate initially to stop hair loss; it is then interesting to link it to the yellow light in order to stimulate new growth and improve the quality of hair.
2 - CONCLUSION

The general ageing approach applies to all the tissues. In androgenic alopecia, the NCTF® 135HA injections combined with LED help us act on all the parameters of ageing, oxidative stress, nutritional deficiencies, cellular metabolism and angiogenesis. The results are convincing with stoppage of hair loss, hair regrowth and repigmentation of the hair when there are functional bulbs and melanocytes.

This therapeutic protocol undoubtedly has major benefits combined with hair transplants and the first results are very encouraging. Further studies are planned in order to objectify the effectiveness of this protocol on the hair bulb and scalp using a histological study on the one hand, and a clinical study whose numbers help validate the protocol combining mesotherapy with the NCTF® 135HA and LEDs on the other, in the prevention and treatment of alopecia, especially the most common among them, androgenic alopecia.

BIBLIOGRAPHY

INTRODUCTION AND OBJECTIVE OF THE STUDY

The objective of this clinical study was to evaluate the effect of transdermal application after mesotherapy using Filorga (NCTF® 135 HA) on subjects suffering from advanced androgenic alopecia of the scalp or on subjects simply affected by a receding hairline in the temple area. The goal is thus to adopt preventive measures to limit hair fall or remedial measures to obtain stimulation of hair regrowth. The hair plays an important role in the overall aesthetics of the face. That is why hair care is essential for both men and women in our society. However, the effectiveness of anti-hair fall products is still limited [1].

From adolescence till the end of life, although hair density at the scalp keeps reducing, it reduces more prominently in the frontal and temporal region. This change corresponds to the inevitable ageing of the hair and is to be differentiated from genuine androgenetic alopecia. Numerous environmental factors could be involved in accelerating the ageing of hair such as stress, tobacco and pollution, sun rays, and food [2, 3, 4]. Oxidative stress at the hair follicle level will therefore shorten the anagen phase and prematurely trigger the catagen phase. The hair follicles are the only organs in the human body to renew themselves cyclically and asynchronously. The hair growth cycle is divided into three phases: the anagen phase (growth, 85% of the hair, duration of 3 years), the catagen phase (1% of the hair, duration of 3 weeks) and the telogen phase (resting and hair fall, 14% of the hair, duration of 3 months).

Androgenetic alopecia affects more than 40% of men and women after the age of 45. It can be considered that in the Hamilton-Norwood classification, actual alopecia starts from type III. There is no destruction but an involution and miniaturisation of hair. For men this alopecia starts by significant receding of hair from the temples and then from the vertex. The crown is not affected because the hair follicles are either less sensitive or not sensitive at all to androgens unlike other areas of the scalp. This form of alopecia is hormonal and genetic in origin with the presence of abnormalities on the chromosome 20 region. The second factor is hormonal with the action of androgens responsible for a shorter anagen phase and hypertrophy of the sebaceous gland significantly increasing the atrophy of the hair follicle [5].
3 - TREATMENT PROTOCOL

The stimulation of hair regrowth and improvement of the hair quality has been evaluated after carrying out the following protocol: 1 mesotherapy session every 15 days for 3 months, followed by 1 session a month for 5 months with transdermal application using a Filorga Roller (NCTF® 135 HA) i.e. a total of 11 sessions.

The sessions were carried out using a Mesoline Roller, a sterile medical device comprising a cylindrical head with 540 fine needles, 0.5 mm long. This device has a triple effect: it causes the stimulation of the scalp skin cells in response to the needles, creates micro-punctures that help optimise the penetration of the applied active ingredients and finally, improves blood circulation of the treated zone [6].

The product used for the mesotherapy was NCTF® 135 HA, a CE approved polyrevitalising solution, medical device, containing 53 active ingredients and free hyaluronic acid with a molecular weight of 1 million Daltons, of a concentration of 5 mg/ml. It also comprises 12 vitamins, vitamin C in particular, B vitamins (B8: biotin, B5: pantothenic acid, B9: folic acid, B1, B2, B3: nicotinamide, B6, B7, B12), vitamins A and E. The formula contains a strong antioxidant and glutathione playing a vital role in preventing oxidative stress on the hair follicles.

The clinical protocol used involved disinfecting the skin using chlorhexidine before every session of mesotherapy on the scalp. The NCTF® 135 HA solution is applied to the surface of the scalp. The roller is then used at a low pressure in multi-directional movements (6 times over each treated area). This treatment was primarily not for the alopecia areas but also for the rest of the scalp.

4 - EVALUATION

The hair regrowth was analysed in alopecia areas by displaying highly enlarged photographs of the subjects. The photographs were taken (front, 3/4 and profile) from a Canon EOS 50D camera with Macro ring MR-14EX and using lights to give the photos a daylight effect.

The quality and quantity of hair on the rest of the scalp other than the alopecia areas were also evaluated. In addition, the tolerance was also evaluated after every session (change in the scalp with the appearance of an erythema or an oedema).
3 RESULTS

In total, 5 men from the age of 31 to 67 (average 50 years ± 14.6) were included in the test with grades from I to V according to the Hamilton-Norwood classification. The evaluation was carried-out after 11 mesotherapy sessions. For subjects (no. 1 and 2) showing a change in the implantation of the scalp of intermediate grade between I and III according to the Hamilton-Norwood classification, an improvement in hair growth on the scalp after mesotherapy is noticed mainly around the temple area. The hair density is the most significant at this level (Figure 1 for subject no. 1, results after 9 sessions). For subjects suffering from genuine type V androgenetic alopecia (subjects 3, 4 and 5) an increase in hair regrowth was observed in the alopecia areas (Figure 2 for subject no. 3, results after only 6 sessions and Figure 3 for subject no. 5, results after only 6 sessions).

The tolerance of all the subjects was excellent.

4 RESULTS

The principle mechanism causing hair growth and resting of the hair is unclear; nevertheless apart from the essential hormonal (androgens) and genetic factors, vitamin and mineral factors must not be overlooked.

The treatment of hair fall and alopecia by mesotherapy aims to inject a mix of active ingredients using intradermal injection with the purpose of stimulating the vitality of the scalp, improving its condition and stopping hair fall by stimulating the bulb. In this test, the supply of vitamins and dietary minerals using mesotherapy has helped supply the hair follicles with elements essential for their growth.

Mesotherapy is a technique involving direct introduction of revitalising substances in the dermis through multiple superficial intradermal injections in small doses using extremely fine needles, a roller or a mesotherapy injector. It recreates a favourable environment to facilitate exchanges and interactions between cells and extracellular space [7]. The product administered for this study (NCTF® 135 HA) using mesotherapy combines hyaluronic acid that is injectable close to the HA of the connective tissue, with a revitalising...
solution containing vitamins, amino acids, nucleic acids, mineral salts and coenzymes. The previous studies carried out by the Filorga laboratory have helped end significant anti-radical activity by limiting the production of hydrogen peroxide and lipid peroxide following irradiation by UVA and B on human lymphoid cells using NCTF®. This compound thus contributes to the protection of cells against oxidative stress. An increase in the proliferation of human fibroblasts of elderly subjects and an increase in type I collagen synthesis were observed after the application of NCTF®. Through this action, the NCTF® compound causes cell renewal and partial renewal of the extracellular matrix. The group of B vitamins is also important since these vitamins are involved in the renewal process of the hair follicle and the production of keratin. Vitamin E and vitamin B3 in particular have a local vasodilator effect.

In this clinical test, an increase in hair density and improvement in aesthetic quality of the hair was observed around the alopecia areas (frontal hairline) in the 5 subjects after transdermal application of the Filorga NCTF® 135 HA product using mesotherapy. Thus, we can certainly foresee the preventive effect of transdermal application of the NCTF® 135 HA product on the receding hairline of the scalp by limiting the loss of hair in the temple area due to ageing and accelerated by environmental factors. Hair strands are keratin shafts produced at the bottom of an epidermal invagination: hair follicle. The hair bulb is the producing area resting on a highly vascularised dermal papilla. In this bulb, the cells essential for hair growth are matrix and germinal cells that multiply very actively and undergo keratinisation during their ascent in the follicle. These cells are in contact with dermal papilla made up of capillaries that will provide them with elements essential for their growth. The product was in contact with the hair bulb to facilitate its stimulating and antioxidant action. For subject no. 3 suffering from type V alopecia, a hair regrowth was also observed on the vertex affected by androgenetic alopecia as well as on the temple area for subjects 4 and 5. A remedial effect of NCTF® 135 HA was thus observed in this study with a regrowth in the scalp areas infected by androgenetic alopecia.

It would be interesting to continue this study with a larger number of subjects. The effectiveness of treatments intended for regrowth on the scalp however still remains very difficult to prove. The most commonly used methods are photographs and phototrichograms facilitating a dynamic study that provides information about the number of hair per surface unit, anagen-telogen distribution and hair diameter, conducted several months apart. In our study the analysis was conducted using photographs to assess the density and quality of hair [8, 9, 10].

5 CONCLUSION

In this test, the transdermal application of the NCTF® 135 HA product using mesotherapy has helped achieve a preventive effect by limiting hair fall in the case of subjects with receding hairlines in the temple area, and stimulating hair regrowth on the androgenetic alopecia areas.

BIBLIOGRAPHY

INTRODUCTION

The diagnosis of alopecia is essentially clinical; it is a result of the reduction or complete loss of hair. The number of hair follicles is 200 per cm², and the number of hair strands is between 80 and 150,000. The development of the hair follicle is cyclic and the phases are not synchronised. We lose 30 to 150 hair strands per day. There are three successive phases: a long growth phase (anagen) of 2 to 6 years (15 to 20 pilary cycles) during which the hair grows by 0.5 to 2 cm per month. Approximately 85 to 90% of follicles are in the anagen phase. The hair then enters an involuting phase (catagen) that lasts for about 3 weeks, followed by the resting phase (telogen) that lasts for 2 to 6 months and involves the development of new hair. The germinating phase of the anagen phase requires growth factors and proper nutrition (iron, proteins, zinc, vitamins). The hair follicle does not synthesise only keratin and melanin but also a significant number of hormones (hormones like: CRF, prolactin, cortisol, melatonin), neurotransmitters, neuropeptides and growth factors (TGF beta 1 &2, IGF 1, HGF) [1]. While hormones like the oestrogens and thyroid hormones are favourable for the growth of hair follicles, the male hormones, particularly dihydrotestosterone (follicular peripheral conversion of testosterone) cause the hair to shrink. An endocrine etiology for hypothyroidism, or less frequently hyperandro genesis can be considered when the alopecia is accompanied by signs of hypothyroidism (weight gain, cold intolerance, psychomotor retardation, etc.) or hyperandrogenism (hirsutism, menstrual problems, acne). A deficiency, of iron for example, could also be considered.

ANDROGENIC ALOPECIA: ETIOLOGICAL APPROACH

Androgenic alopecia is the most frequent cause of alopecia in men or women. The significance of androgens as well as the role played by hereditary factors has been known for a long time. The androgen receptor gene situated on the X chromosome is one of the genes susceptible to androgenic alopecia; the genes situated on chromosome 20 (recently identified as 20p11) would have a prognostic value [3]. With respect to men, it is now understood that there is an increase in the 5-alpha reductase.

*e-mail : gis.beilin@orange.fr
Benefits of combining mesotherapy (NCTF® 135 HA) and LEDs in the treatment of alopecia

activity, resulting in a local increase in dihydrotestosterone (DHT). This would inhibit cellular proliferation at the level of the dermal papilla as well as the local production of the VEGF (Vascular Endothelial Growth Factor). In androgenic alopecia, this local increase of the DHT is associated with the greater sensitivity of the receptor to this hormone conditioned by the genetic factor. When it comes to women, the origin is not definitively known. The oestrogens (pregnancy, contraceptive pill) have a positive effect on this diffuse alopecia, probably due to different mechanisms: anti-androgen effect, effect of increase in the VEGF, proliferative effect on the dermal papilla. As regards therapy, the 5-alpha reductase is ineffective in women; it is likely that the frontal and vertex alopecia sometimes seen in older women is less common than a physiological hypoandrogenism associated with a diffused loss. The combination of oestrogens and an androgenic progestin may provide some unconfirmed results today through clinical study [4].

Androgenic alopecia involves several environmental factors that are also responsible for the ageing of hair. Like the skin, hair also goes through an ageing process, the main two causes of which include intrinsic factors (genetic) and extrinsic factors (environmental): oxidative stress, sun rays (UVA and UVB), tobacco, emotional stress, water and humidity, cosmetics (colorants, curlers and straighteners), tractions for certain hairstyles. Ageing of hair eventually results in reduction of the duration of the hair growth phase, decrease in the diameter of the hair shaft, decreased elasticity and greying. These changes are secondary to the apoptosis phenomena and deterioration of the differentiation process, pigmentary system and vascularisation [5].

Oxidative stress is triggered by several factors like UV rays, certain medicines, pollutants, tobacco, emotional stress, inflammation of the skin and perifollicular inflammation. It will result in cell death since the redox system of the cell will no longer have the capacity to withstand. Glutathione, which comprises three amino acids – glycine, cysteine and glutamate, is at the core of the principal defence mechanism of the cell; it helps eliminate poisonous substances and peroxides. Ageing is accompanied by a drop in glutathione intracellular concentrations in the hair follicle with a decrease in protection against oxidative stress. This triggers an apoptosis of the melanocytes and the depigmentation of the hair [6]. The UVA and UVB rays reduce the adhesion of the cuticular scales, the hair becomes dry and brittle. The oxidation of the keratin caused by the UV rays leads to breaking of the di-sulphur bridges. The UV rays have a direct effect on the keratinocytes and melanocytes that are responsible for the cellular apoptosis and premature triggering of the catagen phase of the pilary cycle. They also have an indirect effect on these 2 types of cells by reducing the effect of glutathione. Tobacco is associated with premature ageing of hair combined with greying in men and women, as well as a more advanced alopecia. Tobacco affects the microcirculation at the level of dermal papillae resulting in deterioration of the trophicity and growth of the hair shaft. The course of action of tobacco is complex as the number of free molecules in cigarette smoke is very high (about 4,000), and includes free radicals and superoxide ions; the cadmium reduces the glutathione concentration. This pro-oxidant effect results in oxidative stress and releases cytokines responsible for inflammation of the hair follicle and perifollicular fibrosis. The endothelial cells are also very sensitive to oxidative stress [7]. Moreover, the follicular activity of the aromatases is reduced by the tobacco which results in follicular hypoestrogenism. Emotional stress changes the pilary cycle by shortening the anagen phase and triggering the catagen phase of the hair prematurely (demonstration on a mouse model). It also causes a perifollicular inflammatory reaction that is secondary to cytokine secretion.
Our experience tells us that mesotherapy is a potent therapeutic weapon against androgenic alopecia in men and women when functional hair follicles are still present, as well as when handling cases of postpartum effluvium. For men the most frequently used classification is that of Hamilton (Figure 2 as per [9]) which classifies alopecia into stages I to VII, in which stage III is the most commonly accepted as the real alopecia stage. The Ludwig classification is used for women (Figure 2 as per [10]). We are not going to review the text on molecules used for treating androgenic alopecia; Supplement no. 6 – 2011 of the Journal of German Society of Dermatology (JDDG)[9]: “Evidence-based (S3) guideline for the treatment of androgenic alopecia in women and men” provides an excellent review of the clinical studies conducted in this regard (Minoxidil, finasteride, Alopecia, 5-alpha reductase inhibitors, etc.). Mesotherapy seems to be more effective than the IV or IM injections [10, 11, 12] containing traditional substances. The substances traditionally used in mesotherapy for treating androgenic alopecia in France are Bepanthen®, biotine, DHE®, Laroscorbin®, polyvitamins. As the results were quite deceptive at times and the extrinsic etiological factors of alopecia were those of the ageing of the skin, it seemed logical to use the product that I injected for a Mesolift, the NCTF® 135 HA (FILORGA Laboratories). The anti-age approach for skin ageing of the aesthetic medicines allows us to consider androgenic alopecia as localised cell ageing of the hair follicle in a dermis and a subcutaneous tissue, which is also a result of the physiological slowdown of cellular functions. A proof of this is slight bleeding of the scalp from an injection shot, difficulty in injecting and dilating the scalp during the first session of mesotherapy, signs of hormonal deficiencies and post-inflammatory fibrosis. As we have seen earlier, it is now accepted that oxidative stress accelerates ageing of the hair follicle, induces genetic mutations and triggers the catagen phase that is associated with reduced peribulbar.
vascularisation. The objective is thus to prevent the catagen phase and extend the anagen phase by using cytoprotective molecules and anti-oxidants.

NCTF® 135 HA is a CE-approved poly-revitalising solution and a medical device which contains 53 active ingredients and free hyaluronic acid with a molecular weight of 1 million Daltons, which is close to that of hyaluronic acid present in the ECM, with a concentration of 5 mg/ml or 15 mg per bottle. The solution is sterilised by double filtration and not by autoclaving in order to preserve the vitamins, amino acids and hyaluronic acid. In autoclaving, saturated steam under pressure at a temperature of 120°C causes protein denaturation by partial hydrolysis of the peptide chains with destruction of vitamins and amino acids in particular. The composition of the NCTF® 135 HA is quite interesting and well-adapted to the actions sought: anti-oxidant action on dermal cells including endothelial cells, stimulation of the cellular metabolism and hydration. The NCTF® is composed of 12 vitamins, especially vitamins C, A, E and B vitamins (B8: Biotin, B5: Pantothenic acid, B9: Folic acid, B1, B2, B3: Nicotinamide, B6, B7, B12). The group of B vitamins is important because these vitamins are involved in the renewal process of the hair follicle, formation of keratin as well as synthesis of many hormones for vitamin B3. Vitamin E and especially vitamin B3 has a local vasodilatory effect which is interesting in this case. The formula contains a powerful anti-oxidant and glutathione which, as we have seen earlier, plays an important role in the fight against the deleterious effects of oxidative stress on the hair follicle and microvascularisation; it reinforces the anti-oxidant action of vitamins C and E. It is a combination of 23 amino acids including those that comprise glutathione, 6 minerals, 5 nucleic acids and 6 coenzymes including coenzyme A, NAD, NADP and UTP. In vitro studies have been conducted on the NCTF® by an independent laboratory (BIOalternatives, Gençay (86), 2007) in order to evaluate its effect on cell proliferation and the synthesis of extracellular matrix and its anti-radical activity. The effect on cell proliferation was measured on normal and older human dermal fibroblasts. The fibroblasts were pre-cultured for 24 hours in plates, 96 wells, containing 2,000 or 8,500 cells per well. After 24 hours of culture, the cells were treated with NCTF® for 72 hours. A radioactive label was incorporated 24 hours before the end of the experiment; the tritiated thymidine (2,000 fibroblasts per well) was used to assess cell multiplication and the tritiated proline (8,500 fibroblasts per well) was used essentially for the synthesis of collagen. The NCTF® significantly stimulated cell multiplication, 147% in the untreated control (p < 0.01) for normal fibroblasts and 148% in the untreated control (p < 0.01) for older fibroblasts. The NCTF® also stimulated the intracellular and extracellular (ECM) collagen synthesis for the normal fibroblasts; 165% in the untreated control (p < 0.01) in intracellular and 200% in the untreated (p < 0.01) in extracellular; for the older fibroblasts, 166% in the untreated control (p < 0.01) in intracellular and 256% in the untreated control (p < 0.01) in extracellular.

The anti-radical action of the NCTF® has been evaluated on the rate of hydrogen peroxide (H2O2) and lipid peroxidation (LP) in human lymphoid cells (Jurkat) that are or are not exposed to UVA and UVB irradiation. The H2O2 and LP were measured using the flow cytometry method involving specific fluorescent probes. The advantage of this method is a very high accuracy in measuring the fluorescence of each cell. Moreover, this measurement is carried out on a large scale (10,000 cells analysed per experimental condition). Culturing the cells on plates, 96 wells, with 10,000 cells per well, whether or not in contact with the NCTF® for 24 hours and 180 mJ/cm2 UVB and 2.4 mJ/cm2 UVA irradiation for H2O2 and 240 mJ/cm2 UVA and 3.2 mJ/cm2 UVA irradiation for LP. The reference anti-oxidant is BHA at 100 μM. The NCTF® significantly decreased the quantity of intracellular hydrogen peroxide with 72% protection and p < 0.01; the effect is equivalent to that observed in case of BHA, the reference anti-oxidant. For the rate of intracellular lipid peroxides, the NCTF® significantly protected the cells from UV irradiation; 90% protection and p < 0.01. Under these experimental conditions, the NCTF® showed considerable anti-radical activity. By allowing the limitation of the production of both hydrogen peroxide and lipid peroxides after UVA+B irradiation, the NCTF® contributes to cellular protection against oxidative stress. In addition to being a lubricating agent (traps 1,000 times its weight in water), the hyaluronic acid also contributes to cell proliferation and differentiation and angiogenesis. It is essential for the visco-elastic balance of the dermis and epidermis. It has anti-radical, anti-inflammatory and anti-bacterial properties [13].
The purpose of photobiostimulation by LEDs in the treatment of alopecia is to slow down or even stop hair loss and stimulate the follicles producing nothing more than a fine down. The work of Dr Daniel Mac has shown mitochondrial cell activation; the cells absorb photons and transform them into ATP, the energy source of the cell necessary for various metabolic processes [14]. The action in the mitochondria is mainly due to two photoreceptors that are cytochrome c oxidase and NADH dehydrogenase. The cytochrome c oxidase plays a crucial role as it is the terminal enzyme of the respiratory chain. Continuous light acts mainly on the mitochondria and pulsed light acts on the DNA.

This phenomenon of photo modulation reinitiates the cellular functions, particularly of the fibroblasts and endothelial cells that become deficient as a person ages. The action of the LEDs on the skin was assessed using immunofluorescence for the anti-radical and anti-inflammatory effect, and using a colorimetric method for increasing the collagen synthesis [15, 16]. The combination of the micronutrient supplementation, antioxidants and activation of mitochondrial functions seemed to be an interesting synergy for effective treatment of androgenic alopecia in both men and women. The action of LEDs improves microcirculation of the blood and oxygen supply, increases ATP production and therefore stimulates cell metabolism including protein synthesis. The anti-inflammatory action is also very interesting in normalising sebum production and reducing hyperkeratosis [17, 18, 19].

4 STUDY PRINCIPLE

1 - MESOTHERAPY: TECHNIQUES AND PROTOCOL

Mesotherapy is a French technique introduced by Dr Pistor in 1952 that involves injecting small quantities of the product next to the area to be treated. Its motto was “away from the right spot”.

A. Injection techniques

There are two main injection techniques – manual or using a jet injector. The traditional manual technique introduced by Dr Le Coz involves injecting microdroplets through micro-punctures that are about 4 mm apart in a very superficial manner. This mechanical technique requires a jet injector and helps prevent technical errors in case of inexperienced doctors as the injection parameters are already configured on the device. Using an injector can be effective for the first few mesotherapy sessions when the scalp is sclerotic and not very vascularised. It allows us to inject with a pressure that is higher than the pressure used for manual injections.

B. Protocol

After carefully disinfecting the scalp, the frontal, temple and if required vertex areas must be uniformly treated. A second round of injections must be carried out on the frontal edges and the temple hair lines. These multipunctures result in local vasodilatation and restart the neo-collagen synthesis. The polyrevitalising used is the NCTF® 135 HA, 1 bottle of 3 ml per session. The injections are performed with 30G needles fixed on a Luer-Lock syringe. The protocol consists of 3 to 4 sessions with a 15-day interval for the first 2 months followed by 1 session per month for 3 months. The frequency is then adapted to every patient depending on the results. In practice, we continued the treatment based on monthly sessions, as the patients did not want to stop the treatment. The treatment does not require pre-testing outside of patients with a known allergic or atopic profile. The contraindications are classic: infectious lesions, zoster, etc., allergies to one of the constituents of the injected substance. In case the patient suffers from itching or oedema during the treatment (which is rare), the treatment must be stopped and the patient must be administered antihistamines or corticosteroids. Consumption of blood-thinning medicines like aspirin should be avoided for 48 hours before the session. Although all prototypes can be treated, special care must be taken for dark skin as it presents a risk of pathological cicatisation: keloid, hypo and hyper pigmented scars. Tanning and using photosensitive products are not contraindications.
Benefits of combining mesotherapy (NCTF® 135 HA) and LEDs in the treatment of alopecia

2 - LEDS: DEVICE, PROTOCOL
An LED is a light emitting diode that produces incoherent monochromatic radiation through energy transformation. The power is measured in milliwatt/cm². This parameter is necessary to exactly determine the irradiation time required for each type of treatment. The time multiplied by the optical power is expressed in Joules per cm² [20, 21]. The device used is a Medical Light System (MLS); wave length is 590 nm for the yellow and 625 nm for the red. For treating alopecia, 12 J/cm² for the yellow and 14 J/cm² for the red. A 10-minute LED treatment can be given to the patient before or after the mesotherapy session.

The programmes used as part of the protocol are:
- For the 1st 3 months: 35 seconds of pulsed red (Mac Daniel) followed by 5 minutes of continuous red (near IR); this is followed by another 35 seconds of pulsed red (Mac Daniel) and 5 minutes of continuous red (near IR).
- From the 4th month during the regrowth of hair, which is still thin and fragile: 35 seconds of pulsed yellow followed by 5 minutes of continuous yellow, 25 seconds of red (near IR) and 5 minutes of continuous red (near IR).

The exclusion criteria were: pregnancy, postpartum effluvium, a pathology-related alopecia (alopecia areata, hypothyroidism, etc.) or alopecia related to nutritional deficiency, anaemia.

The 10 patients included: 5 men and 5 women. All of them had already consulted dermatologists or undergone surgery for treating their alopecia. Among the 5 men, two were siblings suffering from androgenetic alopecia in stage III and IV. Their ages were 32 and 23. The elder sibling had consulted for a transplant while the younger one was already suffering from a reduction of capillary density at the level of the vertex. The 3 other male patients were suffering from androgenetic alopecia that was resistant to local treatments. All of them had consulted a surgeon and considered hair transplants. There was one 68-year-old patient with white hair, the other two men, aged 39 and 45, were dyeing their hair and had already undergone their first hair transplant. The 5 women were menopausal without any hormone replacement therapy. They were aged between 55 and 65; 2 of them had considered a hair transplant and 3 were complaining about strong social embarrassment.

The effectiveness of the treatment was evaluated during each consultation; the evaluation criteria were:
- No hair loss,
- Regrowth,
- Increase in density and quality of hair.

These criteria were clinically evaluated and objectified by photographs using the same frame and magnification. The satisfaction of the patients and doctor was evaluated on a scale of 1 to 5: (1) Dissatisfied, (2) Not very satisfied, (3) Moderately satisfied, (4) Satisfied, (5) Very satisfied.

5 CLINICAL ASSESSMENT

The objectives of this first pilot study conducted on 10 patients was to verify the suitability and effectiveness of the combination of mesotherapy (NCTF® 135 HA) and LEDs in the treatment of androgenic alopecia, either at a very early stage as a preventive measure or at an advanced alopecia stage as a remedial treatment. The objective was also to identify the profile of the "responders" in order to refine the inclusion and exclusion criteria of a multicentre study in the future. The inclusion criteria were: Men and women, stages I to V of Hamilton classification for men and stages I and II of the Ludwig classification for women, the presence of hair follicles in the area to be treated, resistance to all previous treatments.

6 RESULTS

The observations made for the 10 patients treated are as follows:
- No rapid hair loss in 1 month after 1 to 2 sessions.
- A visible regrowth from the 3rd session in 2 months and a progressive increase, with every session, in the density and volume of hair and an improvement in the quality: more shiny, more glossy and re-pigmented.
- A regrowth of re-pigmented hair on the temple hair line and the frontal area (black hair for the treated patients).
Benefits of combining mesotherapy (NCTF® 135 HA) and LEDs in the treatment of alopecia

- During the check-up carried out after the 10th session, it was proposed to the patient to space out the sessions to 1 session every 2 months, 8 of 10 patients wanted to continue treatment at a frequency of one session per month.

Evaluation of the satisfaction: For the 10 patients treated the satisfaction score is 5 (very satisfied) for both the patients and doctor. All the requests for a hair transplant were abandoned.

1 - IN MEN
- All of them observed that the treatment immediately stopped the hair loss, enhanced the density and quality of hair and gave it a darker pigmentation (reappearance of the original hair colour of the patients).
- The areas treated were the vertex, temple hair line and frontal hair line.
- For a hair transplant patient for 4 months with very slow regrowth of the grafts (stagnation), 6 sessions in 6 months have accelerated hair growth.
- The 2nd transplant patient who underwent a transplant 2 years earlier, also observed a marked increase in the quality and density of hair.

2 - IN WOMEN:
- Normalisation of the scalp seborrhoea
- Improvement observed in all women in terms of the quality of facial skin particularly with a smoothing of the peri-orbicular and frontal lines and wrinkles (e.g. patient no. 1), in addition to an improvement in vision, in particular their presbyopia with clearer vision.
- A patient of 56 years observed an increase in the growth of her eyebrows; they were thicker and blacker.
- Sustainability of results after 1 year.

The overall patient compliance is excellent, the 2 least compliant patients are men: the youngest patient aged 23 has androgenic alopecia, lives abroad and motivates himself in his effluvium period. Despite sometimes widely spaced sessions due to his visits to Paris (6 sessions spread over 9 months), there is a significant improvement in the quality and colour of his hair. The second patient aged 68, satisfied with the stoppage of hair loss and the result after 6 sessions over 9 months, continues the treatment but irregularly.