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Intradermal injections of a hair growth factor formulation for enhancement of human hair regrowth – safety and efficacy evaluation in a first-in-man pilot clinical study

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ABSTRACT

Background: Research has shown the efficacy of hair growth factors in hair regrowth. We describe the intradermal injections of a recombinant, bioengineered hair formulation, containing growth factors, into the scalp skin, for enhancement of hair regrowth and evaluate its efficacy. **Objectives**: The objective of this study was to assess the efficacy and safety of the hair growth factor formulation in reducing hair loss and enhancing hair growth. **Materials and methods**: This was an open-label, prospective, single-arm interventional pilot study in which 1000 patients were given intradermal injections of a hair formulation into the scalp skin. The formulation contains vascular endothelial growth factor, basic fibroblast growth factor, insulin-like growth factor, keratinocyte growth factor, thymosin β4, and copper tripeptide-1 suspended in a sterile injectable vehicle. Intradermal injections of this hair formulation were injected into the scalp once every 3 weeks for a total of eight such sessions. Hair pull test was performed before every session. Videomicroscopic and global images were taken at baseline, fourth session, eighth session, and 2 months after the completion of the eight sessions. Relevant safety assessments through physical examination, questionnaires, and appropriate laboratory examination were conducted throughout the study. **Results**: Significant reduction in hair fall was seen in 83% of the patients on hair pull test. Videomicroscopic image evaluation showed that most patients had a decrease in the number of vellus hairs, increase in number of terminal hairs, and increase in shaft diameter. Seventy-five percent of the patients believed that the hair injections were aiding the treatment of their hair loss, and it was also beneficial in post-hair transplant patients. At 1 year, a statistically significant increase in total hair count ($P = 0.002$) continued to be seen. Treatment was well tolerated. **Conclusions**: Intradermal injections of this hair formulation may be a promising option for treating male as well as female patterns of hair loss.

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KEYWORDS
Alopecia; hair loss; growth factors; hair growth; novel formulation

Introduction

The most common cause of baldness or hair loss (95%) is androgenetic alopecia. (1) The current surgical, medical, and cosmetic interventions are limited in approach and success. (2) There are several growth factors which have been found to stimulate or inhibit different stages in the hair growth cycle. Various growth factors studied for hair follicle growth are vascular endothelial growth factor (VEGF) (2), epidermal growth factor (EGF), insulin like growth factor-1 (IGF-1) beta-fibroblast growth factor (bFGF) (3), wingless-related integration site (Wnt), noggin, keratinocyte growth factor, copper tripeptides, and more. These growth factors can be safe, cheap, and nonallergenic tools in the management of alopecia. (4–6)

So, we have prepared a bioengineered, recombinant formulation, consisting of a combination of growth factors, administered intradermally, capable of preventing hair loss and stimulating hair growth. Before conducting this pilot human study, the formulation was tested on animals to establish the concentration of the multiple ingredients, the safety, and the efficacy. (7,8)

Therapeutically acceptable ranges of the growth factors used are as below:

(i) VEGF (human oligopeptide-11) – 0.01–100 mg/L
(ii) Basic FGF (human oligopeptide-3) – 0.01–100 mg/L
(iii) Insulin-like growth factor (human oligopeptide-2) – 0.01–100 mg/L
(iv) Copper tripeptide-1 – 0.1–500 mg/L
(v) Keratinocyte growth factor – 0.01–100 mg/L
(vi) Thymosin β4 – 0.005–100 mg/L

In a preclinical trial experiment, 20 C3H mice were divided into four groups of five mice each The backs of the C3H mice (60 days old, telogen hair growth phase) were closely clipped on day 1 with an electric clipper. Four different solutions were prepared, each containing different proportions of IGF-1, VEGF, bFGF, KGF, thymosin β4, and copper tripeptide-1, in distilled water, labeled as solutions 1 through 4. Each group of mice received intradermal injections (i.e. infiltrated under the skin) of the sterile solution (group 1 received solution 1, group 2 received solution 2, group 3 received solution 3, and group received solution 4) of the respective pharmaceutical composition at two locations within the

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Table 1. Groups of C3H mice injected with different concentrations of the growth factor formulation.

	VEGF (mg/L)	bFGF (mg/L)	IGF-1 (mg/L)	Cu tripeptide-1 (mg/L)	KGF (mg/L)	Thymosin β4 (mg/L)
Solution 1	0.01	0.01	0.01	0.1	0.01	0.005
Solution 2	2	1	1	5	0.5	0.001
Solution 3	5	2	2	10	1	0.01
Solution 4	15	5	5	30	2	0.1

clipped back areas of the mice. Injection at two locations provided two test locations within the clipped area of each mouse. A separate group of five saline-injected mice (0.1 ml of saline per mouse) served as controls.(Table 1)

Following injection of the above intradermal pharmaceutical composition, indications of hair growth were seen within 10 days. The first visual signs were darkening of the skin in a circular region surrounding the injection site. The size of this region is generally dose-dependent, increasing with an increase in dose to a certain extent. The 0.1 ml injections used in this experiment produced a circle of hair growth measuring approximately 0.5–5 cm^2 in diameter. Active hair growth occurred between 14 and 20 days of the injection, with a maximum effect seen on day 30. Both the number of mice growing hair at the injection site and the diameter of hair growth region were determined on day 21. A positive response was observed with respect to the number of mice exhibiting hair growth at the injection sites compared to the total number of mice injected in the study. The results of this experiment are presented in Table 2 (the day of onset is the day at which hair follicle pigmentation was first observed).

Solution 3, comprising 0.0002 mg/0.1 ml of IGF-1, 0.0002 mg of bFGF, 0.0005 mg of VEGF and 0.0001 mg of KGF, 0.001 mg of copper tripeptide, and 1×10^{-6} mg of thymosin β4 in distilled water, gave the best response. Increase in concentration of the ingredients beyond that in solution 4 did not give any significant benefit in terms of number of mice growing hair or the diameter of hair growth region.

This hair formulation showing the best results, called QR 678 by us, is relatively free of untoward effects, as demonstrated by animal studies done to evaluate the safety and efficacy. This formulation, with the concentrations of individual ingredients so predecided, was then used in the human trial described in this paper. A QR Code is a code used in medicine derived from "Quick Response". The number 678 in Morse Code signifies "there is no answer". This formulation has been named QR 678 to signify a "Quick Response to a disease which earlier had no answer", i.e., to alopecia.

Table 2. Results in the groups of mice injected with different concentrations of the growth factor formulations.

Composition	Number of animals growing hair	Day of onset	Area of hair growth around the injection site
Solution 1	2/5	10	<1 cm diameter
Solution 2	3/5	10	>1 cm diameter
Solution 3	5/5	10	>1 cm diameter
Solution 4	4/5	10	>1 cm diameter
Solution 5	0	NA	NA

Materials and methods

This is an open-label, prospective, single-arm interventional pilot study, in which 1000 patients were treated for hair loss. The study was approved by the Institutional Review Board. Written, informed consents were obtained from all the patients.

Inclusion criteria

Indian men and women, 20–60 years of age, were included in the trial. Patients had male pattern/female pattern hair loss (male – Norwood Hamilton grades II–V and females – Ludwig, all three grades) which had not responded to 1 year or more of conventional medical therapy with topical minoxidil (2% in case of females and 5% in case of males) along with oral 1 mg finasteride for males (we specifically excluded any patients who had recently started or stopped these medications to avoid the confounding effects that this might have created) or those who had recent post-hair transplant hair loss (had undergone hair transplant surgeries within a year before study enrollment).

Patients had to agree not to change their hairstyle or use hair color throughout the study. Patients with diabetes, hypertension, and hypercholesterolemia underwent regular monitoring of their relevant parameters. All medications which affect hair growth were required to be withdrawn 6 months before the study and were not permitted during the study.

Exclusion criteria

We excluded patients with a hair loss history of less than 6 months. Patients with a history of multifactorial or serious drug allergy, history of or suspected malignancy, severe seborrheic dermatitis of the scalp, pregnant and lactating women were excluded from the study.

Preparation and physicochemical characterization of the growth factor formulation (QR 678)

In a preferred embodiment, the present formulation utilizes an intradermal pharmaceutical formulation which includes the growth factors in the concentrations, as given in the detailed formulation in Table 3, additionally along with vitamins, minerals, nucleic acids and amino acids, diluents, and/or carriers along with pharmaceutically acceptable diluents and/or carriers.

The present pharmaceutical formulation, as described above, is formulated for intradermal injection to the treatment area. Suitable vehicles for injection include, but are not limited to, saline and distilled water.

Preparation of the formulation

Five milligrams of VEGF, 2 mg of basic FGF, 2 mg of IGF, 10 mg of copper tripeptide-1, 1 mg of KGF, and 0.01 mg of thymosin β4 additionally with pharmaceutically/cosmetically acceptable and appropriate dose of vitamins, minerals, amino acids, and nucleic acids are added to 1 ml of distilled water. The formulation is then biologically sterilized and bottled into

Table 3. Final composition of the novel formulation (QR 678).

S. No.	Ingredients	Quantity
1	Vascular endothelial growth factor	0.01–100 mg/L
2	Basic fibroblast growth factor	0.01–100 mg/L
3	Insulin-like growth factor	0.01–100 mg/L
4	Copper tripeptide-1	0.1–500 mg/L
5	Keratinocyte growth factor	0.01–100 mg/L
6	Thymosin β4	0.005–100 mg/L
7	Vitamins	Vitamin A, vitamin B1, vitamin B2, vitamin B3, vitamin B5, vitamin B6, vitamin B7, vitamin B12, vitamin C, vitamin E, vitamin I, vitamin K
8	Minerals	Calcium, sodium, potassium, magnesium
9	Nucleic acids, essential and nonessential amino acids	

vials of 5 ml each. The formulation of the present invention is stable and can be stored at room temperature (below 25°C).

Injections of the formulation to the scalp

Multiple, tiny, and virtually painless intradermal injections of the solution were administered into the scalp skin. The injections were given using an insulin syringe, with a 31G needle. These injections were given in all areas of visible hair thinning and alopecia. A total of 1.5 ml of solution was injected per session by the nappage technique in the affected areas, once in 3 weeks, for eight such sessions. Approximately 60–70 injections are given per session to the scalp skin in the areas of hair thinning, intradermally by the nappage technique, each injection spaced 1 cm apart, the total volume per injection being 0.02 ml.

Scalp assessment and evaluation

At each visit, patients had to undergo safety assessments, which included physical examination and administration of a non-leading questionnaire about adverse experiences. Hair pull test was performed before every session by an independent observer. The "hair pull test" was performed three times by the same clinician, wherein a bundle of approximately 50–60 hair is grasped between the thumb, index finger, and middle finger and pulled from the base close to the scalp. The hair is firmly tugged away from the scalp, and the extracted hair is counted in every session. Periodic laboratory evaluation was done, along with global photographic assessment and video microscopic assessment to know the condition of hair growth. Subjective analysis was performed by administering the hair growth questionnaire comprising of seven questions, four relating to efficacy of treatment and three to satisfaction with the appearance of scalp hair. The final evaluation was done at 2 months after the eighth session.

Investigator assessments

(1) **Hair pull test**: Assessment to determine improvement in hair loss was done before every session with the Hair pull test. (Figure 1).

(2) **Global photographic assessment**: Standardized clinical photographs of the head for clinical assessment were taken at sessions 1, 4, 8, and 2 months after session 8. The vertex and superior frontal areas of the scalp were photographed using a standardized technique. Photographs were assessed by three independent dermatologists who graded each image from 0 to 10, where 0 represented no growth and 10 indicated full, thick hair growth. The scores were averaged and compared before and after.

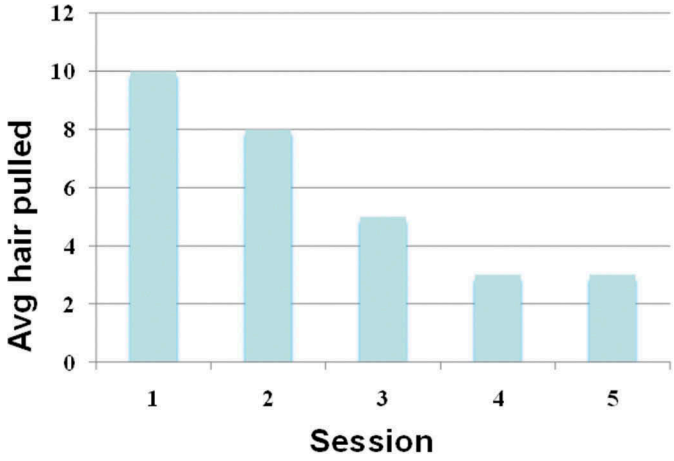

Figure 1. Hair pull test results.

(3) **Videomicroscopic assessment**: Videomicroscopic photographs were taken with a proscope digital handheld camera, at fixed positions on the central scalp, 15 and 20 cm posterior to the glabella. At each fixed position, images were taken through both ¼ and ½ cm windows to calculate hair counts per cm^2 (Figure 2). All videomicroscopic images were analyzed for changes in vellus hair count, terminal hair count, and hair shaft diameter, using specialized software (Trilogic Company, Moscow, Russia; Tricho science version 1.5 has been available since 2008 through Merz Pharmaceuticals, Frankfurt, Germany). Paired *t*-testing of the data was performed using Graphpad Software, an online calculator for statisticians (http://www.graphpad.com/quickcalcs/ttest1.cfm). $P < 0.05$ represents significant difference.

(4) **Patient self-assessments**: Patients completed a validated hair growth questionnaire comprising seven questions, four relating to efficacy of treatment and three to satisfaction with appearance of scalp hair. (9) (Table 4)

(5) **Safety assessments**: Medical history of the patient was recorded at the screening visit and a complete physical examination was performed. Safety assessments included physical examination and non-leading questioning about adverse experiences at each visit as well as periodic laboratory evaluations.

Laboratory evaluations

Hematology and serum biochemical analysis were performed at baseline, sessions 4 and 8, and then 2 months post the eighth session.

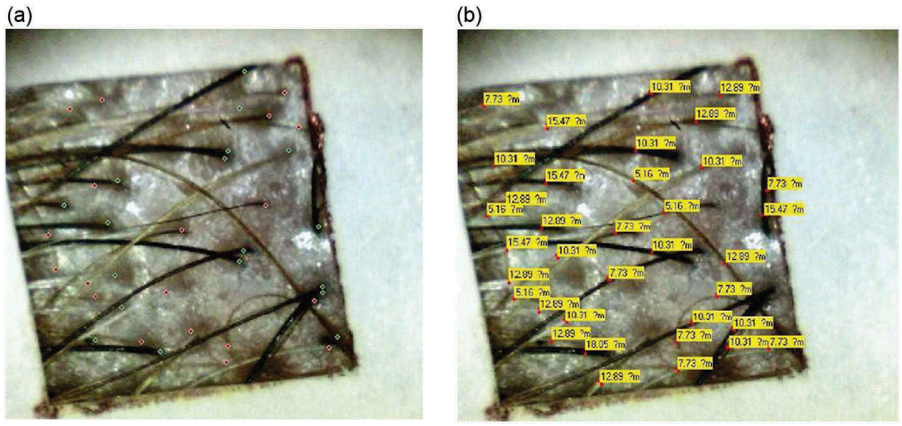

Figure 2. **A**Shows a photograph of ¼ cm cutout of videomicroscopic images showing vellus hair count (in red) and terminal hair count (in green). **2B**: Shows a photograph of ¼ cm cutout of videomicroscopic image showing assessment of mean hair shaft diameter. All measurements shown were multiplied by a factor of 2.77 for conversion to microns.

Table 4. Questionnaire administered to patients, to evaluate efficacy & satisfaction with the QR 678 treatment.

Sr. No	Question	Possible responses
1	Since the start of the study, I can see my bald spot getting smaller	Strongly agree › Strongly disagree
2	Because of the treatment I have received since the start of the study, the appearance of my hair is:	A lot better › A lot worse
3	Since the start of the study, how would you describe the growth of your hair?	Greatly increased › Greatly decreased
4	Since the start of the study, how effective do you think the treatment has been in slowing down your hair loss?	Very effective › Not effective at all
5	Compared to the beginning of the study, which statement best describes your satisfaction with the appearance of:	Very satisfied › Very dissatisfied
	a) the hairline at the front of your head?	Very satisfied › Very dissatisfied
	b) the hair on top of your head ?	Very satisfied › Very dissatisfied
	c) your hair overall ?	

Results

This study was conducted from May 2008 to May 2016. In this study, 1000 (680 males and 320 females) patients were included. The age of the patients ranged from 20 to 60 years. A total of 250 patients withdrew from the study.

(1) The primary factor for patient dropout seems to be the long duration of the treatment and the necessity of treatments every 3 weeks. This was further compounded by the fact that India is a very large country and our patients came from cities that were very far from Mumbai, where the trial center was located. Hence, we had included a large number of patients to begin with, anticipating this issue of dropout.
(2) Some patients realized after a few sessions that they were finding it difficult to commute every 3 weeks for the sessions.
(3) In case of some professionals, their sudden professional commitments entailed outstation travel.
(4) Some women got pregnant.
(5) Some participants could not resist the desire to chemically color the hair or treat the hair with straightening, etc., during the study period. These patients had to be removed from the study as they digressed from the inclusion criteria and requirements of no chemical procedures to be performed on the hair during the study duration.

Hair pull test

Before treatment, the average number of hair pulled out was 10. After four sessions, the average number of hair pulled out was three (which is a negative pull test) in almost 83% of patients and the pull test remained negative henceforth, suggesting a reduction in hair fall, which is apparent around the fourth session (Figure 1).

Videomicroscopic pictures

As seen in Figure 2.

Vellus hair counts

Vellus hair counts for each patient, taken at 15 cm from the glabella, are depicted in Figure 3 (only seven patients have been shown here). Overall, 86% had a decrease in the number of vellus hairs, while remaining patients had an increase. Paired *t*-testing indicated that, on average, after four sessions the patients had 8.57 fewer vellus hairs and after eight sessions they had 11.57 fewer vellus hairs than at baseline. This was statistically significant. Vellus hair counts, taken at 20 cm from the glabella, are depicted in Figure 4. Seventy-one percent of the patients had a decrease in the number of vellus hairs, while the remaining patients had an increase. Paired *t*-testing indicates that, on average, after four sessions the patients had 3.29 fewer vellus hairs, and after eight sessions they had 7.29 fewer vellus hairs than at baseline. Again, this was statistically significant ($P < 0.05$) (Table 5).

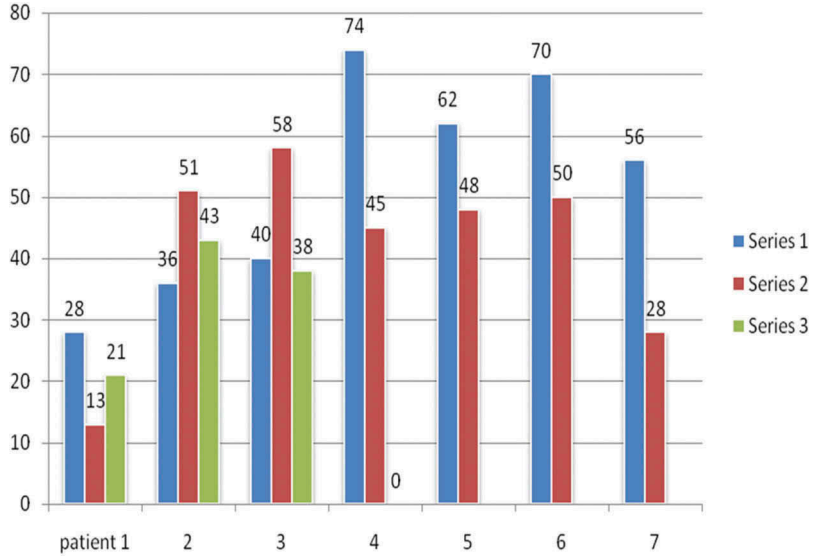

Figure 3. Vellus hair counts at 15 cm from the glabella after fourth session (shown for seven patients) X-axis = patient serial number; Y-axis = number of vellus hairs/cm^2 (series 1 = blue = baseline; series 2 = red = after four sessions; series 3 = green = after eight sessions).

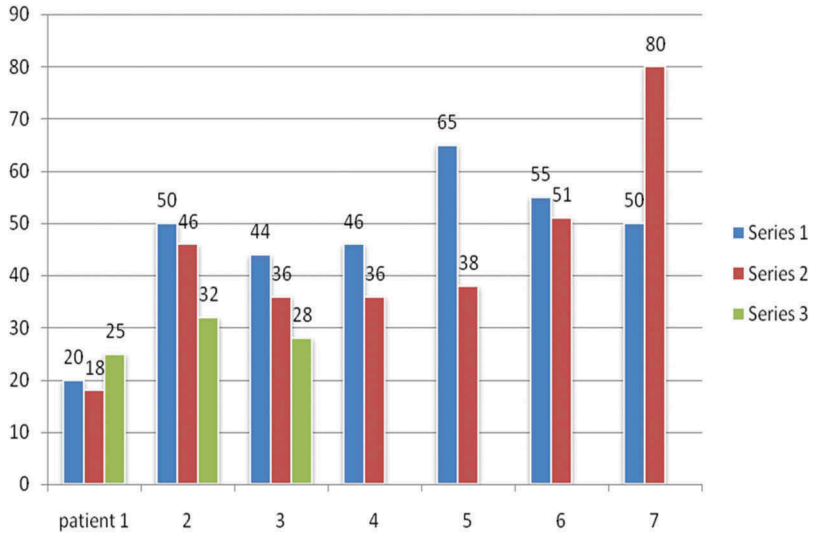

Figure 4. Vellus hair counts at 20 cm from glabella after fourth session (shown for seven patients) (X-axis = patient serial number; Y-axis = number of vellus hairs/cm^2) (series 1 = blue = baseline; series 2 = red = after four sessions; series 3 = green = after eight sessions).

Table 5. Vellus and terminal hair counts and hair shaft diameters, post QR 678 hair injection treatment.

	Mean at baseline	Mean after four sessions	Mean after eight sessions	δ	*P*-value
Vellus hair counts at 15 cm	52.43	43.86	40.86	−11.57	0.003131
Vellus hair counts at 20 cm	48.86	45.57	41.57	−7.29	0.006474
Terminal hair counts at 15 cm	64.14	71.71	78.71	+14.57	0.004183
Terminal hair counts at 20 cm	82.14	88.29	90.29	+8.15	0.004441
Hair shaft diameter at 15 cm (μm)	27.98	28.98	30.98	+3	0.006361
Hair shaft diameter at 20 cm (μm)	29.74	30.71	32.77	+3.03	0.005161

Terminal hair counts

Terminal hair counts for each patient, taken at 15 cm from the glabella, are depicted in Figure 5. Eighty percent of the patients had an increase in the number of terminal hairs, while other patients had a decrease. Paired *t*-testing indicates that, on average, after four sessions the patients had 7.57 more terminal hairs, and after eight sessions they had 14.57 more terminal hairs than at baseline. This was statistically significant. Terminal hair counts for each patient, taken at 20 cm from the glabella, are depicted in Figure 6. Seventy percent of the patients had an increase in the number of terminal hairs, while the other patients had a decrease. Paired *t*-testing indicates that, on average, after

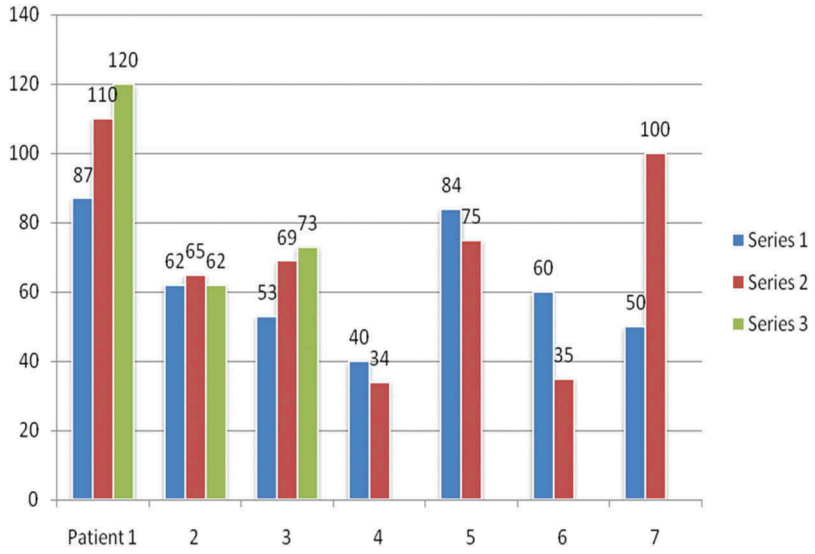

Figure 5. Terminal hair counts at 15 cm from glabella after four sessions (shown for seven patients) (X-axis = patient serial number; Y-axis = number of terminal hairs/cm^2) (series 1 = blue = baseline; series 2 = red = after four sessions; series 3 = green = after eight sessions).

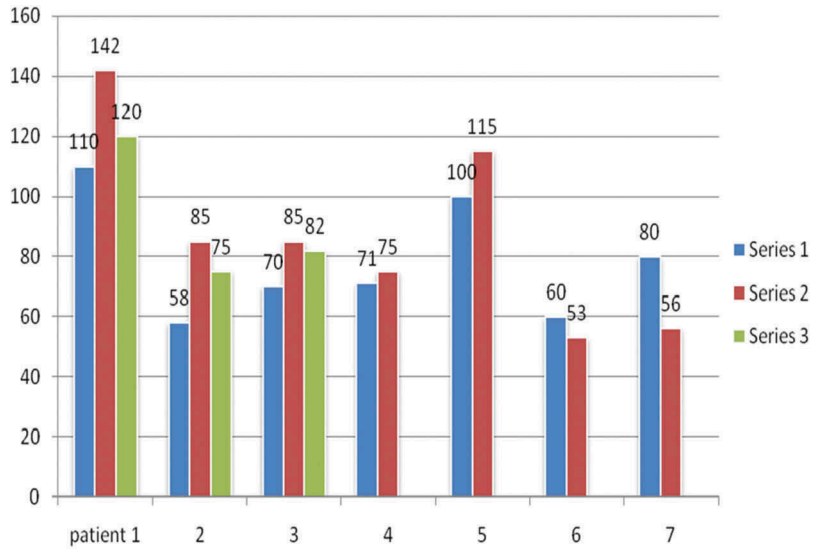

Figure 6. Terminal hair counts at 20 cm from glabella after four sessions (shown for seven patients) (X-axis = patient serial number; Y-axis = number of terminal hairs/cm^2) (series 1 = blue = baseline; series 2 = red = after four sessions; series 3 = green = after eight sessions).

four sessions the patients had 6.14 more terminal hairs, and after eight sessions they had 8.15 more terminal hairs than at baseline (Table 5).

Hair shaft diameter

The average hair shaft diameter for each patient, taken at 15 cm from the glabella, is depicted in Figure 7. Fifty-seven percent of the patients had an increase in the width of their hairs, while the remaining patients had a decrease. Paired *t*-testing indicated that after four sessions the patients had an average hair shaft diameter that was 1.0 μm wider and after eight sessions the average hair shaft diameter was 3 μm wider than at baseline. This was statistically significant. The average shaft diameter, taken at 20 cm from the glabella, is depicted in Figure 8. Paired *t*-testing indicated that after four sessions patients had an average hair shaft diameter that was 0.97 μm wider and after eight sessions the average hair shaft diameter was 3.03 um wider than at baseline. This was statistically significant. (Table 5)

Subjective evaluation of clinical photographs was provided by three blinded reviewers. All images were randomized prior to grading, so the reviewers did not know which was before or after. The results of the clinical photograph evaluation are provided in Table 6. There was an increased score for 71% of the patients, a decreased score for 10% of the patients, and no change in score for 19% of the patients. Figures 9, 10, 11, and 12 show the representative global photographs of patients at baseline and after four and eight sessions of treatment.

Finally, an overall opinion of the patients was assessed. It was observed that 75% of the patients believed it was helping in the treatment of their hair loss, 20% of the patients did not see any benefit, and the remaining 5%

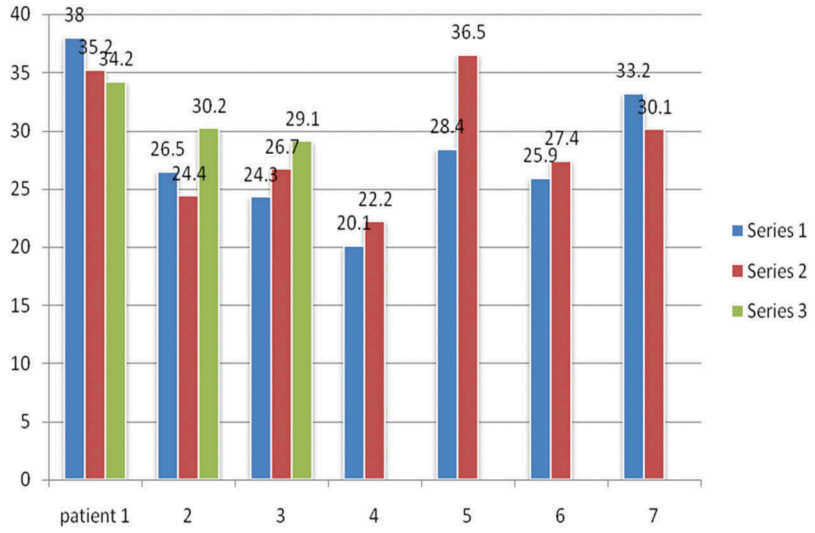

Figure 7. Hair shaft diameter at 15 cm from glabella after four sessions (shown for seven patients) (X-axis = patient serial number; Y-axis = hair shaft diameter in micrometer) (series 1 = blue = baseline; series 2 = red = after four sessions; series 3 = green = after eight sessions).

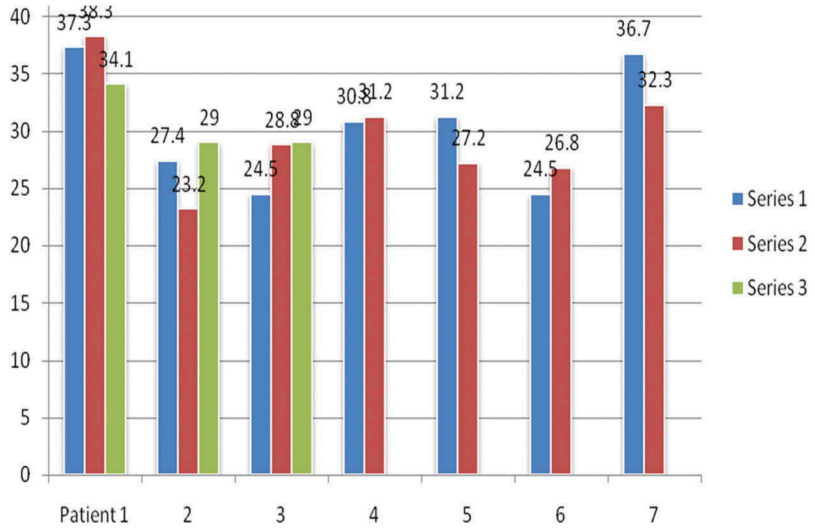

Figure 8. Hair shaft diameter at 20 cm from glabella after four sessions (shown for seven patients) (X-axis = patient serial number; Y-axis = hair shaft diameter in micrometer) (series 1 = blue = baseline; series 2 = red = after four sessions; series 3 = green = after eight sessions).

Table 6. Results of the clinical photograph evaluation.

Patient	Reviewer 1	Reviewer 2	Reviewer 3	Mean score	δ
1. Before	4	4	6	4.67	Increase
After	7	7	7	7.00	
2. Before	9	10	10	9.67	Same
After	9	10	10	9.67	
3. Before	4	6	7	5.67	Decrease
After	5	6	4	5.00	
4. Before	7	5	7	6.33	Same
After	7	5	7	6.33	
5. Before	5	6	5	5.33	Increase
After	6	7	6	6.33	
6. Before	8	7	7	7.33	Increase
After	8	8	9	8.33	
7. Before	5	5	6	5.33	Increase
After	5	5	7	5.67	

were not sure. The proportion of patients reporting improvement from baseline after eight sessions is depicted in Figure 13.

The treatment was effective in improving the appearance of scalp hair and slowing the loss of hair in men and women with patterned hair loss. Improvement in hair growth with therapy was evident as early as after four sessions for all measured end points. At 1 year, a statistically significant increase in total hair count (P = 0.002) continued to be seen.

In order to understand over what distance the QR 678 injections caused hair growth, the scalp was averaged into 48 quadrants, as described by Zimber et al. (2) We found that the QR 678 injections' effect on hair growth was limited to 1–2 mm around the injection sites. This is consistent with other publications demonstrating minimal diffusion of the growth factors when injected into the scalp skin. (2,3,7,8,10,11)

A total of 15% patients reported occasional slight itching of the scalp. Tolerable pain was experienced by most

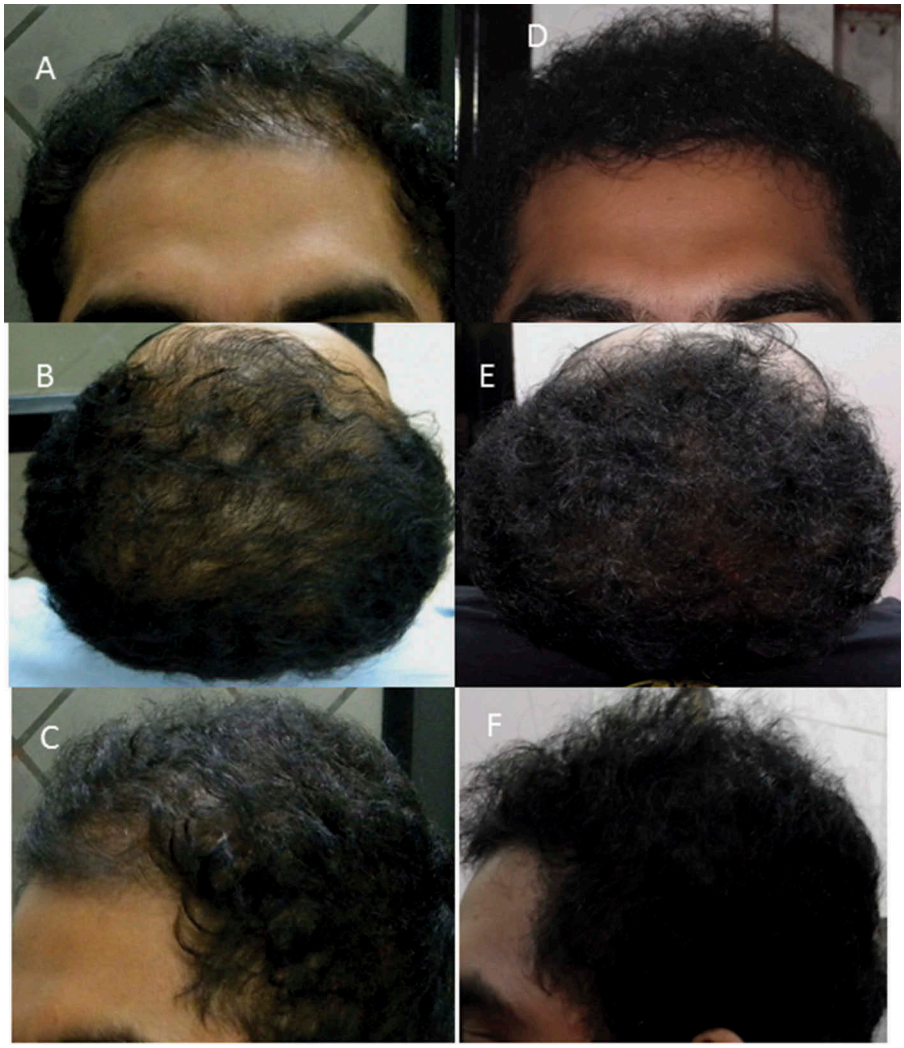

Figure 9. Photographs pre- and post eight sessions of treatment of a representative male patient.

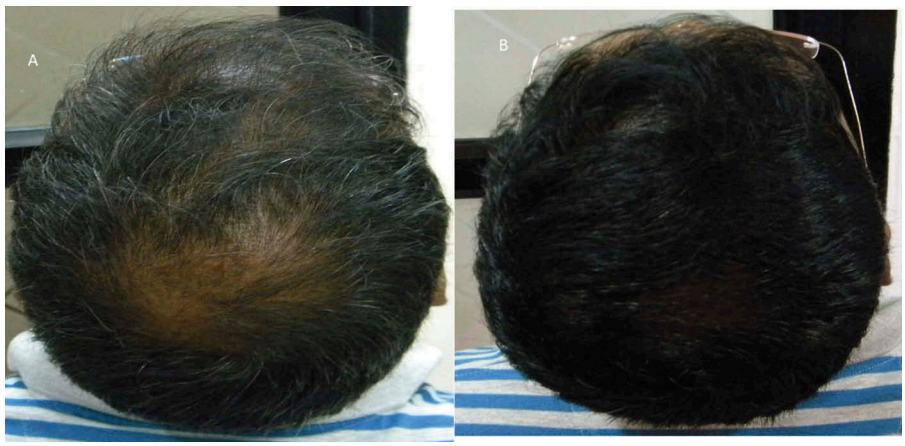

Figure 10. Photographs pre- and post eight sessions of treatment of a representative male patient.

patients while the injections were administered. There were no deaths or serious drug-related adverse experiences and no drug-related adverse experiences that resulted in discontinuation of the study medication during the study. The diabetics, hypertensives, and hypercholesteremic patients included in the study had been monitored, and there was no significant change in the biochemical values due to the injections.

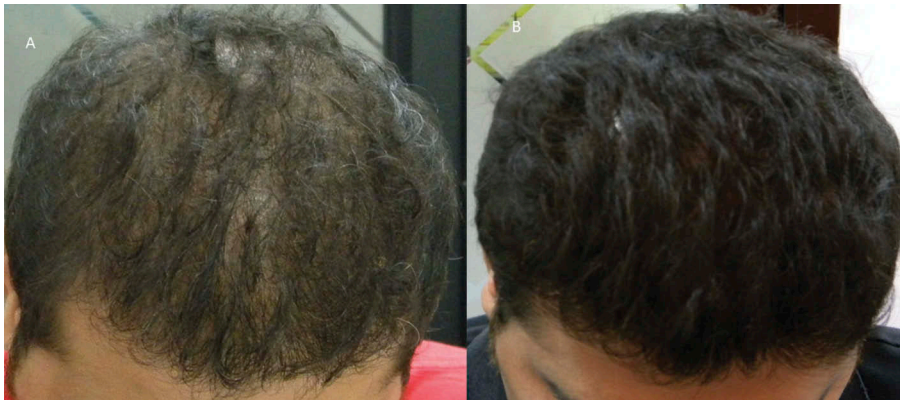

Figure 11. Photographs pre- and post eight sessions of treatment of a representative post-hair transplant male patient.

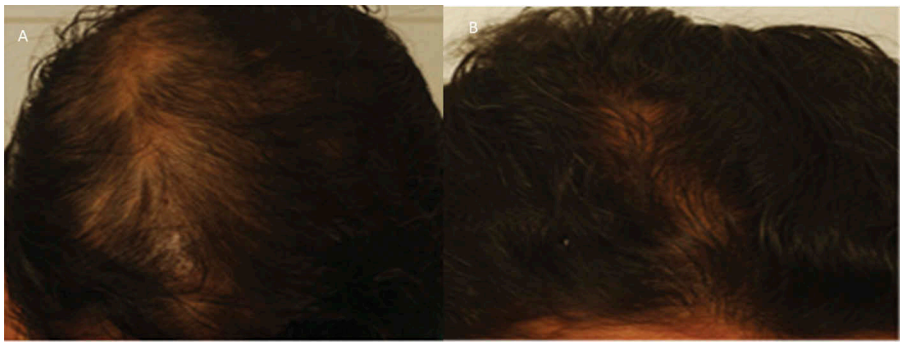

Figure 12. Photographs pre- and post eight sessions of treatment of a representative female patient.

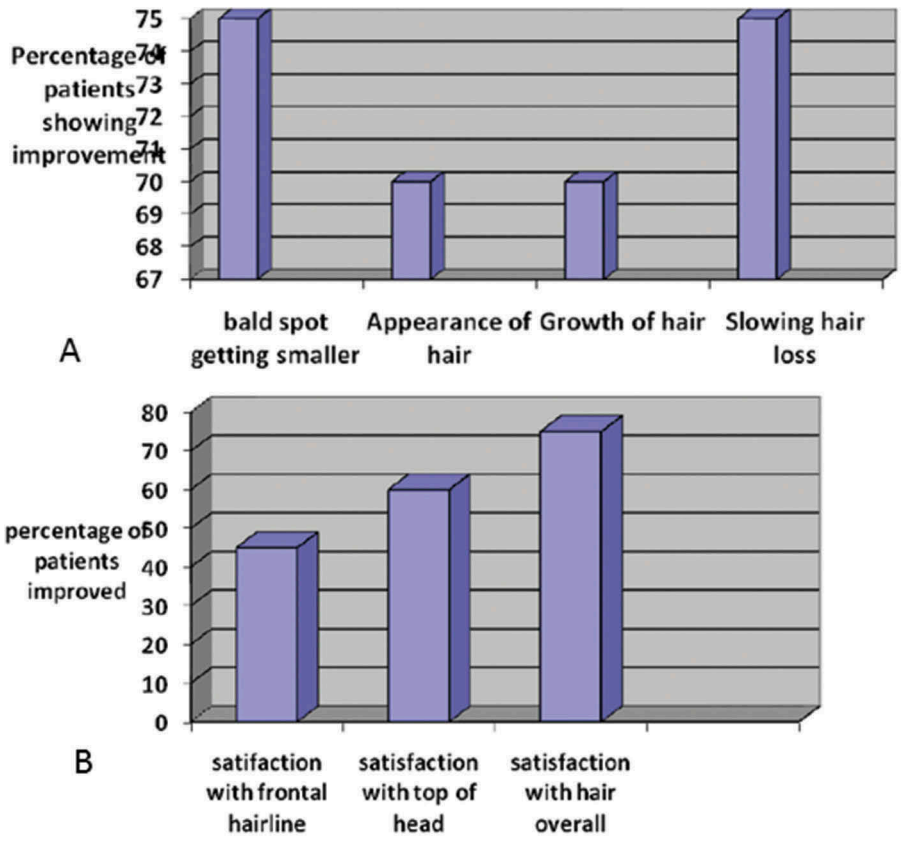

Figure 13. The proportion of patients reporting improvement from baseline after eight sessions (A and B).

The findings of this study suggest that the beneficial clinical effects of this therapy are similar in men and women, across different age groups, and in patients irrespective of the presence of metabolic disorders like diabetes, hypertension, hypercholesterolemia, etc. The differences in results across gender groups are not statistically significant. Moreover, results indicate that therapy was also effective in controlling hair loss in 14 post-hair transplant patients. There was a negative correlation between the duration and stage of hair loss, and the degree of improvement.

Discussion

We have developed a novel formulation which may be a good treatment option in our armamentarium to fight hair loss. Improvement in hair growth with therapy was evident as early as after four sessions, for all measured end points. After eight sessions, global photographs showed improvement from baseline for 71% of the patients, a decreased score for 10% of the patients, and no change in score for 19% of the patients. At 1 year, a statistically significant increase in total hair count (P = 0.002) continued to be seen. In our study, we have shown the benefits of a combination of specific growth factors in stimulating hair growth. Several researchers have studied the effects of various individual growth factors on hair growth.

VEGF, essential for angiogenesis and vascular permeability, may be responsible for maintaining proper vasculature around the hair follicle during the anagen growth phase. (10) KGF is highly capable of counteracting chemotherapy-induced alopecia, and it is one of the components of our formulation. (12) IGF-I is critically involved in promoting hair growth by regulating cellular proliferation and migration during the development of hair follicles. IGF-I has been reported to prevent the follicle from developing catagen-like status. (13,14) Thymosin β4 promotes hair growth in various rat and mice models, including a transgenic thymosin β4-overexpressing mouse, by influencing follicle stem cell growth, migration, differentiation, and protease production. (15) The bFGF has been found to promote hair growth by inducing the anagen phase in resting hair follicles and has been considered to be a potential hair growth-promoting agent. (16) The effects of l-alanyl-l-histidyl–lysine-Cu^{2+} (AHK-Cu) copper tripeptide on human hair growth ex vivo and cultured dermal papilla cells was investigated and shown to promote the growth of human hair follicles. (17)

Platelet-rich plasma (PRP), which contains growth factors, has been studied as a new method for hair regrowth in pattern hair loss. Multiple studies have concluded that PRP injections are a simple and feasible treatment option for androgenetic alopecia, with high overall patient satisfaction. (18–22) However, it is a tedious procedure, as the patient's blood needs to be drawn in every session, requires special equipment, and there is no standardization of the method of preparation, the effective dose, the duration of treatment, and the long-term safety profile, apart from the fact that it is also an expensive procedure.

On performing a detailed literature review, we came across only one small study using growth factors as combination treatment to treat hair loss. (23) The study was carried out in 11 Korean women (mean age, 41.36 ± 2.43 years) with female pattern hair loss. The major components of the topical solution used for treatment were bFGF (2.5 μg/mL), IGF-1 (1 μg/mL), VEGF (2.5 μg/mL), stem cell factor (2.5 μg/mL), KGF-2 (2.5 μg/mL), superoxide dismutase-1 (5 μg/mL), and noggin (2.5 μg/mL). The effects of the topical application of growth factors were suggested to result from the effective penetration afforded by microneedle therapy. (23) Ours is a much larger study, with specific growth factors used through a simple intradermal injection technique and does not require microneedle use, which is very painful for patients.

In total, 15% of the patients reported occasional slight itching of the scalp for a few hours post the injections. Tolerable pain was experienced by most patients while the injections were being administered. There were no deaths or serious drug-related adverse experiences and no drug-related adverse experiences which resulted in discontinuation of the study medication during the trial. The diabetics, hypertensives, and hypercholesteremic patients included in the study had been monitored with respective tests after four sessions and after eight sessions, and there was no significant change in the biochemical values due to the injections of the novel formulation.

A major limitation of this study is the lack of a control group. This was a pilot study and since the results preliminarily seem encouraging, randomized controlled trials are currently underway.

Several factors may have biased the reviewers' blinded analysis of the images. In studies of minoxidil (24) and finasteride (25), tattooing has been used to identify the exact location of the scalp that is being monitored. Likewise, trimming of the hairs in areas of the scalp undergoing treatment can provide greater accuracy in analyzing results. Phototrichogram, the use of which necessitates both the above methods, seems to be more suitable for clinical trials. However, it is not diagnostic, is tedious, time-consuming and subjective, and requires expertise. (26) As this was an independent study, where no patients received monetary reimbursement, it was difficult to convince them to allow us to tattoo their scalps. Unfortunately, we were also not able to convince our female patients to let us trim their hair, especially because their hair was already thinning.

Trichoscan is a digitized phototrichogram that combines standardized epiluminescence microscopy with automatic digital image analysis for the measurement of human hair. The software quantifies the hair parameters within one operation. The use of trichoscan initially involves shaving a scalp area, dyeing it after 3 days, taking a digital photograph, and analyzing the data using a software. Processing of photographs is speedy and results are reproducible. (26) However, use of the trichoscan needs clipping of hair in the study area and dyeing it, which was not feasible in our study. Also, there have been recent disputes regarding the accuracy of Trichoscan (Tricholog GmbH, Freiburg, Germany), and many physicians have observed that the Trichoscan software is error-prone and not precise. (26)

Canfield methodology uses a stereotactic positioning device for global photography on which the patient's chin and forehead are fixed, and on which a given camera and flash device are mounted. It assures that the view, magnification, and lighting are the same at consecutive study visits. (26) In our resource-limited settings, we did not have access to the device; however, our investigators had a separate room dedicated for global photography, where the lighting and the distance were maintained throughout the study. We photographed the scalp at fixed positions of 15 and 20 cm from the glabella and used videomicroscopic assessment and analysis of photographs as the objective assessment test.

More studies are needed to fully assess the role of this formulation containing a combination of growth factors (the QR 678) in hair growth. Whether more sessions will give added benefit, does the time interval between two sessions make a difference, if it would help in other forms of alopecia also, for example, post-chemotherapy hair loss, alopecia areata, etc., are some unanswered questions at present. More randomized, single and multicentric trials with a large number of patients will be required to prove the validity of these results, to obtain these answers, and to get more robust statistical results.

In conclusion, this pilot study represents the first independent study of a unique combination of growth factors and hair growth. The findings of this study suggest that this formulation of multiple growth factors (QR 678) is safe and efficacious in treating male as well as female pattern hair loss and alopecia.

Declaration of interest

The authors have no conflict of interests in any of the materials and methods utilized in this paper.

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