Combination of Minoxidil 5% with Carboxytherapy versus Minoxidil 5% Alone in Treatment of Female Pattern Hair Loss

Fatma Alzahraa Rabea Mohammed Khedr*, Mohamed Khaled Galal Selim, Hanan Fathy Mohamed
Department of Dermatology, Andrology and STDS, Faculty of Medicine, Mansoura University, Egypt
*Corresponding author: Fatma Alzahraa Khedr, **Mobile:** (+20) 01117390050, **E-mail:** fatmaalzahraaderma@yahoo.com

ABSTRACT

Background: Androgenic alopecia (AGA) is a genetically programmed condition brought on by a woman's disproportionate sensitivity to androgens. The frontal hairline is often spared whereas the crown and top of the head experience diffuse hair loss, which is frequently identified by a broader centre part. For women with AGA, topical minoxidil is a popular and successful therapy for hair loss. By reducing the telogen (resting) phase of hair follicles and activating the anagen (growth) phase, minoxidil encourages hair growth. Carboxytherapy is a noninvasive method that has been shown to affect hair development in a number of ways.

Objective: To study the efficacy of combination of both minoxidil 5 % with carboxytherapy versus minoxidil 5 % alone in treatment of female pattern hair loss.

Patients and methods: This study included 40 female patients with AGA, who were randomized into two groups (group A and B); Group A included patients who used minoxidil 5% once daily with carboxytherapy session every 3 weeks for 6 months and Group B who used topical minoxidil 5% only once daily for 6 months.

Results: There was a statistically significant increase in hair diameter during treatment in group A compared to group B (at 3 months and 6 months) (p=0,012, 0.001 respectively). There was a statistically significant increase in hair density during treatment in group A compared to group B (at 3 months and 6 months) (p = 0.004, < 0.001 respectively). There was a statistically significant increase in hair count during treatment in group A compared to group B (at 3 months and 6 months) (P=0.048, 0.002 respectively). Higher satisfaction was recorded in group A compared to group B with a statistically significant difference (P=0.049).

Conclusion: In the context of female pattern hair loss, carboxytherapy seems to be a promising therapeutic modality and it could be used as a helpful adjuvant therapy of AGA in association with minoxidil. In addition, it is recommended for maintenance of the results with minimal side effects.

Keywords: Female androgenetic alopecia, Carboxytherapy, Minoxidil 5%, Hair loss.

INTRODUCTION

Dihydrotestosterone (DHT), an androgen, causes androgenetic alopecia genetically. It is also known as pattern baldness, and it primarily affects males, however it can also affect women[1].

By that point, new discoveries on the genetics, molecular underpinnings, and pathophysiology of androgenetic alopecia (AGA) have led to viable treatment options. Notably, there are several other factors that can contribute to AGA outside a person's genetic make-up, such as food, stress, immunological reaction, allergies, inflammation, poor nutrition, chemotherapy, hormone imbalance, or direct physical tension on the hair follicles [2].

For many years, minoxidil has been used to treat AGA. By promoting hair development and halting hair loss, the drug has an impact on follicular cells[3]. Clinical studies in AGA patients treated with 2% or 5% minoxidil shown a striking increase in hair growth and decrease in hair loss, with the 5% formulation producing superior results[4].

Application of 1 mL of 2% minoxidil twice day and of a half-capful of 5% minoxidil foam once daily are the recommended treatments for female pattern hair loss (FPHL). In FPHL, it has been demonstrated that once daily topical administration of 5% minoxidil foam is just as effective as twice daily treatment of 2% minoxidil with noticeably less side effects[5].

The term "carboxytherapy" describes the therapeutic injection of sterile, medical-grade CO_2 gas intradermally and/or subcutaneously. It causes a local pH drop and relative hypercapnia, both of which cause a significant vasodilator reaction that eventually boosts blood flow to the injection site [6]. It is regarded as a secure, minimally invasive, and therapeutically effective technique for skin rejuvenation, restoration, and reconditioning[7].

In AGA, hair loss has a complicated etiology that is brought on by a number of reasons, including a reduction in the blood supply to the scalp tissue that is impacted. It appeared fascinating to assess carboxytherapy's effectiveness and safety in the treatment of AGA since it enhances circulation at the injection site [8].

The Bohr effect, caused by carboxylic gas, causes a change in the oxygen dissociation curve, which eventually improves oxygenation. Additionally, it interacts with the tissue perfusion regulators to increase lymphatic drainage and perfusion rates in capillary beds, release regional growth factors, and promote angiogenesis[9].

The primary pathophysiology of AGA, which is an androgen-dependent condition, is not directly impacted by carboxytherapy. With improved vascularization of alopecic HFs and consequent activation of hair regrowth, carboxytherapy can be

viewed as a helpful adjuvant therapy for alopecia but cannot be considered a curative therapy for AGA[8].

So, this work was conducted to study the efficacy of combination of both minoxidil 5 % with carboxytherapy versus minoxidil 5 % alone in treatment of female pattern hair loss.

PATIENTS AND METHODS

This randomized clinical trial included 40 female patients with AGA, selected on the basis of inclusion and exclusion criteria. The patients were randomized using block randomization method with sealed opaque envelops.

i. **Group A:** Twenty patients used minoxidil 5% once daily with carboxytherapy session every 3 weeks for 6 months.

ii. **Group B:** Twenty patients used topical minoxidil 5% only once daily for 6 months.

Inclusion criteria:
- Patients with classic and dermoscopic features of AGA with their age range from18–50 years.
- Patients who have not had therapy in at least three months.
- Patients with AGA grade (1 to 4) Sinclaire Scale.
- Patients with AGA who are able to visit for regular follow-up were chosen.

Exclusion criteria:
- AGA patients who have a history of bleeds and/or are taking anticoagulants.
- Female patients with hyperandrogenism-related symptoms and manifestations (such as hirsutism, severe acne, and irregular menstruation).
- Pregnant and lactating females.
- Patients who have an infection that is active locally.
- People who have keloidal tendencies.
- Patients at risk for the Koebner phenomenon include those with a history of psoriasis or lichen planus.
- Renal disease, epilepsy, hepatitis, or any other serious medical condition.
- Patients who have inflated expectations.

Injection technique:

Group A was administered CO_2 gas injections utilising carboxytherapy equipment in an entirely aseptic procedure. It took a few seconds of waiting for the gas to flow fully. The injection was administered slowly, intradermally, with a sterile, disposable 30 G needle, at a 15° angle, with a 2 mm depth adjustment and a 1 cc/s flow rate. 2 mL of gas were injected into each location. The clinical end point of each injection is the occurrence of an erythema and distension of the injected tissue.

We apply in 7 points over the capillitium. On every side of the head 3 points and one in the centre. The distance between each injection was 1-2 cm.
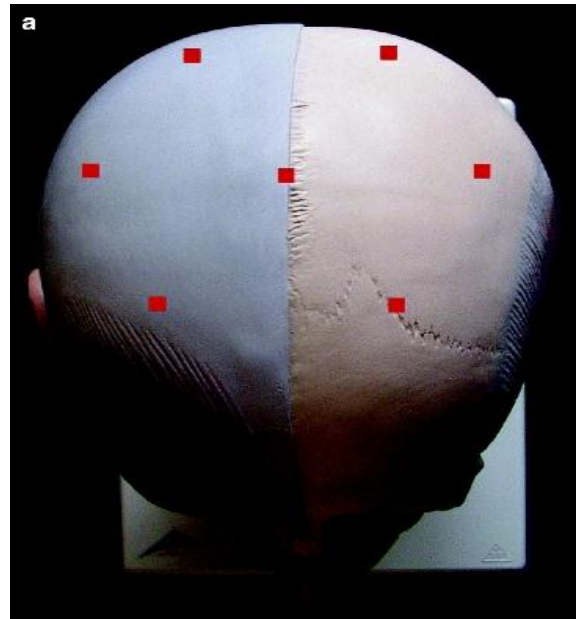
Figure (1): Seven injection points in androgenetic alopecia patients[10]

Evaluation of therapy:
Clinical assessment:

The Sinclaire Scale for Female Pattern Hair Loss (FPHL) was used to evaluate the pattern of scalp hair loss in AGA patients. To represent the progression of the disease, the categorization for the pattern distribution of AGA frequently falls short. Hair density, clinical photography captured by dermoscopic examination, and patient self-assessment were used to evaluate the treatment's effectiveness. Patients were assessed clinically and by dermoscopy at the beginning of therapy, three months into it, and six months at the end of treatment.

Dermoscopic examination; Dermlite 3 (3 Gen, USA): Inspecting patients for:
- The occurrence of >20% variance in hair diameter (a diagnostic indicator of follicle miniaturisation).
- Solitary hair predominates in each follicular unit, as opposed to normal follicles, which may support up to four terminal hairs.

The assessment area was placed at 3 cm in front of the upper end of the right tragus and 10 cm above (perpendicular). The spot was fixed with a stamp that was 1 cm square. Hair density documentation using tricoscan technology (TrichoScan Professional Version 3, Germany), a computer assisted method for determining hair density and hair roots status was performed at three times-points: baseline

(BL), 3 months during treatment and 6 months at the end of treatment. As a result, the evaluation and monitoring of hair regrowth were supported.

Patient self-assessment:

On a scale from 0 to 4, patients' scalp hair growth was evaluated [10]: **0-:** no satisfaction. **1-:** 1-25 % mild satisfaction. **2-:** 26-50 % moderate satisfaction. **3-:** 51-75 % high satisfaction. **4-:** 76-100% very high satisfaction.

Ethical consideration:

All study participants provided their informed permission. This study was taken out from February 2021 to February 2023 after agreement of the local Ethical Committee. Acceptance of the IRB of Mansoura Faculty of Medicine was obtained before starting of the research. (MS.20.09.1). The worldwide medical association's code of ethics, the Declaration of Helsinki for Human Beings, was followed throughout this study.

Statistical Methods

SPSS software version 25 was used for data administration and statistical analysis. When applying the Kolmogorov-Smirnov test to determine if a sample is normal, normality is presumed if the significance level is larger than 0.05. For numerical data, the mean, standard deviation (SD) and range were utilised.

For categorical data, frequency and percentage were employed. To establish the statistical significance of the difference between the means of the two research groups, the Student T-Test was performed. The repeated measure ANOVA test (F-tests) was used to evaluate the relationship between related parametric quantitative variables over many time periods. The Chi-Square test was used to analyse the relationship between two or more qualitative variables.

The Fisher's exact test was used to examine the relationship between two groups and qualitative features when the expected count was less than 5 in more than 20% of the cells. The Monte-Carlo test was used to evaluate the relationship between three or more groups with qualitative features when the expected count was less than 5 in more than 20% of the cells. Significant was defined as P values of 0.05 or less.

RESULTS

Table (1) demonstrates sociodemographic data in the two studied groups. There were no statistically significant differences between both groups regarding age and marital status.

Table (1): Sociodemographic data of the two studied groups

Items	Group A [Combined minoxidil and carboxytherapy] (n= 20)	Group B [Minoxidil alone] (n= 20)	Test of significance
Age (Years)	31.05 ± 8.16	30.40 ± 7.80	t = 0.258 P= 0.798 (NS)
Marital status			
Not married	6 (30%)	7 (35%)	$\chi2 = 0.114$ P= 0.736 (NS)
Married	14 (70%)	13 (65%)	

t: independent samples t-test, χ2: Chi square test, NS: Non-significant

Table (2) shows that, there were no significant differences between the two studied groups regarding disease onset (acute and chronic), disease duration (< 5 years or ≥ 5 years), associated diseases (diabetes mellitus and hypertension), and associated skin diseases (hirsutism, acne and seborrhoeic dermatitis).

Table (2): Patients characteristics and associated skin diseases of the two studied groups

Items	Group A [Combined minoxidil and carboxytherapy] (n= 20)	Group B [Minoxidil alone] (n= 20)	Test of significance
Disease onset			
Acute	5 (25%)	10 (50%)	$\chi2$ = 2.667
Chronic	15 (75%)	10 (50%)	P= 0.102 (NS)
Disease duration			
< 5 years	10 (50%)	11 (55%)	$\chi2$ = 0.100
≥ 5 years	10 (50%)	9 (45%)	P= 0.752 (NS)
Other medical condition			
Diabetes mellitus	2 (10%)	1 (5%)	$\chi2$ = 0.360 P= 0.548 (NS)
Hypertension	2 (10%)	2 (10%)	FET P= 1 (NS)
Associated skin diseases			
Hirsutism	6 (30%)	6 (30%)	$\chi2$ = 0 P = 1 (NS)
Mild to moderate acne	6 (30%)	5 (25%)	$\chi2$ = 0.125 P = 0.723 (NS)
Seborrheic dermatitis	8 (40%)	6 (30%)	$\chi2$ = 0.440 P = 0.507 (NS)

FET: Fischer's exact test, $\chi2$: Chi square test, NS: Non-significant

Table (3) illustrate analysis of grade of hair loss (Sinclair scale) in the two studied groups before treatment. There was no statistically significant difference between both groups as regards hair loss grading. There were no statistically significant differences between both groups as regards vellus hair, single hair, peripilar sign and hair diversity. However, yellow dots were significantly higher in group A compared to group B.

Table (3): Analysis of grade of hair loss (Sinclair scale) and dermoscopic features in the two studied groups before treatment.

Items	Group A [Combined minoxidil and carboxytherapy] (n= 20)	Group B [Minoxidil alone] (n= 20)	Test of significance
Grade of hair loss (Sinclair scale)			
Grade 1	0 (0%)	1 (5%)	
Grade 2	5 (25%)	10 (50%)	
Grade 3	8 (40%)	8 (40%)	MC = 7.467 P= 0.113 (NS)
Grade 4	4 (20%)	1 (5%)	
Grade 5	3 (15%)	0 (0%)	
Dermoscopic features			
Hair diversity	20 (100%)	20 (100%)	_____
Single hair	11 (55%)	15 (75%)	$\chi2$ = 1.758 P = 0.185 (NS)
Yellow dots	9 (45%)	3 (15%)	$\chi2$ = **4.286** **P = 0.038 (S)**
Peripilar sign	9 (45%)	14 (70%)	$\chi2$ = 2.558 P = 0.110 (NS)
Vellus hair	19 (95%)	20 (100%)	$\chi2$ = 1.026 P = 0.311 (NS)

MC: Monte-Carlo test, $\chi2$: Chi square test, NS: Non-significant, S: Significant

Table (4) shows that, there were no significant differences in hair diameter between both groups before treatment. At 3 months during treatment, there was a statistically significant increase in hair diameter in group A compared to group B and such increase became highly significant at 6 months during treatment (p =0.012, 0,001respectively).

Table (4): Comparison of hair diameter (μm) in the two studied groups during treatment

Duration of follow up	Group A [Combined minoxidil and carboxytherapy] (n= 20)	Group B [Minoxidil alone] (n= 20)	Test of significance
Pretreatment	73.30 ± 11.49	74.20 ± 10.44	t = - 0.259 P= 0.797 (NS)
At 3 months during treatment	89.65 ± 11.11	80.90 ± 9.94	**t = 2.624 P= 0.012 (S)**
At 6 months during treatment	97.35 ± 11.49	85.30 ± 9.97	**t = 3.542 P= 0.001 (HS)**
Repeated measures ANOVA	F = 14.548 P1 < 0.001 **(HS)**	F = 5.317 P2 = 0.009 **(S)**	

t: Independent sample t-test, F: Repeated measures ANOVA, NS: Non-significant, S: Significant, HS: Highly significant.

P value: Comparing between group A and group B
P1: Comparing between pretreatment and 3 months during treatment in group A
P2: Comparing between pretreatment and 6 months during treatment in group B

Table (5) shows that, there was no significant difference between both groups in hair density before treatment. Furthermore, during treatment at 3 months, there was a statistically significant increase in hair density in group A compared to group B and such increase became highly significant at 6 months of treatment (p = 0.004, <0.001 respectively).

Table (5): Comparison of hair density (hairs/ cm^2) in the two studied groups along the duration of treatment.

Duration of follow up	Group A [Combined minoxidil and carboxytherapy] (n= 20)	Group B [Minoxidil alone] (n= 20)	Test of significance
Pretreatment	133.55 ± 9.53	130 ± 12.15	t = 1.028 P= 0.311 (NS)
At 3 months during treatment.	158.70 ± 9.53	148 ± 12.54	**t = 3.037 P= 0.004 (S)**
At 6 months during treatment.	170.45 ± 9.60	153.15 ± 12.03	**t = 5.026 P < 0.001 (HS)**
Repeated measures ANOVA	F= 23.423 P2< 0.001 **(HS)**	F = 4.807 P2 = 0.015 **(S)**	

t: Independent sample t-test, F: Repeated measures ANOVA, NS: Non-significant, S: Significant, HS: Highly significant

P value: Comparing between group A and group B.
P1: Comparing between pretreatment and 3 months during treatment in group A.
P2: Comparing between pretreatment and 6 months during treatment in group B.

Table (6) shows that, there were no significant differences between both groups in hair count before treatment. In addition , at 3 months during treatment, there was a statistically significant increase in the hair count in group A compared to group B and such increase became highly significant at 6 months of treatment (p = 0.048, 0.002 respectively)

Table (6): Comparison of hair count/cm^2 in the two studied groups along the duration of treatment.

Duration of follow up	Group A [Combined minoxidil and carboxytherapy] (n= 20)	Group B [Minoxidil alone] (n= 20)	Test of significance
Pretreatment	99.30 ± 15.65	102.80 ± 18.48	t = -0.646 P= 0.522 (NS)
At 3 months during treatment	127.95 ± 15.87	116.85 ± 18.43	**t = 2.041 P= 0.048 (S)**
At 6 months during treatment	145.50 ± 15.37	127.35 ± 18.15	**t = 4.413 P = 0.002 (S)**
Repeated measures ANOVA	F= 28.493 P1< 0.001 **(HS)**	F= 4.922 P2 = 0.011 **(S)**	

t: Independent sample t-test. F: Repeated measures ANOVA, NS: Non-significant, S: Significant
P value: Comparing between group A and group B
P1: Comparing between pretreatment and 3 months during treatment in group A
P2: Comparing between pretreatment and 6 months during treatment in group B

Table (7) show analysis of patient self-assessment in the two studied groups. Higher satisfaction was recorded in group A compared to group B with a statistically significant difference (p= 0.049).

Table (7): Analysis of patient self-assessment in the two studied groups

Items	Group A [Combined minoxidil and carboxytherapy] (n= 20)	Group B [Minoxidil alone] (n= 20)	Test of significance
Patients' assessment			
Mild satisfaction	**7 (35%)**	**8 (40%)**	**MC = 6.037 P= 0.049 (S)**
Moderate satisfaction	**6 (30%)**	**11 (55%)**	
High satisfaction	**7 (35%)**	**1 (5%)**	

MC: Monte-Carlo test, S: Significant

DISCUSSION

In our study, there were no statistically significant differences between the two groups in terms of sociodemographic characteristics or medical history. Before therapy, there was no statistically significant difference between the two groups for any dermoscopic characteristics.

Likewise, analysis of grade of hair loss and clinical assessment demonstrated insignificant differences between both groups.

In the current study, FPHL grading was assessed in all studied cases according to Sinclair scale classification: 25% in group A had grade 2, 40% had grade III and 20 % had grade IV, while in group B 50% had grade II, 40 % had grade III and 5% had grade III.

In the current study, we considered the following trichoscopic parameters before treatment as activity indexes for female pattern hair loss: Hair diversity > 20 % in frontoparietal area, increase number of vellus hair (hair with diameter less than 0,03 mm and shorter than 30 mm), decrease in the number of hairs per follicular unit, the presence of the follicles with peripilar sign, and the presence of yellow dots.

Hair shaft variety was observed in all of the groups we investigated, demonstrating the process of miniaturisation that underlies the pathophysiology of AGA. The increase of the vellus hair rate (95%-100%)

concur that the trichoscopic measure of illness severity based on vellus hair. Decreased number of hair per follicle up to single hair (55%-75%), which is characteristic feature of AGA, the presence of yellow dots (45%-15%), which is characteristic feature of AGA, and the peripilar sign (45%-70%), which is sign of inflammation and progression of the disease; all these finding agree with dermoscopic features of AGA reported previously[11].

Regarding hair diameter, we found that hair diameter increased significantly in both groups at 3 months and 6 months during treatment. Furthermore, the increase in hair diameter was significantly higher in group A (combined minoxidil 5% with carboxytherapy) in comparison with group B (minoxidil only) at 3 months and 6 months (p=0.012, 0.001 respectively).

This result agree with **Doghaim et al.** [8] who treated 40 patients with androgenetic alopecia with carboxytherapy. They reported that after six weekly sessions that compared to the placebo, there was a considerable decrease in the variability of hair diameter and a significant emergence of regrowing hair.

Furthermore, **Nilforooshzadeh et al.** [2] have shown that following treatment (combination of minoxidil (20%) by micro-needling and carboxytherapy mediated by needling) (one and especially three months after treatment), hair diameter increased considerably in all patients.

Regarding hair density (hair/cm^2), in the current study we found that both groups were associated with a significant increase in hair density after three months and six months during treatment. However, as compared to group B (minoxidil alone), group A (combined minoxidil 5% with carboxytherapy) was linked with a statistically significant increase in hair density (p= 0.004, 0,001 respectively).

Our findings are consistent with those of **Doghaim et al.** [8], who showed that carboxytherapy significantly increased hair density. However, they noted that the median hair density had decreased dramatically but was still noticeably better than it had been before to treatment at the conclusion of the follow-up period. These findings indicate that carboxytherapy's therapeutic effects did not last beyond the end of sessions. In order to maintain hair regeneration, the authors advise adding more sessions and combining them with other treatment techniques.

Moreover , in the study of **Nilforooshzadeh et al.** [2] they have reported that the male (n = 4) and female (n = 5) study patients' hair density significantly increased after treatment: in female cases, it was 249±3 and 178±5 vs. 110±3 for 1 and 3 months after treatment versus before treatment, and in male cases, it was 217±3 and 145 ±5 vs. 90 ±3 for 1 and 3 months after treatment versus before treatment.

With regard to hair count, the current study revealed that both groups were associated with a significant increase in hair count at three months and six months during treatment. However, group A (combined therapy) was associated with a statistically significant increase in hair count at 3 and 6 months compared to minoxidil only (p =0.048, 0.002 respectively).

The typical hair follicle's dermal papilla, which is thought to be where follicular stem cells are located, has a well-developed vascularization, which creates the best circumstances for growth[12]. According to a theory, the damaged scalp tissue's vascular supply may be lessened as a result of the hair follicles producing less vascular endothelial growth factor (VEGF), which might lead to inflammatory changes. This theory is reinforced by the observation that revascularization occurs before hair growth and capillary loss are early changes in alopecia [13].

Carboxytherapy has the potential to be an effective treatment for many forms of alopecia because it increases the production of VEGF, which leads to neoangiogenesis and improves oxygenation and nutrition delivery at the injection site [6].

In the current study, after carboxytherapy, statistically significant clinical improvement was seen the form of inducing hair growth and stopping future hair loss, with 35% of patients reporting very high levels of satisfaction, 30% moderate satisfaction and 35% mild satisfaction in group A (combined minoxidil with carboxytherapy). Patient satisfaction was significant higher in group A (combined minoxidil 5% with carboxytherapy) in comparison with group B (minoxidil only) (P=0,049).

At the conclusion of the follow-up period, **Doghaim et al.** [8] found a slow loss of the regrowing hair, but it was still better than it was before to treatment. Only 30% of the investigated patients had very good responses, and 10% reported exceptional responses.

The treated patients in the current study experienced moderate discomfort, a burning feeling, erythema, and edema as relatively minor side effects. This is in line with the findings of a research by **Doghaim et al.** [8], who found that the adverse effects of carboxytherapy in both groups were mild and brief and manifested as erythema, edema, slight discomfort, and burning at the injection site before spontaneously dissipating within 10 to 15 minutes. Hematomas or ecchymosis were not previously documented.

Limitations: Despite the promising findings of the current study, small sample size is considered the main limitation as well as the short period of follow up.

Supporting and sponsoring financially: Nil.
Competing interests: Nil.

CONCLUSION

Carboxytherapy appears to be a potential treatment approach for treating female pattern hair loss, and it could be utilised as an effective adjuvant therapy for AGA when combined with minoxidil. Additionally, it is advised for maintaining the outcomes with little adverse effects.

REFERENCES

1. **Kaliyadan F, Nambiar A, Vijayaraghavan S (2013):** Androgenetic alopecia: an update. Indian J Dermatol Venereol Leprol., 79(5):613-25.

2. **Nilforooshzadeh M, Lotfi E, Heidari-Kharaji M *et al.* (2020):** Effective combination therapy with high concentration of minoxidil and carboxygas in resistant androgenetic alopecia: Report of nine cases. Journal of Cosmetic Dermatology, 19(11): 2953-2957.

3. **Rossi A, Cantisani C, Melis L *et al.* (2012):** Minoxidil use in dermatology, side effects and recent patents. Recent Pat Inflamm Allergy Drug Discov., 6(2):130–136.

4. **Price V, Menefee E, Strauss P (1999):** Changes in hair weight and hair count in men with androgenetic alopecia, after application of 5% and 2% topical minoxidil, placebo, or no treatment. J Am Acad Dermatol., 41(5 Pt 1):717-21.

5. **Blume-Peytavi U, Shapiro J, Messenger A *et al.* (2016):** Efficacy and safety of once-daily minoxidil foam 5% versus twice-daily minoxidil solution 2% in female pattern hair loss: a Phase III, randomized, investigator-blinded study. J Drugs Dermatol., 15(7):883–889.

6. **Varlaro V, Manzo G, Mugnaini F *et al.* (2007):** Carboxytherapy: effects on microcirculation and its use in the treatment of severe lymphedema. Acta Phlebol., 8:79-91.

7. **Pianez L, Custódio F, Guidi R *et al.* (2016):** Effectiveness of carboxytherapy in the treatment of cellulite in healthy women: a pilot study. Clin Cosmet Investig Dermatol., 9:183-190.

8. **Doghaim N, El-Tatawy R, Neinaa Y *et al.* (2018):** Study of the efficacy of carboxytherapy in alopecia. Journal of Cosmetic Dermatology, 17(6): 1275-1285.

9. **Ahramiyanpour N, Shafie'ei M, Sarvipour N *et al.* (2022):** Carboxytherapy in dermatology: A systematic review. Journal of Cosmetic Dermatology, 21(5): 1874-1894.

10. **Koutná N (2011):** Carboxytherapy in aesthetic medicine. Aesthetic Medicine, pp. 547-76. DOI: 10.1007/978-3-642-20113-4_43

11. **Rossi A, Ferranti M, Magri F *et al.* (2022):** Clinical and trichoscopic graded live visual scale for androgenetic alopecia. Dermatol Pract Concept, 12(2): e2022078. doi: 10.5826/dpc.1202a78

12. **Li W, Man X, Li C *et al.* (2012):** VEGF induces proliferation of human hair follicle dermal papilla cells through VEGFR-2-mediated activation of ERK. Experimental Cell Research, 318(14): 1633-1640.

13. **Yano K, Brown L, Detmar M (2001):** Control of hair growth and follicle size by VEGF-mediated angiogenesis. The Journal of Clinical Investigation, 107(4): 409-417.