Clinical and Sonographic Assessment of Carboxytherapy Efficacy in Treatment of Skin Aging: A 2-split Randomized Clinical Trial

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ABSTRACT

Introduction: Aging is a continuous and irreversible process which affects the skin. During the aging process, intrinsic progressive degenerative changes in the skin impair its structure, which makes it prone to different dermatoses, resulting in impaired quality of life in the elderly.

Objective: Carboxytherapy is considered as a safe, minimally invasive modality applied for skin rejuvenation, restoration, and recondition. Herein, we have assessed the efficacy of carboxytherapy in the treatment of skin aging through clinical and sonographic studies.

Materials and methods: Our study was a prospective 2-split clinical trial in which the efficacy of carboxytherapy was assessed in the treatment of abdominal skin aging through clinical and sonographic evaluations.

Results: Twenty-eight patients with skin-intrinsic aging manifestations in the abdomen completed the study. Their mean age was 44.13 years. The mean weight, BMI, and waist circumference of the subjects significantly decreased after the treatment. The clinical subjective and objective evaluations revealed statistically significant improvement of skin wrinkles, laxity, and skin pigmentation and overall satisfaction by carboxytherapy. Upon sonographic investigation, a significant increase in epidermis and dermis thickness was observed. No significant side-effect was reported by the subjects.

Conclusion: Carboxytherapy is an effective and safe modality in treating intrinsic skin aging.

KEY WORDS: skin aging, carboxytherapy, treatment

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INTRODUCTION

The skin comprises about 17% of the human body weight and acts as a protective and defensive line against water loss, physical stressors, and aggressive environmental factors such as sun exposure and pollution (1). It also has a role in controlling body temperature and perception of temperature, pressure, and pain (2).

Aging is a continuous and irreversible process which affects the skin as well as the other organs (3). Skin aging, which is mainly the result of changes in the function and structure of the dermis, is characterized by thinning, drying, and loss of elasticity (4). It significantly adversely influences the role of skin as barrier against all aggressive exogenous factors (1). Skin aging-related conditions result in significant morbidity in the elderly, particularly if associated with other comorbid systemic diseases (3).

During the aging process, intrinsic progressive degenerative changes in the skin impair its structure, which predispose the skin to different dermatoses, resulting in impaired quality of life in the elderly. On the other hand, the high prevalence of chronic diseases such as hypertension, diabetes mellitus, thyroid disorders, etc., in the elderly predisposes them to a high risk of the cutaneous problems (3). Skin aging manifests in photo-aging, laxity, sagging, wrinkling, fragility, insufficient sweating, increased sensitivity to temperature, xerosis, pruritus, altered skin pigmentation, stasis dermatitis, pressure ulcers, impaired wound healing, increased risk of skin malignancy, and skin problems due to systemic disorders (4-6).

Table 1. Calculation of the sample size using the "G*Power" software

Exact - Proportions: Inequality, two independent groups (Fisher's exact test)
Options: Exact distribution
Analysis: A priori: Compute required sample size
Input: Tail(s) One
Proportion p1 = 0.5
Proportion p2 = 0.9
α err prob = 0.05
Power (1-β err prob)= 0.90
Allocation ratio N2/N1 = 1
Output: Sample size group 1 =25
Sample size group 2 = 25
Total sample size = 50
Actual power = 0.9114300 Actual α = 0.0144020

During the past few decades, a worldwide rise in the elderly population that is the result of changes in socioeconomic development has been reported. It is estimated that the number of people aged 65 or older will increase from 524 million in 2010 to approximately 1.5 billion in 2050, with a greater increase in developing countries. In comparison with young skin, geriatric skin is more prone to different dermatoses, with 13.3% of skin disorders being observed in elderly persons over 60 years of age. Additionally, there is an association between systemic disorders and skin problems in the general population. Hence, in elderly persons with a higher prevalence rate of systemic disorders, skin conditions due to these systemic disorders are expected to be common (3).

Skin aging is a complicated process resulting from intrinsic factors (chronologic aging with a genetic basis) and extrinsic factors (such as detrimental influences from the environment, mainly UV radiation) (4). Intrinsic skin aging is defined as the inevitable physiological process resulting in thinness of the skin, fine wrinkles, dry skin, and progressive dermal atrophy. Extrinsic skin aging is induced by external environmental factors leading to coarse wrinkles, rough appearance, loss of skin elasticity, and skin laxity (7).

Skin appearance certainly has an impact on social behavior and the reproductive status of those affected. Many individuals, particularly women, expend a significant amount of daily expenses for modalities and procedures to prevent or even reverse skin aging (7). The main aim in the treatment of aging is enhancing the quality of life in the elderly and preventing age-relate disorders. Moreover, the management of aged skin is especially important because the skin presents the most obvious signs of the aging mechanism and general health (8). Medications, light and laser therapy, and aesthetic surgical procedures have been used for treating skin aging, with different levels of success.

Carboxytherapy is defined as intradermal and/or subcutaneous microinjections of sterile purified carbon dioxide into different sites of the body to achieve therapeutic goals (9,10). It has been considered a safe and minimally invasive modality applied for rejuvenation, restoration, and reconditioning of the skin (9).

Carboxytherapy improves blood circulation and skin elasticity, reduces localized fat deposition, and

improves the cosmetic effects of other cosmetic procedures such as liposuction (11). It has been used in dermatology and cosmetology for the treatment of skin aging, alopecia areata, androgenetic alopecia, cellulite, localized fat deposits, striae distensae, infraorbital hyperpigmentation, scars, lymphedema, psoriasis, morphea, en coup de sabre, vitiligo, and other conditions.

There have been only a few studies with low numbers of recruited subjects examining the efficacy of carboxytherapy in the treatment of skin aging. Herein, we have assessed the efficacy of carboxytherapy in the treatment of skin aging through clinical and sonographic studies.

PATIENTS AND METHODS

Our study was a prospective right-left sided randomized clinical trial and represents a portion of a larger project with the ethical code IR.TUMS.MEDICINE.REC.1400.1077 and ChiCTR2200055185.

In this study, the sample size was calculated using the "G*Power" software based on "skin aging" as a qualitative variable and referring to a clinical trial by Nassar *et al.* (12) (Table 1). Regarding the acquired sample size, we selected 30 subjects among cases with intrinsic aging features in the abdominal skin who were referred to the Department of Dermatology at Arak University of Medical Sciences and were candidates for cosmetic procedures. The inclusion and exclusion criteria for selecting the cases are listed in Table 2.

After explaining the planned steps of the project, written informed consent was obtained from all subjects. In each case, the abdominal wall was symmetrically divided into 2 sides, namely the right and left. Carboxytherapy was performed on one side (case group), while the other side was left without therapy (control group). The case and control groups in each case were selected randomly via a coinflip. This procedure was performed every week for 10 sessions, maintaining constant treatment and control sides.

In our study, we used a digitally-controlled carboxytherapy device ("MEDAION", Nik Fannavaran Plasma Co., Tehran, Iran). This machine injects CO2 through the bolus method. CO2 was injected intradermally to achieve the aim of carboxytherapy, namely alleviating the aging process in our patients. Superficial injections can result in lifting of the skin, particularly when applied for managing skin imperfections and uneven appearance (13). For this reason, the needle was inserted at an angle of 15 to 30 degrees. The needle used was a 30G mesotherapy needle 4 mm in length. On the treated side, CO2 was injected in the volume of 150 cc. The machine parameters for gas injection include input pressure of 238 kPa, output pressure of 50 kPa, volume per shot of 2.0 cc, and gas temperature of 40 °C. We initiated the treatment from the most lateral part of the abdomen in order to decrease the potential risk of CO2 penetration from the case side to the control side.

High-resolution digital photography was performed at time 0, before each session, and two weeks after the last session. This allowed us to evaluate and compare improvement of the skin wrinkles (including striae distensae), laxity, pigmentation, and overall signs of aging due to the treatment.

The clinical study was based on assessment of objective and subjective findings acquired from direct visual and photographic investigation of any skin changes or improvement in terms of skin wrinkles, laxity, and lightening, and on overall patient satisfaction. Objective assessments were performed by 3 evaluators blinded to the process of treatment. We used a separate scoring system for each of these parameters (14) (Table 3). These parameters were evalu-

Table 2. Inclusion and exclusion criteria for selecting the subjects

Inclusion criteria
- Ages >40 years old
- Both genders
- Presence of aging features in abdominal skin (including wrinkle, laxity, pigmentary alteration, stria distensae, etc.)
Exclusion criteria
- Active skin infections at the biopsy site
- Connective tissue diseases or genodermatoses
- Keloid tendency
- Any systemic disorder such as diabetes mellitus, kidney diseases, cardiac diseases, respiratory diseases, liver diseases, severe anemia, etc.
- Medication (also including supplementary agents) within 6 weeks before the study initiation
- Any antiaging treatment or procedure at the site of study within one year before study initiation
- Cigarette smoking and alcohol and drug abuse
- Pregnancy
- Breast feeding

Table 3. Scoring system for subjective or objective evaluation of skin wrinkles, laxity, and lightening, and overall satisfaction based on visual and photographic findings

1-	No improvement (0%)
2-	Mild response (1%-25%)
3-	Moderate response (26%-50%)
4-	Good response (51%-75%)
5-	Very good response (76%-100%)

Table4. The comparison of epidermal, dermal and subcutaneous thickness between the treated and control sides

Variables	Group	N	Mean	Std. Deviation	Std. Error Mean
Epidermal thickness	Treatment	28	.6975	.12468	.02356
	Control	28	.5443	.10465	.01978
Dermal thickness	Treatment	28	2.1596	.40580	.07669
	Control	28	1.8829	.38943	.07360
Subcutaneous thickness	Treatment	28	26.3482	8.55468	1.61668
	Control	28	28.6564	8.68774	1.64183

ated before every session of the treatment and 2 weeks after the last session.

We measured the thickness of the epidermis, dermis, and subcutis to investigate changes due to carboxytherapy. This measurement was performed by a radiologist through classical ultrasonography, who was blinded to the project. It was performed on symmetrical points on both sides of the abdomen, 10 cm from the right and left, in the midway of the line linking the umbilicus to the caesarean section line. For every subject, after registering their name, sonography was performed by a linear probe applied in a superficial mode in which the abdominal muscular tissue was in the deepest part of the sonographic view. The procedure was performed without including pressure on the skin and soft tissue, by measuring the thickness of the epidermis, dermis, and subcutis. The sonography machine used was the Samsung ws80a device.

RESULTS

We recruited 30 subjects, all of whom were female. Only 28 patients completed the study, whereas 2 exited the study for personal reasons unconnected to the treatment. Patient mean age was 44.13 years (SD=4.53), with a maximum and minimum age of 40.0 and 56.5 years, respectively. Carboxytherapy was performed on the right side of the abdomen in 15 cases (53.6%) and on the left side in 13 cases (46.4%).

The mean weight of the subjects was 80.35 kg (SD=11.58) and 79.39 kg (SD=11.71) before and after the carboxytherapy, respectively. This difference was statistically significant (Paired t-Test, *P*-value<0.001, t=7.76).

The mean BMI among the studied patients was 29.78 kg/m^2 (SD=4.08) and 29.26 kg/m^2 (SD=4.01) before and after the treatment, respectively, and this difference was statistically significant (Paired t-Test, *P*-value<0.001, t=6.58).

Table 5. The frequency of manifestations and side-effects reported by the subjects during the study

Variable		Frequency	Percentage
Immediate pain	Positive	28	100.0
	Negative	0	0.0
Long lasting pain	Positive	5	17.9
	Negative	23	82.1
Bruising	Positive	28	100.0
	Negative	0	0.0
Heat sensation	Positive	2	7.1
	Negative	26	92.9
Fullness sensation	Positive	3	10.7
	Negative	25	89.3
Skin stiffness	Positive	24	85.7
	Negative	4	14.3
Apparent decrease in waist circumference size based on trouser size	Positive	19	67.9
	Negative	9	32.1
Asymmetric abdomen	Positive	28	100.0
	Negative	0	0.0

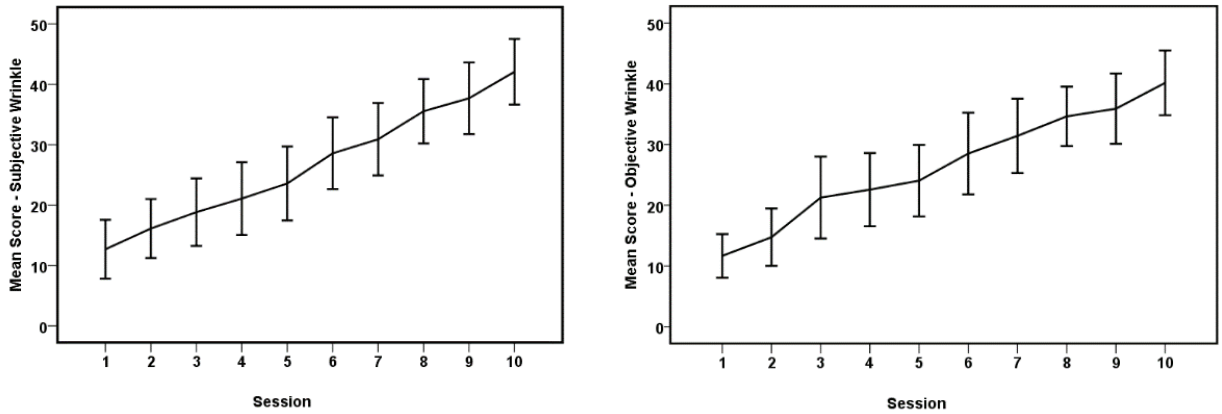


Diagram 1. The mean and standard deviation for improvement in skin wrinkles and striae distensae assessed subjectively (left) and objectively (right) during and 2 weeks after carboxytherapy.

The mean waist circumference of the subjects was 98.51 cm (SD=12.09) and 97.78 cm (SD=12.02) before and after the carboxytherapy, respectively, and the difference was once again statistically significant (Paired t-Test, *P*-value<0.001, t=7.74).

Diagram 1 shows the mean and standard deviation of improvement of skin wrinkles and striae distensae due to carboxytherapy assessed subjectively and objectively after the first session for 9 sessions, and 2 weeks after the last session. The mean scores demonstrated a progressively increasing pattern during this period for both of these variables. After performing Mauchly's Test of Sphericity (*P*-value<0.001), the results of the RM-ANOVA test revealed that the mean scores had statistically significantly increased during these 10 sessions for both the subjective and objective assessments (*P*-value<0.001, F=34.195, and *P*-value<0.001, F=34.538, respectively).

Diagram 2 shows the mean and standard deviation for the improvement of skin laxity due to carboxytherapy evaluated subjectively and objectively after the first session for 9 sessions, and 2 weeks after the last session. The mean scores progressively increased during this period for both variables. After performing Mauchly's Test of Sphericity (*P*-value<0.001), the results of the RM-ANOVA test showed that the mean acquired scores had statistically significantly increased during the 10 sessions for both the subjective and objective assessments (*P*-value<0.001, F=36.75, and *P*-value<0.001, F=26.216, respectively).

The mean and standard deviation for skin lightening due to carboxytherapy were assessed subjectively and objectively after the first session for 9 sessions, and 2 weeks after the last session are shown in Diagram 3. The mean scores did not show a serial increase in the severity of these parameters; in the both assessments, the skin darkened until the 4th session, after which skin lightening progressive was observed, but not in a stepwise manner. For instance, the severity of skin lightening was higher in the 7th session in comparison with the 8th and 9th sessions in the subjective assessment and higher than the 9th session in the objective assessment. However, the results of the RM-ANOVA test showed a statistically significant increase of the mean score during these 10 sessions for both the subjective and objective assessments

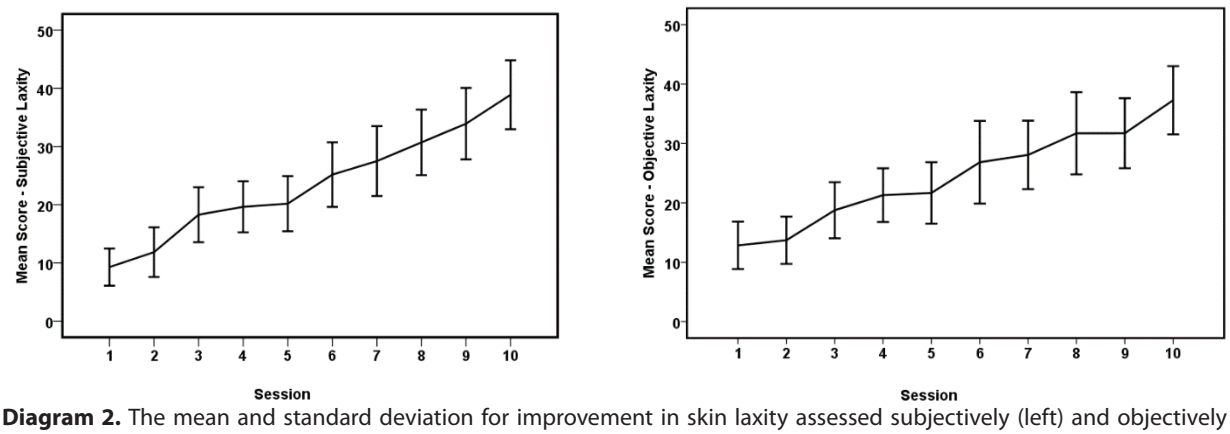


Diagram 2. The mean and standard deviation for improvement in skin laxity assessed subjectively (left) and objectively (right) during and 2 weeks after carboxytherapy.

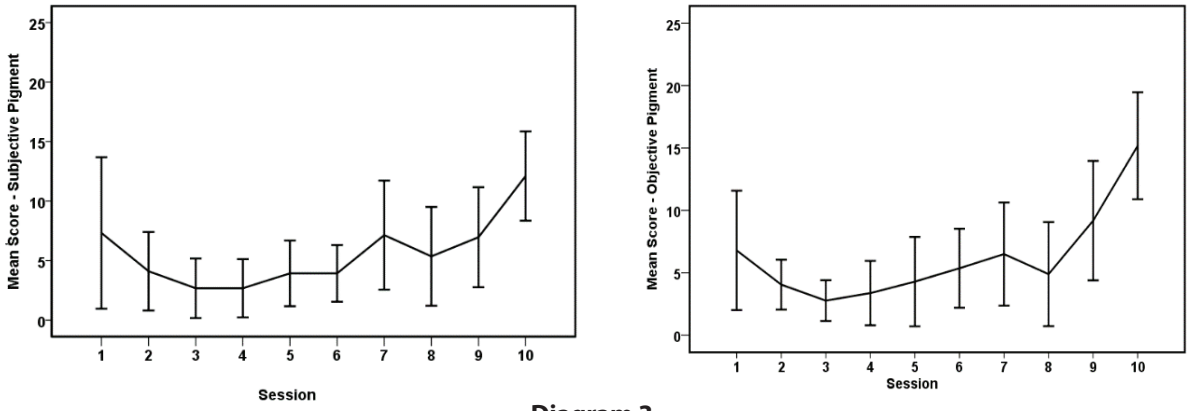


Diagram 3

Diagram 3. The mean and standard deviation for skin lightening assessed subjectively (left) and objectively (right) during and 2 weeks after carboxytherapy.

(*P*-value<0.001, F=4.00, and *P*-value<0.001, F=7.015, respectively).

The mean and standard deviation for subjective and objective overall satisfaction due to carboxytherapy after the first session for 9 sessions, and 2 weeks after the last session are shown in Diagram 4. The mean scores progressively increased during this period. After performing Mauchly's Test of Sphericity (*P*-value<0.001), the results of the RM-ANOVA test showed that the mean acquired scores had statistically significantly increased during these 10 sessions for both subjective and objective evaluations (*P*-value<0.001, F=31.194, and *P*-value<0.001, F=30.739, respectively).

Comparison of epidermal, dermal, and subcutaneous thickness between the treated and control sides is shown in Table 4. The result of an independent T-test showed a statistically significant difference in the epidermal (*P*-value<0.001, t=4.98) and dermal (*P*-value<0.001, t=2.60) thickness between the 2 groups, but, no statistically significant difference was seen between these 2 groups regarding subcutaneous thickness (*P*-value=0.321, t=-1.002) (Diagram 5).

Table 5 lists the frequency of other clinical manifestations and side-effects reported by the subjects during the study. The majority of subjects experienced conditions that included immediate pain, bruising, skin stiffness, decreased apparent waist circumference size, and asymmetry of the abdomen. In all cases, the pain was mild and insignificant.

Some accessory manifestations were reported by 6 subjects. These included a decrease in scalp hair loss in a patient with long-lasting therapeutic-resistant telogen effluvium in 1 case (3.6%), improvement of eczema and pruritus at the site of the procedure in 1 case (3.6%), decreased severity of lentigo at the site of the procedure in 1 case (3.6%), improvement in a caesarian section scar in 2 cases (7.1%), and very severe pain at the site of treatment after the 5th session of therapy that lasted for 2 days together with severe bruising in 1 case (3.6%).

DISCUSSION

As a continuous and irreversible process, aging affects the skin as well as the other organs. During this

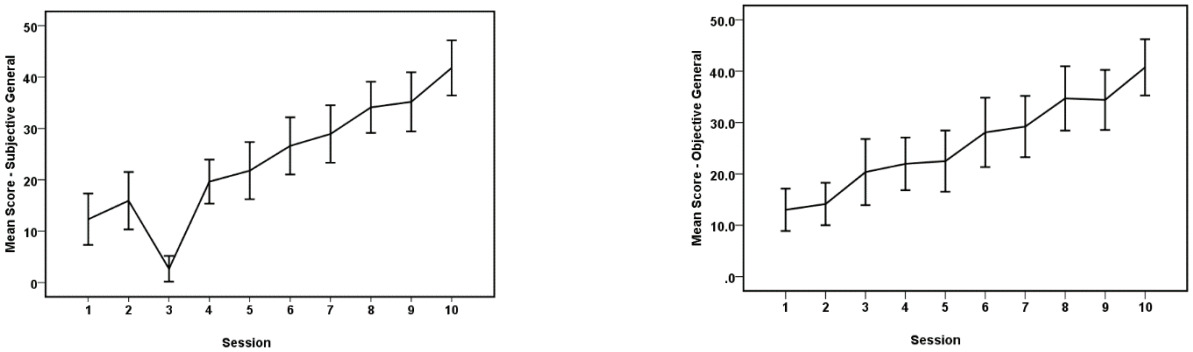
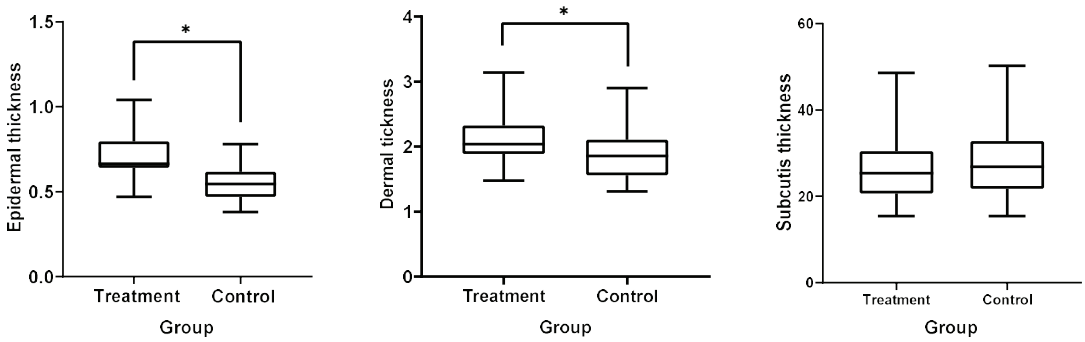


Diagram 4. The mean and standard deviation for subjective (left) and objective (right) overall satisfaction during and 2 weeks after carboxytherapy.



*: Significant difference at p<0.05 *: Significant difference at p<0.05

Diagram 5. The mean and standard deviation for changes in epidermal (left), dermal (middle), and subcutaneous (right) thickness after carboxytherapy.

process, intrinsic progressive degenerative changes involving the skin result in its structural impairment, which predisposes it to different dermatoses (3).

Skin aging is characterized by various signs and symptoms, of which photo-aging, skin laxity, sagging, wrinkling, fragility, xerosis, pruritus, and pigmentation are most important (4-6). Photo-aging, as a manifestation of extrinsic aging, is a dermatosis that develops due to prolonged exposure of the skin to solar ultraviolet (UV) radiation and is characterized by fine and coarse wrinkles, roughness, erythema, lentigines, and dyspigmentation (15). The intrinsic skin aging process results in a reduced number of dermal fibroblasts, decreased synthesis of collagen and elastin in the extra cellular matrix, particularly collagen types I and III, and thinning of the dermis, which induce skin laxity, wrinkling, fragility, and loss of elasticity (16).

With progressing age, the skin presents with histopathological changes mostly due to chronic UV exposure. These changes mainly affect the epidermis and dermis. With advanced age, the epidermis becomes progressively thinner, while the dermal changes mainly manifest as UV-induced damage to the dermal connective tissue and supporting tissue (17). Many of the other histologic changes in the dermis are due to disruption of the connective tissue infrastructure; a decrease in the number of mast cells and fibroblasts (7) and degradation of the collagen and elastic fibers associated with aging (1). In the abdomen of men and on the thighs of women, subcutaneous fat thickness increases as part of the aging process.

The main goal of treatment for aging is improving of the life quality in the elderly and prevention of age-related diseases. The management of aged skin is particularly important because the skin presents the most clinically evident signs of the aging mechanism and general state of health (8). Carboxytherapy is considered a safe, minimally-invasive modality applied for skin rejuvenation, restoration, and recondition (9). It improves blood circulation and skin elasticity and reduces localized fat deposition (11). It acts by causing increased permeability of the capillary walls, vasodilatation, increased blood flow (9-11,18), promotion of angiogenesis (10,19), improvement of tissue drainage (10), promotion of dermal fibroblasts, production of collagen and elastin in higher quantities and of better quality (19), increased collagen remodeling (18), increased flexibility and decreased firmness of collagen fibers (9-11,18), increased temperature (10,11), skin regeneration, lipolysis (19), and increase of muscle trophicity (10).

The effectiveness of carboxytherapy in the treatment of skin aging has been demonstrated in only a few studies with a limited number of cases. Nassar *et al.* compared the efficacy of carboxytherapy, radiofrequency skin tightening, and intense pulsed light in treating facial wrinkles and skin rejuvenation in 60 cases. Their study demonstrated the superiority of intense pulsed light treatment, followed by carboxytherapy and radiofrequency skin tightening. Their findings were confirmed by immunohistochemical assessment of MMP1. In this study, a statistically significant increase was found in MMP-1 expression after treatment (12). MMP-1 acts as a collagenase 1 through initiating catalyzation of α chains of the type I, II, and III collagens. It is effective in destruction of damaged collagen and replacement of the matrix in the processes. It plays a role in wound healing, skin response to UV irradiation, and prevention of aging (20). In a study by Oliveira *et al.*, the effectiveness of carboxytherapy in managing skin laxity was demonstrated

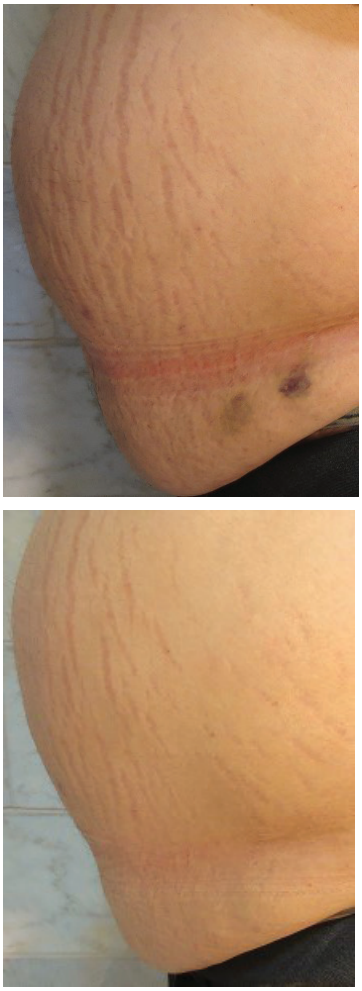


Figure 1. Decrease of striae distensae in a subject who received carboxytherapy on the left side of the abdomen; (A) before treatment and (B) after treatment.

Figure 2. Decrease of skin wrinkles, sagging, and striae distensae in a subject who received carboxytherapy on the right side of the abdomen; (A) before treatment and (B) after treatment.

through histological investigation of the status of collagen and elastin fibers on 9 patients who received treatment on the left side of the infraumbilical region. After 60 days of treatment, the findings revealed the effectiveness of this modality in promoting collagen and elastic fiber synthesis and significantly improving the morphometry of collagen fibers (21). In a 2-split study by Pinheiro *et al.*, the effectiveness of carboxytherapy and radiofrequency skin tightening on skin aging after abdominoplasty was compared in 8 cases. In this clinical trial, a single treatment of carboxytherapy and radiofrequency was performed on the right and left infra-umbilical regions, respectively, and skin biopsies were taken 120 days later. The pathological assessment revealed collagen remodeling in both group in comparison with untreated skin as the control group; however, this remodeling was more prominent and lasted longer in the radiofrequency group. An increase in elastic fibers was observed with carboxytherapy, whereas no change in elastin fibers was seen with radiofrequency therapy (22). In a clinical trial by Medrano *et al.*, the objective effectiveness and subjective satisfaction for a carboxytherapy gel mask were demonstrated with regard to the acceleration of wound healing after treatment with nano-fractional radiofrequency in 9 subjects with facial photoaging. Improvement of photodamage, pigmentation, and wrinkles was observed in all subjects. Furthermore, significantly less erythema, dryness, edema, crusting, and healing, as side-effects of radiofrequency, were observed at 24-hour follow-up in patients who received this gel mask (23).

The effectiveness of carboxytherapy has been demonstrated in the treatment of striae distensae. In a clinical trial, Podgórna *et al.* studied the efficacy of carboxytherapy in striae distensae by investigating cutometric parameters in 15 women. Their findings demonstrated that this modality significantly

improved skin elasticity. In this study, the analysis of photographic findings showed 58% improvement in striae distensae visibility, with a decrease in the width and length of the lesions and conversion of the lesion color to the natural skin tone (24). In a 2-split clinical trial, the efficacy of carboxytherapy and platelet-rich plasma (PRP) was compared in the treatment of striae albae in 20 cases. Based on clinical examination and patient satisfaction, no significant difference in response was reported between the 2 groups. Upon immunohistochemical study, staining with fibronectin demonstrated significantly higher expression of this protein in the carboxytherapy group (25). El-Domyati *et al.* studied the efficacy of carboxytherapy in treating striae distensae by clinical and pathological assessments. Statistically significant improvement was demonstrated upon clinical assessment. A statistically significant increase in epidermal thickness was demonstrated based on histometric analysis. Histochemical assessment of elastic and collagen fibers showed an increase in content and density of collagen fibers, which were organized in bundles parallel to the epidermis. On pathologic examination, better arrangement of curled and fragmented elastic fibers was observed after therapy (14). Farouk *et al.* demonstrated the effectiveness and safety of carboxytherapy in treating striae distensae in a clinical trial on 30 women (26).

The present study demonstrated the statistically significant effectiveness of carboxytherapy in treating skin laxity, wrinkles, and striae distensae as manifestations of skin aging, determined both subjectively and objectively on clinical evaluation (Figure 1 and Figure 2). We speculate that carboxytherapy was effective in treating manifestations of aging through increased vascular permeability (11), vasodilatation (9-11,18), promotion of angiogenesis, stimulation of dermal fibroblasts, increased synthesis of collagen and elastin with better quality (19), increased collagen remodeling (18), and increased flexibility and decreased stiffness of collagen fibers (9-11,18). Our sonographic findings confirmed the efficacy of carboxytherapy in reduction of skin aging manifestations. Based on our findings, the thickness of epidermis and dermis was significantly increased (Figure 3).

The statistically significant effectiveness of carboxytherapy was generally demonstrated with regard to skin lightening; however, skin darkening was observed in the first 4 sessions. We hypothesized that this could be attributed to high blood perfusion at the site of treatment in the primary stages of the treatment, which was obscured by increased skin thickness in later stages.

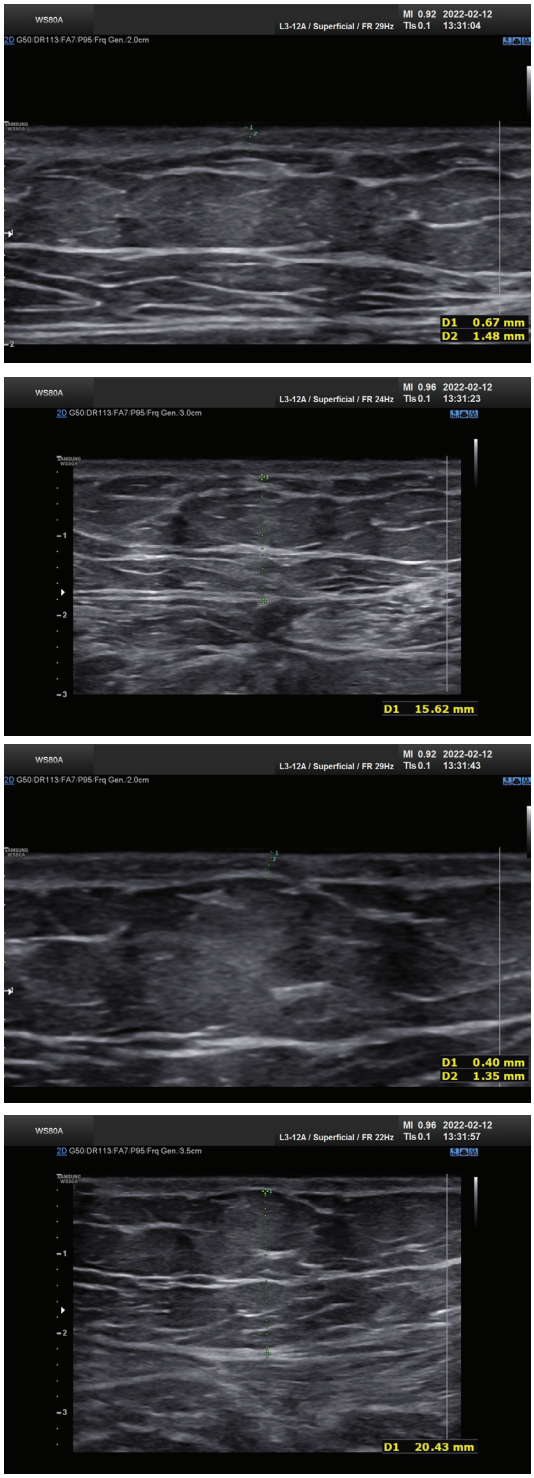


Figure 3. Sonographic findings in a subject who received carboxytherapy on the right side of the abdomen; (A) and (B) the thickness of the epidermis, dermis, and subcutis on the right side; (C) and (D) the thickness of the epidermis, dermis, and subcutis on the left side. As shown in the figures, an increase was observed in thickness of the epidermis and dermis of the treated side, while the subcutaneous thickness showed a decrease.

Overall, our study demonstrated the statistically significant effectiveness of carboxytherapy in treating skin aging and its manifestations through subjective and objective clinical evaluations.

The efficacy of carboxytherapy in treating cellulite has been demonstrated. In a study by Lee, the effectiveness of carboxytherapy in treating cellulite was shown by measuring the weight and the circumference of the treated site. In this study, 101 women and 10 men received treatment for abdominal cellulite, and 57 women underwent therapy for thigh cellulite. The author demonstrated statistically significant weight loss and reduction of abdominal and thigh circumference in women, while the results in men were not significant (27). Pianez *et al.* evaluated the efficacy of carboxytherapy in treating 10 women with cellulite. In this study, standardized digital photography and ultrasonography showed a statistically significant reduction of the cellulite from degree III to degree II, and improved organization of the fibrous lines and disposal of adipose tissue lines (18). A comparison between carboxytherapy and mesolipolysis using phosphatidylcholine in a study by Eldsouky *et al.* showed no difference in the management of cellulite in 48 women after evaluation using a cellulite grading scale and thigh circumference measurements (28).

Different studies have shown the effectiveness of carboxytherapy in treating localized adiposities as demonstrated by measurable reduction in the subcutaneous fat thickness (18, 29). In a study on 42 patients by Brandi *et al.*, carboxytherapy was introduced as a complement modality to liposuction. The authors demonstrated the effectiveness of this modality in improving skin irregularities and cutaneous elasticity after liposuction (30). In a study on 28 female Wistar rats, Balik *et al.* demonstrated the effectiveness of carboxytherapy in treating local adiposity. The authors reported a statistically significant decrease in adipocyte diameters by calculating the maximal diameter of 50 adjacent adipocytes during both the early and late phases of their study (31). In a left-right sided clinical trial on 10 women, Lee demonstrated the effectiveness of carboxytherapy in lipolysis on the bra line, upper arms, abdomen, and thighs. In this study, caliper and ultrasonography were applied for measuring subcutaneous fat thickness (32).

In the present study, although a decrease in the subcutaneous fat thickness was seen on sonography in the treatment group compared with the control group, this decrease was not statistically significant (Figure 3). This finding was expected because the volume of CO2 injection was not sufficient for the promotion of lipolysis. Additionally, in the present study, the level of gas injection was intradermal, not subcutaneous. Thus, the effect of the gas on the dermis was greater than on the subcutis. However, the decrease in waist circumference was statistically significant in the case group, which did not appear to be due to the decreased subcutaneous fat thickness. Given the mechanism of action of carboxytherapy, the decrease in waist circumference can be attributed to improvement of tissue drainage resulting in decreased cellulite, improvement of skin laxity resulting in skin lifting and increased abdominal wall stiffness, and increase of muscle trophicity resulting in decreased abdominal sagging (10). Abdominal wall stiffness as a symptom of muscle trophicity was reported in 24 cases (85.7%) of cases.

A statistically significant decrease was observed in the weight and BMI of our subjects after treatment. We hypothesize that the weight loss and consequently decreased BMI may be due to increased blood flow to the abdominal wall, promotion of angiogenesis, and increased temperature of the skin, all of which can result in activation of fat oxidation in the fatty cells (10,11). Additionally, our treatment may induce our patients to follow a diet in order to lose weight.

The pain due to carboxytherapy is the main limiting factor for its application, with negative effects on the tolerance of this procedure (33). In the present study, immediate pain that was mild and insignificant was reported by all our subjects (100%). In 5 cases (17.9%), long-lasting pain (pain lasting more than 48 hours) that was mild and tolerable, was reported in only one session during the study.

A sensation of heat, tingling, and burning sensations have been reported as complications immediately after therapy. These sensations often resolve quickly, however, prolonged sensation of heat due to vasodilatation, which resolves within 10 to 20 minutes, has been reported. In sensitive persons, these sensations can persist for up to 24 hours after therapy (13). In the present study, immediate sensation of heat that was insignificant and tolerable was reported in 2 cases (7.1%).

Ecchymosis and hematoma formation, which are reported by 5% of patients, are other side-effects of carboxytherapy. Persistent edema lasting for more than 48 hours is another side-effect of this therapy, particularly commonly reported when the procedure is performed in the periorbital area (13). In the present study, bruising was observed in all patients (100%) in at least one session during the study, but disappeared within 5 to 7 days. No edema was reported by our subjects.

With regard to minor complications, a sensation of fullness was reported in 3 cases (10.7%) in at least one session during the treatment. Asymmetry in the abdomen was observed in all the cases (100%), which was due to decrease in the skin laxity, wrinkles, and striae distensae on the treated side.

The low number of cases, an imprecise method of subjective and objective clinical assessment, and low precision of conventional sonography machines are the most important limitations of our study. Thus, a robust study with more patients, more precise methods of clinical investigation, and high frequency ultrasonography would be necessary to confirm the effectiveness of carboxytherapy in the treatment of skin aging.

CONCLUSION

Skin aging is a complicated process resulting from intrinsic and extrinsic factors (4) and is defined as an inevitable physiological process leading to skin thinness, fine wrinkles, dry skin, progressive dermal atrophy, loss of skin elasticity, and skin laxity (7).

Carboxytherapy is considered a safe, minimally invasive modality applied for skin rejuvenation, restoration, and reconditioning (9). In the present study, we demonstrated the effectiveness of this modality in the treatment of skin aging through clinical and sonographic evaluation.

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