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ORIGINAL CONTRIBUTION

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Comparative study of the efficacy of Platelet-rich plasma combined with carboxytherapy vs its use with fractional carbon dioxide laser in atrophic acne scars

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Summary

Background: Acne scars are a major concerning problem to all acne patients affecting their quality of life. Platelet-rich plasma (PRP) and fractional CO_2 laser are innovative treatment modalities for acne scars. Carboxytherapy can also be used to improve scar tissue through the increase in collagen deposition and reorganization, and the improvement in skin texture and tone.

Aims: The aim of this work was to compare the efficacy, safety, and complications of the intradermal injection of PRP combined with carboxytherapy and PRP combined with fractional CO_2 laser, in the treatment of atrophic acne scars.

Patients and Methods: Forty patients with atrophic acne scars were divided into 2 groups. Group A included 20 patients and was subjected to three fractional CO_2 laser sessions combined with PRP injection. Group B included 20 patients and was subjected to three sessions of carboxytherapy combined with PRP injection.

Results: Both fractional CO_2 laser and carboxytherapy combined with PRP showed improvement in acne scars and patients' satisfaction but the improvement with fractional CO_2 laser was significantly better than carboxytherapy but with more side effects.

Conclusions: Improvement of acne scars was noted in both treatment modalities with obvious higher and statistically significant results in favor of fractional CO_2 laser but with more side effects. Carboxytherapy is a promising tool in treatment of acne scars with less complication.

KEYWORDS
acne scars, carboxytherapy, CO_2 laser, PRP

1 | INTRODUCTION

Acne scarring is an unfortunate, permanent complication of acne vulgaris, which may be associated with severe psychological distress. Although it may occur in up to 95% of patients with acne vulgaris, the precise incidence of acne scarring is not well studied.[1]

It could be a risk factor for suicide and also may cause poor self-esteem, depression, anxiety, impaired social interactions, body image alterations, embarrassment, anger, lowered academic performance, and unemployment.[2]

Why some acne patients develop scars while others do not is still a matter of debate. The scarring process can occur at any stage of acne with no correlation to severity of acne.[3]

Many different treatments are available to acne scars but with limited success including chemical peels, surgical excision, punch

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grafting, dermabrasion, and tissue augmentation. The clinical utility of these treatments is limited due to complications such as incomplete scar removal, poor intraoperative visualization, scar worsening, tissue fibrosis, and permanent pigmentary alteration.[4]

New techniques have been added to improve the outcome of acne scars treatment, and older ones have been modified to improve risk-benefit profiles. Moreover, combined modalities are often required to achieve best results.[5]

Platelet-rich plasma (PRP) treatment is performed via the autologous injection of high concentration of platelets in a small volume of plasma. PRP is commonly used in dermatology and plastic surgery, especially for treating chronic wounds, ulcers, and burns. Lately, PRP has also started to be used in volumetric filling, skin rejuvenation, acne scars, and alopecia.[6]

The most important contents of platelets are present in the α-granules. There are more than 30 bioactive substances in these granules such as platelet-derived growth factor (PDGF), transforming growth factor (TGF)-β1, 2, epidermal growth factor, and mitogenic growth factors such as platelet-derived angiogenesis factor and fibrinogen.[7]

Fractional ablative carbon dioxide (CO_2) laser (FCL) therapy is based on the theory of fractional photothermolysis creating microscopic channels of thermal injury in the skin, skin tightening and smoothening through ablation and re-epithelialization, and elevation of the floor of depressed scars through collagen remodeling. The CO_2 laser wavelength is 10 600 targeting water in epidermis and superficial dermis. Fractional ablative lasers have been widely used in acne scars, with favorable results despite the possible complications.[8]

Carboxytherapy refers to the cutaneous and subcutaneous administration of carbon dioxide gas [CO_2] for therapeutic purposes.[9]

Carboxytherapy has the same effect as needle subcision as it has the ability to improve blood flow, vascularization, and collagen synthesis. Moreover, carboxytherapy was found to improve skin elasticity, circulation, encourages collagen repair, improves the appearance of fine lines and wrinkles, and destroys localized fatty deposits.[10]

Intradermal injection of CO_2 has many advantages, especially because of the clinically favorable results observed, its low cost, and safety. However, there is a lack of scientific evidence regarding the histological modifications that occur with the intradermal injection of CO_2.[9]

2 | SUBJECTS AND METHODS

This study was carried out after approval from the Department of Dermatology and Andrology and The Research Ethics Committee in Benha University. All patients were recruited from Dermatology Outpatient Clinic, Benha University Hospital in the period March 2015-August 2016. An informed consent was obtained from all participants.

This study included forty patients with atrophic acne scars. Patients using contraceptive pills, anticoagulant, aspirin, skin type 5 or 6, patients who have been treated with lasers or chemical peels within the last month and patients known to be HIV or hepatitis B or C positive or immunocompromised were excluded from the study. Patients were subjected to complete history taking (including personal history, acne vulgaris history as well as history of other skin diseases or drug intake) and general clinical and cutaneous examination.

Local anesthetic cream (5% lidocaine) was applied to the area to be treated in all patients and under occlusion for 60 minutes before the procedure to minimize pain or discomfort. Then, the whole face was cleansed using a mild cleanser and dried with sterile gauze.

Patients were divided into two groups:

2.1 | Group A

Twenty patients were subjected to three sessions of PRP intradermal injection after fractional CO_2 laser with 1 month apart.

The face was subjected to fractional CO_2 laser (Smartxide DOT, Advanced CO_2 Fractional technology, DEKA, Florence, Italy). The treatment parameters were power, 15 W; dwell time, 600 ms; spacing, 700 μm; smart stack, level 3. Ice packs were used to minimize heat and pain during and after the procedure. Afterward, the treated areas were injected with autologous PRP.

2.1.1 | Platelet-rich plasma preparation

A 15 mL volume of autologous blood was withdrawn from each patient into tube containing 4% sodium citrate. The separation of the blood cell elements was performed using a laboratory centrifuge (Regen Lab, CA, USA). The tubes were centrifuged at 1500 revolutions per minute for 6 minutes at room temperature resulting in three basic components, red blood cells (bottom of the tube), PRP (middle of the tube), and platelet-poor plasma (PPP) (top of the tube). Separated PRP with PPP was collected with the help of pipette in another test tube. This tube was rotated in a second centrifugation, which was longer and faster than the first, called "hard spin," at 2500 revolution per minute for 15 minutes. This allows the platelets (PRP) to settle at the bottom of the tube. The upper layer containing PPP was discarded, and the lower layer of PRP was loaded in an insulin syringe.

2.2 | Group B

Twenty patients were subjected to three sessions of PRP intradermal injection, and then, CO_2 gas (Carboxytherapy machine (Cell-Q CARBO, Seoul, South Korea)) was injected intradermally in the area of scars using a 30G needle with flow 80 cc/min. The clinical endpoint of each injection is at the occurrence of an erythema and distension of the injected tissue.

Follow-up for all patients was carried out after 1 week of each session for early complications (edema, hyperpigmentation, and pain) and then for 3 months after the last session. Photographs were taken before and after last session.

Patients' evaluation by two dermatologists and patients' satisfaction were given an improvement score:{+:0%-25% (minimum),++: 25%-50% (moderate),+++: 50%-75% (good),++++:75%-100% (very good),where +/- means no change}.

2.3 | Statistical analysis

Results were expressed as number (%). Comparison between categorical data was performed using chi-square test. The data were considered significant if *P* value was equal to or less than .05. Statistical analysis was performed with the SPSS computer program (version 16 Windows).

3 | RESULTS

Both genders were included in the study as group (A) included 5 males and 15 females, while group (B) consists of only one male and 19 females. While the range of ages of patients in group A was from 18 to 47 years with a mean of 28.65 ± 7.74 years, the range of ages of group B was from 17 to 40 years with a mean of 25.10 ± 6.70 years. Five patients in group A were smokers, while in group B, three patients were smokers. There was no statistically significant difference in all demographic data between the two groups.

As regards occupation of patients, in group (A), nine (45%) patients were employee, six (30%) were students, and five (25%) were housewives with only four (20%) patients reporting a history of exacerbation of their acne lesions with sun, while in group (B), eight (40%) patients were employee, eight (40%) were students, and four (20%) were housewives with only four (20%) patients reporting a history of exacerbation of their acne lesions with sun. In group (A), five (25%) patients reported family history of acne and only three (15%) patients reported family history of acne scars, while in group (B), six (30%) patients reported family history of acne and four (20%) patients reported family history of acne scars with no statistically significant difference in occupation, sun exposure, family history of acne, and acne scars between the two groups.

Patients included in this study had different skin types. In group (A), 45% had skin type II, 35% had skin type III, and 20% had skin type IV, while in group (B), 45% had skin type II, 40% had skin type III, and 15% had skin type IV with no statistically significant differences between the two groups.

According to the predominant scar, 4 (20%) patients in group (A) had boxcar scar and 16 (80%) had rolling scar, while in group (B), 5 (25%) patients had boxcar scar, 14(70%) had rolling scar, and only one patient had icepick scar with no statistically significant differences between the two groups (*P* value = .275).

Evaluation of patients' improvement indicated a statistically significant improvement of acne scars in group A compared to group B (Table 1)(Figures 1-3), with no statistically significant differences between the two groups in patients' satisfaction (Table 2).

As regards pain, in group (A), most of the patients had pain (70%), while in group (B), most of patients had no pain (60%). In group (A), most of the patients had no post-treatment hyperpigmentation (80%), as well as in group (B) most of patients had no post-treatment hyperpigmentation (95%) with no statistically significant differences between the two groups in pain and post-treatment hyperpigmentation. As regards edema, in group (A), most of the patients had edema (90%), while in group (B), half of patients had edema (50%) with statistically significant differences between the two groups.

TABLE 1 Comparison between the two groups as regards evaluation of treatment

Evaluation	Group A	Group B	Total
Very good, N (%)	9 (45.0)	2 (10.0)	11 (27.5)
Good, N (%)	5 (25.0)	7 (35.0)	12 (30.0)
Moderate, N (%)	5 (25.0)	5 (25.0)	10 (25.0)
Minimum, N (%)	1 (5.0)	6 (30.0)	7 (17.5)
Total, N (%)	20 (100.0)	20 (100.0)	40 (100.0)
Chi-square (*P*-value)	8.359 (.039*)		

*Statistical significance was accepted at *P*-value < .05.

In both groups, there was a statistically significant improvement in treatment as regards skin type, with more improvement in patients with skin type II (Tables 3 and 4).

In group (A), there was a statistically significant difference between smokers and nonsmokers in response to treatment, with more improvement in nonsmoker patients (Table 5).

There was no statistically significant difference in response to treatment as regards predominant scar in groups (A) and (B). *P* value = (.074) (.052), respectively.

4 | DISCUSSION

Multiple treatment modalities with different protocols for acne scars have been tried, either singly or in combination with different results. The best treatment option for acne scars is still unavailable, so dermatologists have tried to put protocols, but still there is no consensus on the best treatment.[11]

In this study, evaluation was assessed with grading scale to evaluate the degree of improvement of the scars after therapy. Patients' evaluation in group (A) shows that 45% had very good evaluation score, 25% had good evaluation score, 25% had moderate evaluation score, and 5% had minimum evaluation score.

A study done by Lee et al[12] comparing the effect of fractional CO_2 laser alone and the effect of combining fractional CO_2 laser with PRP in acne scars, showed that treatment with PRP after ablative CO_2 fractional resurfacing improves recovery of laser-damaged skin and synergistically improves the clinical appearance of acne scarring.

In another study done by Shin et al[13] twenty-two Korean women underwent three sessions of fractional laser; 11 were treated with topical application of PRP combined with fractional laser. PRP combined with fractional laser had more patients' satisfaction with much

FIGURE 1 Before and after fractional CO_2 laser with Platelet-rich plasma (PRP) showing very good improvement

FIGURE 2 Before and after carboxytherapy with Platelet-rich plasma (PRP) showing good improvement

FIGURE 3 Before and after carboxytherapy with Platelet-rich plasma (PRP) showing moderate improvement

TABLE 2 Comparison between two groups as regards patient's satisfaction

Patients satisfaction	Group A	Group B	Total
No, N (%)	0 (0)	0 (0)	0 (0)
Minimal, N (%)	0 (0)	0 (0)	0 (0)
Moderate, N (%)	7 (35.0)	9 (45.0)	16 (40.0)
Good, N (%)	8 (40.0)	8 (40.0)	16 (40.5)
Very good, N (%)	5 (25.0)	3 (15.0)	8 (20.0)
Total, N (%)	20 (100.0)	20 (100.0)	40 (100.0)
Chi-square (*P*-value)	0.750 (.687)		

better improvement of skin elasticity with decrease in the erythema index. PRP had the advantage of increasing the length of the dermo-epidermal junction, the amount of collagen, and the number of fibroblasts. The ability of PRP to increase dermal elasticity is due to keratinocyte and fibroblast proliferation and collagen production.

Faghihi et al[14] investigated the potential effect of platelet-rich plasma combined with fractional laser therapy in the treatment of acne scarring and concluded that overall clinical improvement of acne scars was higher on sites treated with combined platelet-rich plasma-fractional carbon dioxide laser than those treated with ablative fractional carbon dioxide laser alone.

Gawdat et al[15] used topical and intradermal platelet-rich plasma after ablative fractional carbon dioxide laser for acne scar treatment in 30 patients. One group underwent treatment with fractional carbon dioxide laser-intradermal platelet-rich plasma on one side and fractional carbon dioxide laser-intradermal normal saline on the other side. The other group underwent fractional carbon dioxide laser with intradermal platelet-rich plasma on one side and fractional carbon dioxide laser with topical platelet-rich plasma on the other side. In addition to reporting synergistic effects on clinical response and patient satisfaction after adding platelet-rich plasma to fractional carbon dioxide laser, they also showed that side effects were fewer on the platelet-rich plasma sides (both intradermal and topical) than the other sides. Other studies also reported less erythema after adding platelet-rich plasma to laser therapy.[13,16]

Platelets help wound healing, not only through matrix formation, but also through cell differentiation and proliferation as well as angiogenesis.[17] Upon platelet activation during the PRP preparation, 30% of the growth factors are released and the 70% is released over the following 8 days in the tissues, which is the platelet life span, where they are all in physiological amounts, thus better than recombinant growth factors.[18]

As regards group (B), 35% of patients had good evaluation score, 30% had minimum evaluation score, 23% had moderate evaluation score, and 10% had very good evaluation score with statistically significant differences between the two groups in favor of CO_2 laser.

The tissue stretching during infusion induces a subclinical inflammation, which triggers tissue regeneration processes that induce the activation of macrophages, fibroblasts, and endothelial cells that stimulate remodeling of the extracellular matrix.[9]

TABLE 3 Comparison between responses to treatment according to skin type in group (A)

Skin type (A)	Very good	Good	Moderate	Minimum	Total
II, N (%)	7 (77.8)	2 (40.0)	0 (0)	0 (0)	9 (45.0)
III, N (%)	2 (22.2)	3 (60.0)	2 (40.0)	0 (0)	7 (35.0)
IV, N (%)	0 (0)	0 (0)	3 (60.0)	1 (100.0)	4 (20.0)
Total, N (%)	9 (100.0)	5 (100.0)	5 (100.0)	1 (100.0)	20 (100.0)
Chi-square (*P*-value)	16.575 (.011*)				

*Statistical significance was accepted at *P*-value < .05.

TABLE 4 Comparison between responses to treatment according to skin type in group (B)

Skin type(B)	Very good	Good	Moderate	Minimum	Total
II, N (%)	2 (100.0)	5 (71.4)	2 (40.0)	0 (0.0)	9 (45.0)
III, N (%)	0 (0)	2 (28.6)	3 (60.0)	3 (50.0)	8 (40.0)
IV, N (%)	0 (0)	0 (0)	0 (0)	3 (50.0)	3 (15.0)
Total, N (%)	2 (100.0)	7 (100.0)	5 (100.0)	6 (100.0)	20 (100.0)
Chi-square (*P*-value)	13.837 (.032*)				

*Statistical significance was accepted at *P*-value < .05.

TABLE 5 Comparison between smokers and nonsmokers in response to treatment in group (A)

Smoking (A)	Very good	Good	Moderate	Minimum	Total
No, N (%)	8 (88.9)	5 (100.0)	2 (40.0)	0 (0)	15 (75.0)
Yes, N (%)	1 (11.1)	0 (0)	3 (60.0)	1 (100.0)	5 (25.0)
Total, N (%)	9 (100.0)	5 (100.0)	5 (100.0)	1 (100.0)	20 (100.0)
Chi-square (*P*-value)	8.859 (.031*)				

*Statistical significance was accepted at *P*-value < .05.

Carboxytherapy can also be used to improve scar tissue through the increase in collagen deposition and reorganization, and the improvement in skin texture and tone.[19]

In group B, subcutaneous injection of CO_2 gas (carboxytherapy) adds an adjuvant effect to the PRP by its effect on increasing the circulation and improving the collagen remodeling under the scar in addition to its subcision effect. Patients of group B showed improvement but less than laser group.

As far as we know, this study was the first to compare the efficacy of intradermal injection of PRP with carboxytherapy and PRP with fractional CO_2 laser in treatment of acne scars.

When patients were questioned about their overall satisfaction, no statistically significant differences were found between the two groups. This may mean that the two treatment modalities showed improvement in acne scars, no complete healing was achieved causing dissatisfaction of patients, and although patients on laser therapy much improved than patients on carboxytherapy, they had more side effects affecting their satisfaction of treatment.

In this study, there was a statistically significant difference in response to treatments regarding skin type, with better improvement of patients with skin type II than darker skin types. Also, nonsmokers in group A showed better improvement in their scars than smokers, and this may be due to less vascularity and oxygenation of the blood.

The toxic constituents of cigarette smoke; particularly, nicotine, carbon monoxide, and hydrogen cyanide have harmful effects on wound healing. Nicotine reduces blood flow to the skin, resulting in tissue ischemia and impaired healing of injured tissue, increases platelet adhesiveness, raising the risk of thrombotic microvascular occlusion and tissue ischemia, and reduces proliferation of red blood cells, fibroblasts, and macrophages. Carbon monoxide decreases oxygen transport and metabolism, whereas hydrogen cyanide inhibits the enzyme responsible for oxygen transport at the cellular level.[20]

5 | CONCLUSION

Fractional CO_2 laser combined with PRP is a very good tool for treatment of acne scars, while carboxytherapy combined with PRP is an innovative and promising treatment of acne scars with fewer side effects. All patients should be advised to stop smoking to improve treatment outcome of acne scars.

CONFLICT OF INTERESTS

No conflict of interests.

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