

# Evaluation of the Response of Fractional Carbon Dioxide (CO<sub>2</sub>) Laser and Punch Elevation Technique in Treatment of Acne Scars: A Split-face Comparative Clinical, Histopathological, and Immune Histochemical Study

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## ABSTRACT

**Background:** Acne is the most common skin disease affecting adolescents and young adults, with associated scarring and its sequelae. Treatment options are either lifting procedures raising scar base closer to normal skin surface, or resurfacing ones injuring epidermis and superficial dermis with neocollagenesis and epidermal repair. Punch elevation (PE) method is better for improving deep atrophic acne scars, that can be combined with depth resurfacing. CO<sub>2</sub> laser stimulates new collagen formation, tightens skin and raises scar to surface. **Aim of the Work:** to assess efficacy and safety of combination of PE technique and fractional co<sub>2</sub> laser (Fr co<sub>2</sub> L) compared to fr co<sub>2</sub> L alone. **Subjects and Methods:** 20 atrophic post acne scars patients were subjected to history taking, general and dermatological examinations, identifying scars types and grades. Treatment response at start, before each fr. co<sub>2</sub> L session and 4 weeks after last laser session was clinically evaluated by Goodman and Baron scale, and recording patients' photographs, satisfaction, and complications. Histopathological examination of scar skin biopsies before and 4 weeks after the last fr. co<sub>2</sub> L session was done using H&E, Mallory trichrome, sliver stain and immunohistochemical VEGF. **Results:** combined treatment with fr co<sub>2</sub> L and PE was more effective in improving post acne scars than fr. co<sub>2</sub> L resurfacing alone. Histopathological examination before combined fr. co<sub>2</sub> L and PE treatment and 1 month after the last fr. co<sub>2</sub> L session showed high significant increase in epidermal thickness (H&E), increase in collagen fiber type III (Mallory trichrome stain), decrease in collagen fiber type I (sliver stain) and increase in fibroblasts' activity (VEGF). **Conclusion:** both PE technique and fr. co<sub>2</sub> L resurfacing show promising efficacy and safety in treating atrophic post acne scars, complementing each other to improve skin texture and appearance, with excellent cosmetic outcomes. **Keywords:** Acne scar, Punch elevation, Fractional CO<sub>2</sub> Laser, Efficacy, Safety.

## INTRODUCTION

Acne is an inflammatory disease of the pilosebaceous units in the skin of the face, neck, chest and upper back<sup>(25)</sup>. While the reported prevalence rates of acne vulgaris vary from 35 % to 90 % in adolescents, and it persists into adulthood in approximately 12% of cases with psychological and social implications<sup>(8)</sup>. In some patients with acne, the inflammatory response results in permanent, disfiguring scars from either increased tissue formation or due to loss or damage of tissue. Hypertrophic scars and keloids are examples of scars that result from increased tissue formation. Atrophic scars are caused by loss or damage of skin tissue, occur in 80-90%, and can be classified into icepick, rolling and boxcar scars<sup>(6)</sup>.

Many treatment modalities are available that reduce the appearance of acne scars, however acne scars are often difficult to be treated and require the use of many various approaches over multiple sessions. Medical management of atrophic scars can be done using topical retinoids. Surgical management includes punch excision or elevation, skin grafting and

Subcision<sup>(25)</sup>. Procedural management includes microdermabrasion, chemical peels, and percutaneous collagen induction by microneedling<sup>(3)</sup>. Various ablative and non-ablative lasers and light energies are also available for treatment of atrophic acne scars<sup>(26)</sup>.

Fractional carbon dioxide lasers have now been considered the gold standard treatment for atrophic acne scars and skin rejuvenation<sup>(28, 27, 18, 16)</sup>. They ablate the skin surface and help in tightening the collagen fibers underneath. Furthermore, they treat only fractions or columns of the affected skin, leaving skin intervening areas untreated which stimulate rapid skin re-epithelialization, with minimal chance of prolonged and serious adverse effects<sup>(13, 11)</sup>.

Another common treatment approach for atrophic acne scars is Punch elevation, utilizing a circular blade applied only to the base, not the walls of the scar yielding a more even skin after healing with reduction of pitted or pockmarked look<sup>(7)</sup>.

## AIM OF THE WORK

The current work aimed at comparing the efficacy of combined use punch elevation and fractional carbon

dioxide (CO<sub>2</sub>) laser resurfacing, with fractional CO<sub>2</sub> laser resurfacing alone, for treatment of atrophic acne scars.

## PATIENTS AND METHODS

The present study is a split face comparative study which included 20 adult patients ranged from 18 to 55 years old (8 males and 12 females). The study was approved by the Research Ethical Committee, Faculty of Medicine, Ain Shams University and fulfilled all the ethical aspects required in human research. All patients received full information about description of the procedure of treatment, possible side effects, photo documentation and they all provided written consent. Patients with moderate to severe atrophic post acne scars, classified on the basis of **Goodman and Barons's** quantitative classification with nearly equal scores on both halves of the face and skin. phototype II-IV were included in the study, while patients excluded from the study were those with pregnancy or lactation, active inflammatory acne, immunoincompetence, use of isotretinoin in the previous 6 months, active infection in the treatment area, premalignant lesions or malignant lesions in the treatment area, bleeding tendencies, history of herpes simplex or herpes zoster infection on the face, and history of hypertrophic scars or keloids. All patients were subjected to full history taking, including onset, course and duration of acne, previous acne scar treatment and post-treatment complications such as hyperpigmentation or keloid formation, general and dermatologic examination, and post acne scars evaluation via Clinical evaluation; before start of treatment, before each fractional laser treatment session and 4 weeks after the last fractional laser session according to Goodman and Barons' quantitative global acne scarring grading system that evaluates acne scars according to their type, severity and number(10). Assessment of patients' photographs was performed independently using (Sony Cyber-shot DSC-W320 14.1 mega pixel camera). In addition to patients' satisfaction with the treatment using a visual analog scale (VAS; A rating of 0 was no satisfaction, and a rating of 10 was the best possible satisfaction<sup>(4)</sup>. A histomorphometric examination of skin biopsies taken from a specific scar area before treatment and 4 weeks after the last fractional laser treatment session was done. All slides were examined using an image analysis system which included a Leica Qwin 500 IW (Leica microsystems imaging solutions Ltd, Cambridge, UK, 2002), photo-microscope was taken with Leica DFC295 camera. Ten non-overlapping

fields were randomly selected from the slides stained with H&E, Mallory trichrome and silver stains respectively. H&E stain was used to compare the epidermal thickness. Scale bar microscopic measurement was used to measure the epidermal thickness in micro-meter ( $\mu\text{m}$ ) before and after treatment. Mallory trichrome stain was used to compare the Area percentages of type I collagen fibers before and after treatment. The degree of collagen remodeling was evaluated, Silver stain used to compare area percentages of type III collagen before and after treatment. Moreover, immunohistochemistry was done for evaluation of fibroblasts activity using vascular endothelium Growth Factor (VEGF). Lastly, an assessment of treatment complications considered; Pain, when patients were examined immediately after each session to evaluate post procedural pain which was rated using numerical rating scale (NRS). Each patient is asked to rate the pain intensity as a number from zero to ten (16), Post procedural erythema that was classified into mild (1-2 hours), moderate (2-5 hours), marked (5-12 hours) and severe (>12 hours) based on the time taken until erythema had completely resolved (17), and Other complications like scarring due to overaggressive needling and dyspigmentation.

**Methods:** Pretreatment preparation was done by injection of lidocaine with epinephrine was done before punch elevation, while local anesthesia with EMLA cream was applied before each laser session to the whole face and covered with cellophane tape for approximately 45-60 minutes. The face was disinfected and degreased with alcohol. Procedure was carried out where one side of the face was subjected to fractional co<sub>2</sub> laser alone while the other side was treated with punch elevation combined with Fractional co<sub>2</sub> laser. Our approach to treatment of acne scars consisted of two operative steps. Initially, punch elevation using 2.5 or 3 mm disposable biopsy punches (KAI Medical, Japan) was performed on one side of the face and the punched scars are covered with steri strips. Secondly, 5 days after punch elevation, a full face CO laser treatment session was performed by (BISON Fire-xel, Korea). A second full face laser treatment session was performed 4 weeks later. The laser device used on each side of the face was the same in the two treatment sessions. The parameters set on the fractional CO<sub>2</sub> laser were (scanner mode: pulse width 1.106 ms, repeat delay single, overlap 4 times, density 0.8mm, energy 66.3mj/cm<sup>2</sup>, wave length 10600nm and spot size of 300 $\mu\text{m}$ ). Post treatment advices given after punch elevation that punched scars

are to be covered with sterile strips bands for 5 days secondly, an SPF +50 sunscreen cream was put after each treatment session and to be used daily before sun exposure and to be re-applied every 2 hours and topical antibiotic was prescribed to avoid any secondary bacterial infection. Statistical analysis of the results: data were analyzed using the SSPS- version 20.0, released 2011, IBM Corp, NY).

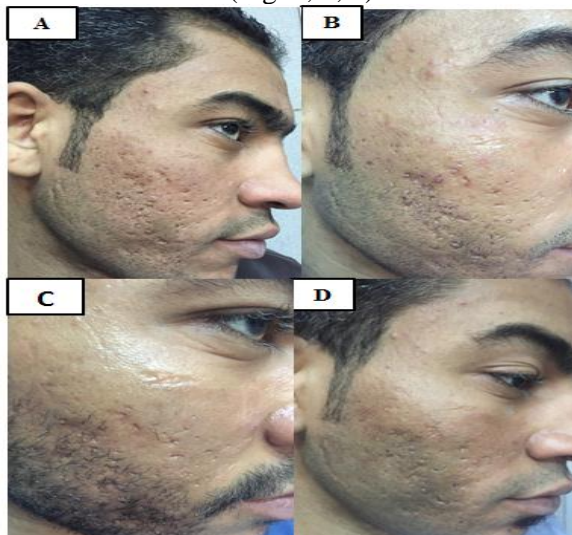
The study was approved by the Ethics Board of Ain Shams University.

## RESULTS

### I. Clinical evaluation

**1. Demographic data:** The present study included 20 patients with different types of atrophic post acne scars (10 moderate and 10 severe) based on Goodman & Baron’s qualitative classification. They were 8 males (40%) and 12 females (60%), attending the Dermatology outpatient clinic of Ain Shams University Hospitals from December, 2016 to May, 2017. The age of patients ranged from 18 to 55 years old, with the mean age of 28.6 years  $\pm$  7.34 SD. Positive family history of atrophic post acne scars accounted for 35% of all patients. Patients’ skin photo types according to Fitzpatrick were; type II for 3 patients (15%), type III for 13 patients (65%), and type IV for 4 patients (20%).

**2. Clinical improvement:** All patients exhibited improvement in skin texture, tone as well as reduction in scar severity after every treatment session. It was shown that the maximum percent of improvement was 4 weeks after the last session. Combined treatment with fr.co2 laser and punch elevation technique showed better resolution in comparison to treatment with fr. co2 laser alone (Fig. 1, 2, 3).



**Fig. (1):** Right side of a male patient, 26 years old, with multiple severe boxcar and icepick scars treated

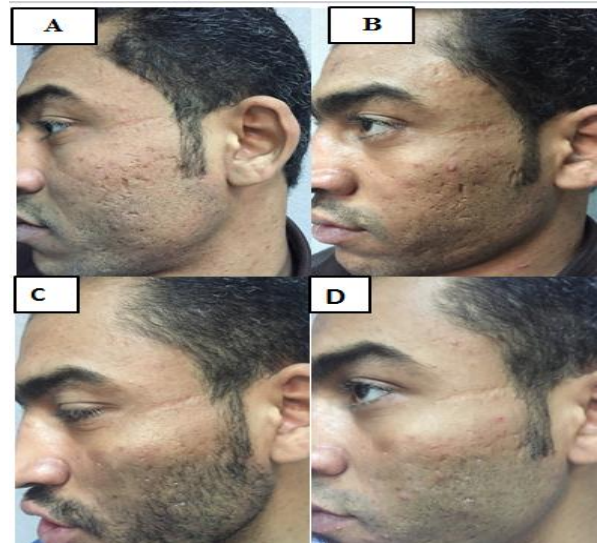
with PE technique and 2 sessions of fr. co2 laser, one month interval. Response was rated as excellent by the patient.

(A) Before treatment (total score was 20).

(B) After 5 days with PE technique

(C) 1 month after the first fr. co2 laser session

(D) Four weeks after the second fr. co2 laser session (total score was 14).



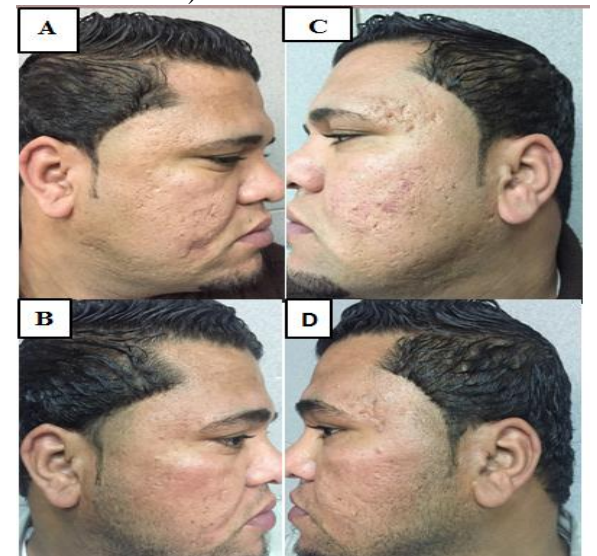
**Fig. (2):** The left side of the same patient, with multiple rolling, boxcar and icepick scars treated with 2 sessions of fr. co2 laser, one month interval. Response was rated as good by the patient.

(A) Before treatment (total score was 16).

(B) After 5 days without PE technique

(C) 1 month after the first fr. co2 laser session

(D) Four weeks after the second fr. co2 laser session (total score was 14).



**Fig. (3):** male patient, 27 years old, with multiple icepick, boxcar, and rolling scars in both halves of the

face. Response was rated as good by the patient to the right side and excellent to the left side.

(A) Rt side before treatment with PE and fr. co2 laser (total score 20).

(B) Rt side 4 weeks after the last treatment session (total score was 14).

(C) Lt side before treatment with fr. co2 laser alone (total score was 20).

(D) Lt side 4 weeks after the last treatment session (total score was 17).

Assessment of the treatment response according to Goodman's quantitative global acne scarring scale showed treatment response according to Goodman's quantitative global acne scarring scale on both sides of the face from 12.3 +/- 4.9 before PE/fr.CO2 L to 7.15 +/- 3.96 after that combined approach, compared to 12.1 +/- 5.27 before fr.CO2 L and 10.25 +/- 5.25 after fr.CO2L alone. There was a highly significant change (P<0.001) in the mean score from 12.30 points to 7.15 points with 10% minimal, 70% moderate and 20% good improvement (Tab.1), and a highly significant change (P<0.001) in the mean score from 12.10 points to 10.25 points, with 80% minimal, 15% moderate and 5% good response.

**Table (1):** Description of grade of improvement according to Goodman's scale after punch/fr. co2 laser and fr. co2 laser alone:

		n	%
Improvement in Goodman scale after punch/frac co2	Minimal	2	10.0%
	Moderate	14	70.0%
	Good	4	20.0%
Improvement in Goodman scale after frac co2 alone	Minimal	16	80.0%
	Moderate	3	15.0%
	Good	1	5.0%

As regards to the change in the mean Goodman's qualitative scale score and the percentage of change 1 month after the last fractional co2 laser session, both punch elevation combined with fractional co2 laser and fractional co2 laser alone showed highly significant change in of the score being higher with combined punch elevation technique and fractional co2 laser with 5.15 points and 44.40% compared with 1.85 points and 18.89% with fractional co2 laser alone.

**Table (2):** Description of percent of change in Goodman's scale after punch/fr. co2 and fr. co2 treated sides:

	Mean	±SD	Min	Max	Med	IQR	
Percent of change in Goodman after punch/frac co2	44.40	15.18	15	75	45.45	35.42	50
Percent of change in Goodman after frac co2 alone	18.89	14.26	0.	60	16.67	10.26	21.1

Comparing response to combined fr.CO2 and PE (mean = 5.15 +/- 2.13) with fr.CO2 alone (mean = 1.85 +/- 0.87) by scores of Goodman's scale and percent of change in the same scale scores yielded highly significant difference (P<0.001).

Assessment of treatment response according to patient's satisfaction: when patients were asked to rate their treatment satisfaction on each side of the face 1 months after the last fr.co2 laser session in a scale of 1-10; the mean satisfaction score was 6.80 points on the side of the face treated with combined punch elevation technique and fr. co2 laser which was higher than 5.65 points on the side treated with fr. co2 laser alone, also with highly significant difference (P<0.001). In addition, an assessment of the treatment complications on asking patients to rate the pain in a scale of 1-10, could coin that the mean was 6.30 points after fr. co2 laser sessions while there was no pain felt with punch elevation as local anesthesia was injected into the site of the procedure. Only five patients had post inflammatory hyper pigmentation, with no other complications were recorded as secondary infection, scarring keloid and prolonged erythema.

There was no significant relation (P>0.057) between the patients sex and the change in the Goodman's quantitative score and percentage of change with combined PE technique and fr. co2 laser or with fr. co2 laser alone 1 month after the last fr. co2 laser session, while family history in relation to Goodman's quantitative score change and percentage of change was significant (P< 0.24) with PE and fr. co2 laser. However, there was no significance on relation between family history and change in Goodman's quantitative scale score (P>0.26) or percentage of change with fr. co2 laser alone (P>0.14). In correlating between age and the change



in Goodman’s quantitative score and percentage of change; no significance ( $P > 0.157$ ) was found nor with PE combined with fr. co2 laser or with fr. co2 laser alone.

**II. Histopathological evaluation:**

Results of histopathological study made for 6 patients before treatment with combined PE and fr. co2 laser showed; i) fibroblast activity had a mean of 5.87 area % using VEGF immunohistochemical marker, ii) the mean epidermal thickness was 635.65 mm using H&E stain, iii) type I collagen had a mean of 45.76 area % using Mallory trichrome stain, and

iv) type III collagen had a mean of 4.20 area% using sliver stain.

While results of histopathological study made for the same of patients 1 month after the last fr. co2 laser session from the side treated with combined PE and fr.co2 laser showed; i)the mean of fibroblast activity increased to 19.14 area % using VEGF immune -histochemical marker, ii) the mean epidermal thickness was 1497.35 mm using H&E stain, iii) the mean of type I collagen decreased to 29.01 area % using Mallory trichrome stain, and iv) the mean of type III collagen increased to 9.86 area% using sliver stain (**Tables 3, 4**).

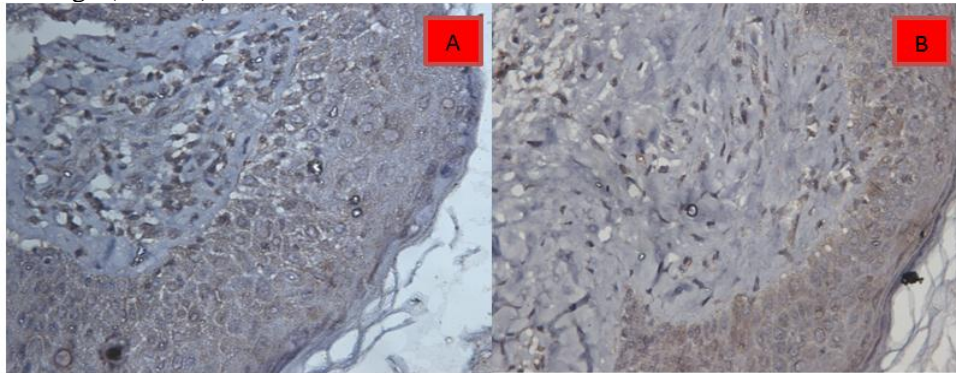
**Table 3:** Description of histopathological results before treatment in punch/fr. co2 laser treated side:

	Mean	±SD	Minimum	Maximum
Fibroblast activity before	5.87	1.87	2.79	8.21
Epidermal thickness before	635.65	62.63	530.33	703.36
Type I collagen before	45.76	4.37	40.20	51.47
Type III collagen before	4.20	1.64	2.46	6.35

**Table (4):** Description of histopathological results after treatment in punch/fr. co2 laser treated side:

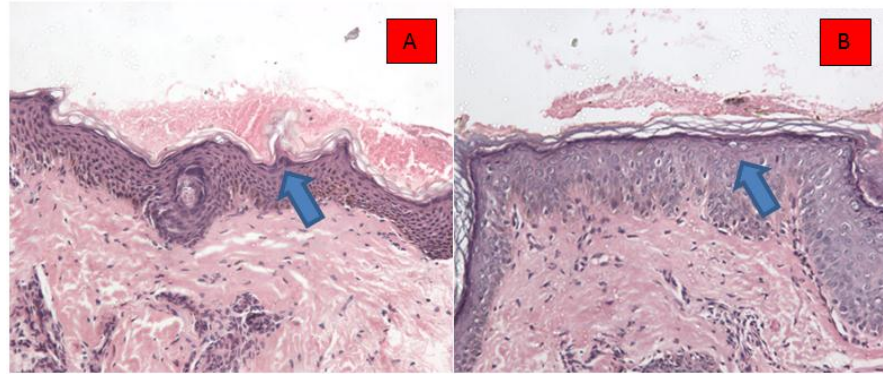
	Mean	±SD	Minimum	Maximum
Fibroblast activity after	19.14	4.47	12.10	25.30
Epidermal thickness after	1497.35	61.76	1442.74	1607.93
Type I collagen after	29.01	4.92	23.99	35.86
Type III collagen after	9.86	2.34	7.69	13.40

On Comparison between fibroblast activity using VEGF immunohistochemical marker before treatment and 1 month after the last fr. co2 laser session showed at the side treated with combined PE technique and fr. co2 laser highly significant change ( $P < 003$ ) in the score from 5.86 area % to 19.13 area %.



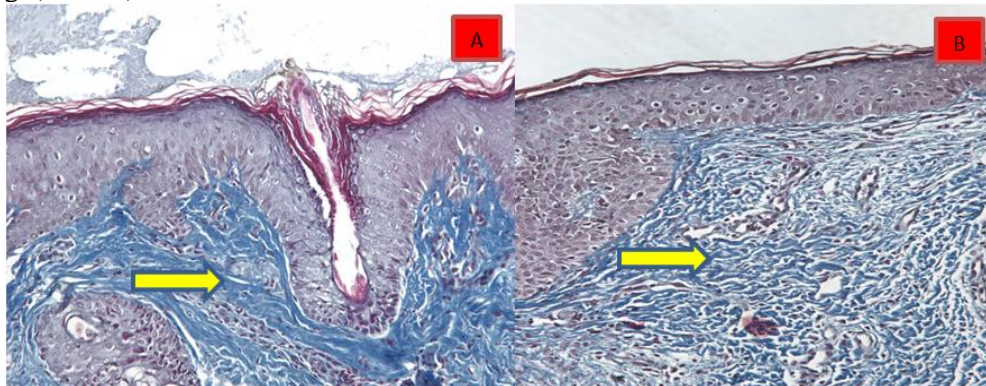
**Fig. (4):** A-B histopathological pictures showing fibroblast activity before and after punch/fr. co2 laser treatment using VEGF Immunohistochemical marker.

Comparison between epidermal thickness using H&E stain before treatment and 1 month after the last fr.co2 session at the side treated with combined PE technique and fr.co2 laser showed highly significant change ( $P < 0.001$ ) with increased in mean score from 635.64 mm to 1497.34 mm (Fig. 4 A & B, and 5 A & B).



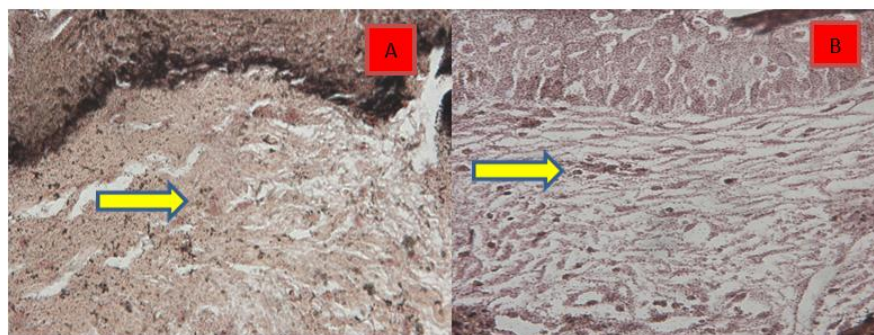
**Fig. (5):** A & B histopathological pictures showing epidermal thickness before and after punch/fr. co2 laser treatment using H&E.

Comparison between type I collagen content using Mallory trichrome stain before treatment and 1 month after the last fractional co2 session at the side treated with combined PE technique and fr. co2 laser showed highly significant change ( $P < 0.01$ ) with decrease in the score from 45.76 area% to 29.00 area%.



**Fig. (6):** A&B histopathological pictures showing Type I collagen before and after punch/fr. co2 laser treatment using Mallory trichromes stain.

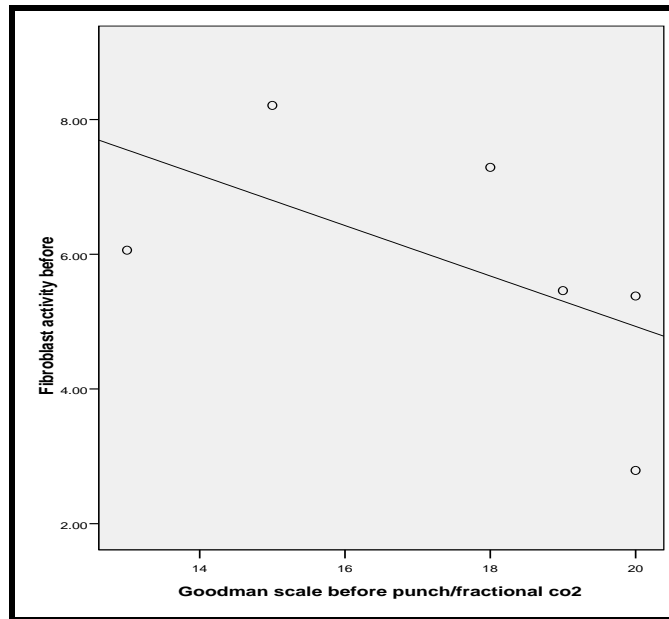
Comparison between type III collagen content using silver stain before treatment and 1 month after the last fr.co2 session at the side treated with combined PE technique and fr. co2 laser showed highly significant change ( $P < 0.02$ ) with increase in the score from 4.19 area % to 9.85 area % (Figures 7A & B).



**Fig. (7):** A & B histopathological picture showing Type III collagen before and after punch/fr. co2 laser treatment using silver stain.

Correlations done between the histopathological results and the Goodman's quantitative score before treatment with combined PE and fr. co2 laser, showed that there was a significant negative correlation between Goodman's scale and fibroblast activity

( $P < 0.05$ ) as the higher the Goodman's score the lower the fibroblast activity. However no significant correlations were found between epidermal thickness ( $P > 0.78$ ), type I collagen ( $P > 0.82$ ), and type III collagen ( $P > 0.57$ ) with Goodman's quantitative score.



**Fig. (8):** Correlations between Goodman’s quantitative scale and fibroblast activity before punch/fr. co2 laser treatment.

On the other hand, correlation between the histopathological results done and the Goodman’s quantitative score after treatment with combined PE and fr. co2 laser, indicated that there were no significant correlations between Goodman’s scale and any of histopathological results (**Table 5**).

**Table (5):** Correlations between Goodman’s quantitative scale and histopathological results after punch/fr. co2 laser treatment:

		Fibroblast activity after	Epidermal thickness after	Type I collagen after	Type III collagen after
Goodman scale after	r	.771	-.314	.143	-.371
	P	.072	.544	.787	.468
	Sig	NS	NS	NS	NS

\* Pearson Correlation

**DISCUSSION**

Acne is a multifactorial chronic inflammatory disease of pilosebaceous unit. Unfortunately, acne scarring is common and occurs easily in the course of the disease. Treating acne scars is one of the most challenging cosmetic procedures<sup>(20)</sup>. Acne scars come in a wide variety of structures and depths, and each of the available treatments is ideally suited to address a subset of this spectrum<sup>(24)</sup>. Many therapeutic modalities have been developed to attempt cosmetic correction of acne scarring, including ablative laser resurfacing, chemical peeling, dermabrasion, punch techniques, subcuticular incision (subcision), injection of dermal fillers, and fractional photothermolysis<sup>(19)</sup>. Most treatment modalities can be grouped into the broad categories of lifting procedures and resurfacing

procedures Lifting procedures raise the base of the scar closer to the normal skin surface level. Resurfacing procedures cause an injury to the epidermis and superficial dermis with resulting neocollagenesis and epidermal repair<sup>(5)</sup>.

A number of studies have been performed and demonstrated the benefits of fractional CO2 laser treatment in acne scarring and have promoted the advantage of selective ablation<sup>(29)</sup>. Although CO2 lasers allow for more precise ablation control, some deeper scars require multiple treatment passes that could produce additional scarring. Another limitation of the CO2 laser is its depth of penetration. The laser is only effective in approximately the upper 30 μm of the skin, which limits the area that can be treated by the laser<sup>(21)</sup>.

The punch elevation method is better for improving

deep acne scars than depth resurfacing and it can be combined with depth resurfacing depending on the type of acne scar. Therefore, punch elevation techniques have increased the efficacy of treatment of atrophic acne scars<sup>(13)</sup>.

The aim of our study was to compare between the use of fractional co2 laser alone and combined use of fractional co2 laser resurfacing with punch elevation technique in atrophic acne scarring treatment. The study included 20 patients (8 males and 12 females) with atrophic post acne scars. The age of patients ranged from 18 to 54 years old, with the mean age of 28.60 years  $\pm$  7.34 SD. One side of the face was subjected to fractional co2 laser alone and the other side was treated with punch elevation and Fractional co2 laser resurfacing. Patients had punch elevation treatment on one side of the face then 5 days later 2 fractional co2 laser treatment sessions for the whole face at 4-weeks interval

With punch elevation technique the scar is punched but not discarded. The tissue cylinder may be freed up at the level of the subcutaneous fat or transected from its fat base. The scar floats up and is held in place with forceps so the scar sits at the same level as the surrounding skin. The cylinder of tissue will become fixed in place by the patient's serum and sit as a flap attached at its subcutaneous base or as a free graft. It is then immobilized with surgical tape for several days<sup>(9)</sup>. This method is used on deep boxcar scars that have sharp edges and bases that appear normal<sup>(4)</sup>.

For the CO2 laser, the absorption of its wavelength (10,600 nm) in water is high. However, because the chromosphere of CO2 laser is water that exists uniformly in soft tissues<sup>(25)</sup> it stimulates new collagen formation and causes tightening of the skin resulting in the scar being raised to the surface<sup>(3)</sup>.

This study confirms the efficacy of fractional co2 laser resurfacing in acne scarring treatment and suggests that the combined use of fractional co2 laser resurfacing and punch elevation technique is more effective in improving acne scars than skin fractional co2 laser resurfacing alone. Combined treatment with fractional co2 laser resurfacing and punch elevation technique showed early resolution in comparison with treatment with fractional co2 laser resurfacing alone in many sessions. All patients noticed improvement in skin texture, tone and reduction in scar severity after every treatment session, it was shown that the maximum percent of improvement seen 4 weeks after Histopathological examination carried out before treatment with combined fractional co2 laser and

the last session during follow up. Our results showed that combined treatment with fractional co2 laser and punch elevation resulted in highly significant improvement as the total mean change in Goodman quantitative scale was 44.40% at follow up period and treatment with fractional co2 laser alone resulted in significant improvement as the total mean of improvement at follow up period was 18.89 %. This finding could be attributed to the synergistic effects of both modalities on all types of atrophic post acne scars. Goodman's quantitative global scale showed excellent response in 20% of patients, good response in 70% of patients and poor response in 10% of the patient over the side treated with fractional co2 laser and punch elevation, while the side treated with fractional co2 laser alone showed excellent response in 50% of patients, good response in 15% of patients and poor response in 80% of patients. The patient satisfaction rate of treatment for the side of face treated with fractional co2 laser and punch elevation was high as 6.8 mean while the fractional co2 laser only treated side was 5.65 (out of 10). There is an agreement with the results found by *Faghihi et al.*<sup>(4)</sup> who had conducted a split-face trial to compare the efficacy of fractional co2 laser resurfacing combined with punch elevation technique and fractional co2 laser alone for the treatment of atrophic acne scars and concluded punch elevation efficacy in the management of atrophic acne scars that can be combined with fractional co2 laser to enhance the final clinical outcomes in comparison with fractional co2 laser alone. Goodman's quantitative global scale showed excellent response in 40% of patients and good response in 60% of patients over the side treated with fractional co2 laser and punch elevation, while the side treated with fractional co2 laser alone showed excellent response in 10% of patients, good response in 60% of patients and poor response in 30% of patients. Furthermore, another work done by *Jacob and colleagues*<sup>(14)</sup>, reported that the combined use of punch elevation technique with fractional co2 laser resurfacing in the treatment of atrophic post acne scars was more effective in improving acne scars than fractional co2 resurfacing alone.

To our knowledge, using fractional co2 laser and punch elevation in treatment of atrophic post acne scars in a split face trial and histopathological evaluation has not been studied in the past.

punch elevation and one month after the last treatment session showed high significant increase in the



epidermal thickness from a mean of 635.64 $\mu$ m before to a mean of 1497.34 $\mu$ m after treatment using H&E stain, highly significant increase in the collagen fiber type III content from a mean of 4.19 area% before to a mean of 9.85 area% after treatment using Mallory trichrome stain, highly significant decrease in the collagen fiber type I from a mean of 45.76 area% before to 29.00 area% after treatment using Sliver stain and highly significant increase in the fibroblasts activity using VEGF immune- histochemical marker from a mean of 5.86 area% before to a mean of 19.13 area% after treatment. These results correlates to those of *Dong et al.* <sup>(2)</sup> who found increased fibroblast activity and collagen type III production after fractional co2 laser resurfacing in acne scars. Dermal ECM is comprised mainly of type I collagen fibers, interweaved with other fibers, including type III collagen and elastic fibers <sup>(2,3)</sup>. While during the remodeling phase, type III collagen is gradually replaced by type I collagen, which is typically the most abundant collagen in the dermis <sup>(1)</sup>. This analogues to collagen change (amount and quality) seen in our study after treatment with combined fractional co2 laser and punch elevation.

Furthermore, there was a significant negative correlation between Goodman's scale and fibroblast activity at the side treated with combined fractional co2 laser and punch elevation as the higher the Goodman's score the lower the fibroblast activity. This further emphasizes the efficacy of punch elevation combined with fractional co2 laser in treatment of post acne scars. However no significant correlation were found between epidermal thickness, type I collagen and type III collagen with Goodman's quantitative score. This could be attributed to the low number of patients or short follow-up duration. Also There was no significant relation between the patients age or sex and the change in the Goodman's quantitative score and percentage of change with combined punch elevation technique and fractional co2 laser or with fractional co2 laser alone 1 month after the last fractional co2 laser session, while family history in relation to Goodman's quantitative score change and percentage of change showed significance with punch elevation and fractional co2 laser. However, there was no significance on relation between family history and change in Goodman's quantitative scale score or percentage of change with fractional co2 laser alone. These concluded that no change in treatment response with combined fractional co2 laser and punch elevation or fractional co2 laser alone based on age or sex change, However taking

family history of atrophic acne scars before start of the treatment is highly valuable. *Grevelink et al.* <sup>(12)</sup> implied that an obvious advantage to the combination of punch elevation and laser ablation is that the laser acts superficially and punch elevation eliminates the need for multiple laser passes over deep scars Moreover, another advantage of combination is the ability of the laser to effectively ablate the superficial portion of the scar and remove the epidermal component. This means that punch grafting is not necessary when using this technique, which eliminates complications of transplantation that often requires redo grafting. The combination of punch elevation with fractional co2 laser enhances the rate of improvement seen with punch elevation as it enhances collagen production and fibroblast activity in order to enhance the wound healing response.

Patients were examined immediately after each session to evaluate post procedural pain which was rated by a pain score (0 – no pain, to 10 – the highest pain) <sup>(4)</sup>. *Pain scale* after treatment showed a mean of 6.30 out of 10. The most commonly reported adverse effects were transient erythema and crusting seen in all patients lasting for an average of 3–4 and 4–7 days, respectively and resolved without any treatment. Moreover, five of the patients had post inflammatory hyper pigmentation and resolved after applying whitening agents and sunscreen, no other complications were recorded as secondary infection, scaring keloid and prolonged erythema.

#### Conclusion

Punch elevation technique is a simple, affordable office procedure with minimal downtime, better combined with fractional co2 laser resurfacing. It increases the efficacy of treatment of deep acne scars and lowers the need of more fractional co2 laser sessions which are expensive. The two treatment modalities showed a promising efficacy and safety in the treatment of atrophic acne scars, they complement each other to improve skin texture and appearance that give excellent cosmetic results.

#### Recommendations

The existing work recommends extending these lines of the studies to cover a large number of patients with longer term follow up as collagen remodeling processing continue up to several months after fractional co2 laser. It is also recommended to increase the number of sessions, prolong the time interval between sessions as well as the follow up period. These factors may help detecting the differences in the rate of treatment improvement. Finally, procedures combining

more treatment modalities are further recommended as scar subcision, microneedling and PRP with punch elevation and fractional CO<sub>2</sub> laser resurfacing.

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