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Comparison Between Er: Yag and Co2 Ablative Fractional Lasers in The Treatment of Keloid and Hypertrophic Scars: Histopathological, Immuno-Histochemical and Ultrastructural Study

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Abstract

Background: Scarring is a frequent occurrence, since it results from skin damage in individuals of all ages. This work aimed compare clinical results and effect on histopathological architecture and expression of CTGF of the ablative fractional 10,600-nm CO_2 and 2,940 nm Er:YAG lasers in keloid and hypertrophic scars treatment. **Methods:** that randomized, prospective, double-blinded comparative study included 60 individuals (Fitzpatrick skin type II to V), that are diagnosed clinically and histopathologically with hypertrophic scars. Those were split into 2 groups; 30 patients in group I (Fractional Er:YAG laser), and 30 patients in group II (fractional CO_2 laser). **Results:** A substantial decrease in post treatment VSS with fractional ablative Er:YAG and Co2 lasers mostly as a result of improved vascularity, pliability and to a lesser extent in height with Er:YAG ,while after CO_2 improvement was significant in height and pliability only. Histopathological examination of skin biopsies in current study, showed that there were increased thickness of epidermis and irregular collagen bands replacement with parallel new collagen fibril organisation after fractional ablative Er:Yag. Immunohistochemical staining of CTGF showed significant reduction 6 months follwoing fractional Erbium YAG and CO2 laser treatment with prominent decrease after Fr Er: YAG. **Conclusions:** Fractional ablative lasers including both CO2 and Er:YAG have combined impressive ablative laser results with greater protection profile of nonablative lasers. Fractional ablative Er:YAG laser mainly improve vascularity and pliability of hypertrophic scars while Co2 improvement was significant in height and pliability.

Keywords: Er: Yag, CO_2 Ablative Fractional Lasers, Keloid, Hypertrophic Scars.

1. Introduction

Scarring is a frequent occurrence, since it results from skin damage in individuals of all ages. Even though majority of scars do not represent a health concern, they may be very traumatising, leading to a decline in life quality [1].

Keloids are a frequent fibrotic condition caused by skin damage following burns, surgical excision or trauma. They are marked histologically by extensive collagen synthesis in the dermis. Unlike hypertrophic scars, keloids grow beyond the primary injury's boundary and can cause symptoms such as discomfort, swelling and itching [2].

Their pathophysiology is not fully understood, but it is thought that the aberrant biological activity of keloid fibroblasts and the complicated process of wound healing abnormalities are major causes **[3]**.

Recent study reveals that the formation of keloids is controlled by various growth factors, including CTGF (connective tissue growth factor), VEGF (vascular endothelial growth factor) and TGF-b(transforming growth factor b), that all increase fibroblasts multiplication and production of collagen in keloids. Therefore, the aforementioned elements play a significant and intricate role in the origin and evolution of keloids [4].

CTGF is a newly identified, highly profibrotic growth factor implicated in the development of pathological scar through the TGF/SMADs (Sma and Mad-related protein) pathway. It has been demonstrated that CTGF is mostly represented as dermal fibroblasts and sometimes as epidermal basal cells [5].

Importantly, CTGF is specifically and strongly stated as fibrotic tissue pathology like keloids and hypertrophic scars, nevertheless, expression is absent in normal tissue [6].

CTGF rises proportionally with keloid multiplication. Igarashi et al. discovered that CTGF mRNA-positive fibroblasts were unevenly dispersed in keloid tissue, particularly in the peripherally growing lesions. In the development of scar fibrosis, several physical and chemical stimuli, including excessive glucose, dexamethasone, 5-serotonin, environment, Angiotensin (Ang), TGF-b and signalling pathways, regulate CTGF production [7].

Preventing the production of CTGF and its profibrotic action may thus be a novel and possibly successful therapy for keloids.

Relative to other growth factors, its biological role is quite unique [8].

Hypertrophic and Keloid scars are very challenging to heal and continue to be a therapeutic obstacle. Treatment options involve intralesional corticosteroids alone or in conjunction with excision or cryosurgery, pressure therapy, intralesional fluorouracil, and silicon sheet therapy. However, each of these treatments has its own restrictions [9].

Several studies have shown the effectiveness of lasers in the treatment of scars since the introduction of lasers in dermatology, with the strategy selected depending on the kind of scarring.

Initially, hypertrophic scars were treated with argon, carbon dioxide (CO2), and neodymium-doped yttrium aluminium garnet lasers, but these lasers exhibited high recurrence rates [10].

Pulsed dye laser therapy (PDL) at the 585–585 nm wavelength, which is based on the principle of selective photothermolysis, has a large body of evidence confirming its effectiveness in the treatment of hypertrophic scars. Nevertheless, total clearance of scar thickness and pigmentation is seldom accomplished, and many treatment sessions are often necessary for good outcomes [11]**.**

Fractional lasers are considered one of the mainstays of therapy due to their ability to improve all scar types (Anderson et al., 2020). AFLT with wavelengths varying between 10,600 nm fCO2 lasers and 2940 nm Er:YAG lasers gave high efficacy and safety [12].

However, there are no comparative reports about therapeutic efficacy of the ablative fractional CO_2 and Er:YAG lasers for keloid and hypertrophic scars treatment.

This study purposed to clinically compare results and effect on histopathological architecture, expression of CTGF and ultrastructure of the ablative fractional 10,600-nm CO_2 and 2,940 nm Er:YAG lasers in hypertrophic and keloid scars treatment.

2. Patients and methods

The present study was a randomized, double-blinded comparative, prospective research aiming to compare clinical effectiveness and effect on histopathological architecture, expression of CTGF and ultrastructure of the ablative fractional 10,600-nm CO2 and 2,940 nm Er:YAG lasers in hypertrophic and keloid scars treatment.

This research was done between December 2015 and September 2016 on patients recruited via the outpatient clinic of the Dermatology and Venereology Department at Benha University Hospitals (EGYPT), and in St. Andrews laser Centre, Broomfield Hospital (UK), between September 2016 to April 2018. The Benha Faculty of Medicine Research Ethical Committee and the Broomfield Hospital Research Ethical Committee authorised the research. Before treatment, all patients were informed of the nature and specifics of the procedure and provided signed informed permission.

A total of sixty patients from both sexes (Fitzpatrick skin type II to V) divided randomly into two groups 30 patients each (Group I altered with Er:YAG laser fraction and Group II altered with CO2 laser fraction) were included in that work. Those participants were clinically diagnosed with keloid or HTS resulting from burns. They were categorized as having keloids or HTS which were confirmed by histopathological examination.

The study included patients at least 16 years old with post-burn hypertrophic and keloid scars that were at least six months old and varied in form, surface area, and body location. None of the treated scars exceeded 3percent of the total body surface area (TBSA). Each group's scars were comparable in size and appearance within the same anatomical location.

Exclusion criteria were all participants suffering any of the coming conditions: Concomitant treatment to the scar area, Immunocompromised patients, Severe autoimmune disease, Past drug history of oral retinoids within the last six months, Haemorrhagic disease or current anticoagulant medication, Pregnancy or lactation, Active infection or inflammation in studied area, Lesions suspicious for malignancy especially those diagnosed with Squamous cell carcinoma or melanoma , Patients with a previous history of laser therapy related adverse outcomes.

Pathological evaluation: Before and six months after the last laser session, a 2.5 mm punch biopsy was performed to evaluate changes in scar tissue.

The specimens were divided into two groups: The first was formalin 10% fixed for 24h at room temperature and processed to prepare 5 µm thick paraffin sections for H&E stain and immunohistochemistry (IHC) examination of CTGF expression was performed using Anti-CTGF antibody - C-terminal ab135812 (Abcam PLC., Cambridge, UK). Histological interpretation and photographs were carried out using a Leica light microscope in Pathology Department, Faculty of Medicine, Tanta University, Egypt and utilizing a Primo StarTM microscope (Carl Zeiss AG, Oberkochen, Germany) with an

integrated camera, photomicrographs of the different histopathological and histochemical results were acquired in the United Kingdom. Four normal skin samples were collected from the upper and lower limbs. H&E staining was used to evaluate epidermal thickness on tissue sections. This procedure was performed utilizing sections of 5-µm-thickness and H&E stained at magnification of 100 & 400-fold. The thickness of the epidermis was determined by measuring the distance between the crest (ridge) of stratum basal and the interface of stratum granulosum. The thickness of the epidermis was measured in five distinct locations of a single tissue slice. According to the following criteria, routinely stained H&E sections were independently scored based on the look and pattern of dermal collagen and perforation of collagen fibres.

Immunohistochemistry: Sections (4 mm-thick) were subjected to IHC for CTGF. IHC examination of CTGF expression was performed using Anti-CTGF antibody -6C-terminal ab135812 (Abcam PLC., Cambridge, UK). Deparaffinization and rehydration of sections were done. Endogenous peroxidases were inhibited by immersing slides for 15 minutes at room temperature in a 10:1 solution of water and 30 percent hydrogen peroxide. For antigen healing, they were then rinsed three times with water and once or twice with phosphate-buffered saline (PBS). Five percent BSA was dropped at room temperature for twenty minutes and extra liquid was swept away. The primary antibody was diluted overnight at 48C and then washed twice for two minutes in PBS. Following hematoxylin counterstaining, the slides were dried. After that, the cover slip was closed.

Evaluation of immunohistochemistry: Three fields were chosen for picture analysis in each region and then calculated the average of the positive cells as the final result in the three fields. The Olympus BH2 optical microscope and Nikon 4500 digital camera were used to capture these images.

Ultrastructural evaluation: The second set of tissue samples was set in 2.5 percent glutaraldehyde neutralized with 0.1 M phosphate buffer at pH 7.4 for two hours at 4 °C, rinsed with the buffer, and then treated in 1 percent osmium tetroxide in the same buffer for one hour at 4 °C. Following rinsing in phosphate buffer, samples were dried with rising grades of ethanol, placed in propylene oxide at room temperature for 30 minutes, saturated in a combination of propylene oxide and resin (1.1) for 24 hours, and then in a clear resin for another 24 hours. The samples were subsequently encapsulated with Embed-812 resin in BEEM capsule at 60 °C for 24 hours. 1 mm thick semithin slices were dyed with 1% toluidine blue for light microscopy inspection. Utilizing Leica ultracut UCT, ultrathin slices were sliced and dyed with uranyl acetate and lead citrate. These were studied using JEOL JEM 1010 electron microscopy at the Tanta University Faculty of Medicine Electron Microscopic Research Laboratory. The ultrastructure of the biopsies was assessed by an independent pathologist with no prior specific knowledge of the patients.

Statistical analysis:

Data were entered into the computer and analysed using version 20.0 of the IBM SPSS software suite. (Armonk, New York: IBM Corporation) Quantitative and percentage descriptions of qualitative information. The Kolmogorov-Smirnov test was conducted to determine the distribution's normality. Range (minimum and maximum), mean, standard deviation, and median were used to characterise quantitative data. Chi-square test was performed to compare various groups based on category data. Monte Carlo correction was utilised for chi-square adjustment when more than 20percent of the cells had predicted counts of less than 5. Student t-test for normally distributed quantitative variables, to compare between two studied groups. Paired t-test for normally distributed quantitative data comparing two times. Mann Whitney test for quantitative variables with an irregular distribution to compare two groups. Wilcoxon signed ranks test for quantitative variables with an irregular distribution, to compare two periods. At the 5% threshold, the significance of the acquired findings was determined.

3. Results

No significant variation in demographic features was found between study groups. All patients had hypertrophic scars and it was proved by clinical perspectives and histopathological findings (13**).** According to Fitzpatrick classification, Skin type II and III were the most common types in the total study group (73.3%). Scars duration (from burn to the time of first evaluation) was 60 months in median average. Boiling fluids were the predominant cause of scars (n=26, 43.3%).

No critical change was seen between studied groups regarding their baseline scar characteristics. **Table 1**

Table (1) Comparison between the two studied groups according to Scar characteristics

	Erbium (n = 30)		CO_2 (n = 30)		Test of Sig.	P
	No.	%	No.	%		
Skin type						
II	12	40.0	10	33.3	χ_2=	MCp=
III	8	26.7	14	46.7	2.866	0.456
IV	6	20.0	4	13.3		
V	4	13.3	2	6.7		
Scar duration (month)						
Min. – Max	6.0 – 180.0		6.0 – 180.0		U =	0.146
Mean ± SD	59.13 ± 50.72		45.0 ± 49.65		352.0	
Median	36.0		24.0			
Scar site						
Face	8	26.7	10	33.3	χ_2=	MCp=
Upper limb	8	26.7	10	33.3	1.365	0.807
Lower limb	4	13.3	2	6.7		
Trunk	10	33.3	8	26.7		

A significant change was seen between scar characteristics at baseline and 6 months following treatment protocol completion as featured by mean value of Vancouver scale score in Group I of the studied groups as regards height, pliability and vascularity, while in Group II there was a critical change in mean value of pliability and height. There was a significant difference (<0.001) between VSS total score at baseline and 6 months following last laser session in both studied groups. While POSAS mean values at baseline and 6 months following completion of therapy protocol, gave a critical difference as regards color, stiffness, thickness, irregularity, itching and pain in both studied groups.
Table 2
Comparison of the Extent of Improvement in Scar Characteristics between the two Groups: A significant change was present (p<0.05) between mean reduction height score, pliability and vascularity among studied groups regarding VSS.

Table (2) Comparison between the two studied groups according to results of Vancouver scar assessment scale (VSS) at baseline and total score of Vancouver scar assessment scale (VSS) and % of change of Vancouver scar assessment scale (VSS)

	VSS	Erbium (n = 30)	CO2 (n = 30)	U	P
Pigmentation	**Pretreatment**				
	Median (Min. – Max.)	2.0 (0.0 – 3.0)	2.0 (0.0 – 3.0)	438.0	0.849
	Mean ± SD.	2.17 ± 0.76	2.20 ± 0.78		
	Post-treatment				
	Median (Min. – Max.)	2.0 (0.0 – 3.0)	2.0 (0.0 – 3.0)	426.0	0.705
	Mean ± SD.	2.10 ± 0.77	2.17 ± 0.82		
	p_1	0.157	0.157		
Height (mm)	**Pretreatment**				
	Median (Min. – Max.)	2.0 (1.0 – 3.0)	2.0 (1.0 – 3.0)	450.0	1.000
	Mean ± SD	1.93 ± 0.69	1.93 ± 0.69		
	Post-treatment				
	Median (Min. – Max.)	1.73 ± 0.69	1.33 ± 0.48	310.0*	0.019*
	Mean ± SD.	2.0 (1.0 – 3.0)	1.0 (1.0 – 2.0)		
	p_1	0.014*	<0.001*		
Pliability	**Pretreatment**				
	Median (Min. – Max.)	2.0 (1.0 – 4.0)	2.0 (1.0 – 4.0)	450.0	1.000
	Mean ± SD	2.47 ± 0.99	2.47 ± 0.99		
	Post-treatment				
	Median (Min. – Max.)	1.50 (0.0 – 3.0)	1.0 (0.0 – 1.0)	216.0*	<0.001*

	Mean ± SD.	1.50 ± 0.77	0.90 ± 0.28		
	p_1	<0.001*	<0.001*		
Vascularity	**Pretreatment**				
	Median (Min. – Max.)	0.0 (0.0 – 3.0)	0.0 (0.0 – 3.0)	450.0	1.000
	Mean ± SD	0.77 ± 0.96	0.77 ± 0.96		
	Post-treatment				
	Median (Min. – Max.)	0.0 (0.0 – 1.50)	0.0 (0.0 – 3.0)	302.0*	0.009*
	Mean ± SD	0.17 ± 0.40	0.77 ± 0.96		
	p_1	0.001*	1.000		
Vancouver Scar Scale (VSS)		Erbium (n = 30)	CO_2 (n = 30)	t	p
Pretreatment					
Median (Min. – Max.)		7.0 (4.0 – 10.0)	7.0 (4.0 – 10.0)	0.062	0.951
Mean ± SD		7.33 ± 2.07	7.37 ± 2.11		
Post-treatment					
Median (Min. – Max.)		5.50 (3.0 – 8.0)	5.0 (2.0 – 8.0)	0.806	0.423
Mean ± SD		5.50 ± 1.69	5.17 ± 1.50		
p_1		**<0.001***	**<0.001***		
% of change VSS		Erbium	CO2	U	P
Pigmentation		**(n = 28)**	**(n = 28)**		
Median (Min. – Max.)		0.0 (-33.33 – 20.0)	0.0 (-33.33 – 0.0)	366.0	0.523
Mean ± SD.		-3.33 ± 11.90	-2.38 ± 8.74		
Height (mm)		**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)		0.0 (-50.0 – 0.0)	-33.33 (-50.0 – 0.0)	270.0*	0.002*
Mean ± SD.		-8.89 ± 18.43	-26.67 ± 22.99		
Pliability		**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)		-40.0 (-100.0 – 0.0)	-66.67 (-100.0 – 0.0)	204.0*	<0.001*
Mean ± SD.		-39.89 ± 26.70	-60.11 ± 21.26		
Vascularity		**(n = 14)**	**(n = 14)**		
Median (Min. – Max.)		-100.0 (-100.0 – -50.0)	0.0 (0.0 – 0.0)	0.0*	<0.001*
Mean ± SD		-82.14 ± 22.85	0.0 ± 0.0		
Total Score		**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)		-25.0 (-44.44 – -11.11)	-27.27 (-50.0 – 0.0)	362.0	0.192
Mean ± SD.		-24.85 ± 9.17	-28.87 ± 13.56		

U, p: U and p values for **Mann Whitney test** for comparing between the two groups, p_1: p value for **Wilcoxon signed ranks test** for comparing between Pre and Post treatment in each group, *: Statistically significant at $p \leq 0.05$.

A significant change was found (***p*<0.05**) between mean reduction score of color and stiffness among studied groups according to Pt scale of POSAS. **Table 3**

Table (3) Comparison between the two studied groups according to POSAS (Pt scale)

	POSAS (patients)	Erbium (n = 30)	CO2 (n = 30)	U	P
Color	**Pretreatment**				
	Median (Min. – Max.)	6.0 (2.0 – 9.0)	6.0 (2.0 – 9.0)	440.0	0.880
	Mean ± SD.	6.13 ± 2.13	6.20 ± 1.86		
	Post treatment				
	Median (Min. – Max.)	4.0 (1.0 – 7.0)	5.0 (1.0 – 8.0)	310.0*	0.034*
	Mean ± SD.	4.33 ± 1.77	5.20 ± 1.97		
	p_1	<0.001*	<0.001*		
Stiffness	**Pretreatment**				
	Median (Min. – Max.)	5.0 (2.0 – 9.0)	5.0 (3.0 – 8.0)	442.0	0.904
	Mean ± SD.	5.13 ± 1.89	5.07 ± 1.76		
	Post treatment				
	Median (Min. – Max.)	3.0 (1.0 – 7.0)	2.0 (1.0 – 4.0)	198.0*	<0.001*
	Mean ± SD.	3.13 ± 1.53	1.80 ± 0.85		

	p_1	<0.001*	<0.001*		
Thickness	**Pretreatment**				
	Median (Min. – Max.)	6.0 (1.0 – 8.0)	5.0 (1.0 – 8.0)	418.0	0.632
	Mean ± SD.	5.07 ± 2.30	4.93 ± 1.95		
	Posttreatment				
	Median (Min. – Max.)	3.0 (1.0 – 7.0)	3.0 (1.0 – 6.0)	360.0	0.173
	Mean ± SD.	3.47 ± 1.89	2.80 ± 1.24		
	p_1	<0.001*	<0.001*		
Irregularity	**Pretreatment**				
	Median (Min. – Max.)	5.0 (2.0 – 7.0)	5.0 (2.0 – 7.0)	412.0	0.565
	Mean ± SD.	4.67 ± 1.37	4.47 ± 1.38		
	Post treatment				
	Median (Min. – Max.)	3.0 (1.0 – 6.0)	3.0 (1.0 – 4.0)	396.0	0.413
	Mean ± SD.	3.20 ± 1.54	2.80 ± 1.0		
	p_1	<0.001*	<0.001*		
Itching	**Pretreatment**				
	Median (Min. – Max.)	2.0 (0.0 – 7.0)	2.0 (1.0 – 6.0)	402.0	0.466
	Mean ± SD.	2.93 ± 1.98	2.53 ± 1.48		
	Post treatment				
	Median (Min. – Max.)	1.0 (0.0 – 4.0)	1.0 (0.0 – 3.0)	394.0	0.370
	Mean ± SD.	1.13 ± 1.22	1.27 ± 1.01		
	p_1	<0.001*	<0.001*		
Pain	**Pretreatment**				
	Median (Min. – Max.)	1.0 (0.0 – 5.0)	1.0 (0.0 – 4.0)	382.0	0.297
	Mean ± SD.	1.60 ± 1.52	1.20 ± 1.30		
	Post treatment				
	Median (Min. – Max.)	0.0 (0.0 – 2.0)	0.0 (0.0 – 1.0)	438.0	0.836
	Mean ± SD.	0.47 ± 0.63	0.40 ± 0.50		
	p_1	<0.001*	<0.001*		

There were no differences among groups as regarding POSAS total score of patient scale and % of change in PSOAS (patient scale) respectively. **Table 4**

Table (4) Comparison between the two studied groups according to total score pt scale of POSAS and % of change of POSAS (pt scale)

Total Score POSAS	Erbium (n = 30)	CO2 (n = 30)	t	P
Pretreatment				
Median (Min. – Max.)	26.0(13.0 – 42.0)	25.0(16.0 – 37.0)	0.667	0.508
Mean ± SD.	25.53 ± 7.18	24.40 ± 5.93		
Post treatment				
Median (Min. – Max.)	15.0(6.0 – 29.0)	14.0(9.0 – 20.0)	1.286	0.204
Mean ± SD.	15.73 ± 5.35	14.27 ± 3.23		
p_1	<0.001*	<0.001*		
% Of change POSAS	Erbium	CO2	U	p
Color	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	-25.0 (-60.0 – 0.0)	-16.67 (-66.67 – 0.0)	228.0*	0.001*
Mean ± SD	-29.65 ± 14.78	-19.27 ± 17.84		
Stiffness	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	-40.0(-66.67 – -20.0)	-66.67(-85.71 – -33.33)	146.0*	<0.001*
Mean ± SD	-40.35 ± 14.24	-62.49 ± 15.95		
Thickness	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	-33.33 (-66.67 – 0.0)	-50.0 (-66.67 – 0.0)	330.0	0.074
Mean ± SD	-30.53 ± 19.62	-38.98 ± 21.88		
Irregularity	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	-33.33 (-66.67 –	-40.0 (-66.67 – -	382.0	0.310

	0.0)	20.0)		
Mean ± SD.	-34.35 ± 17.99	-37.86 ± 11.90		
Itching	**(n = 28)**	**(n = 30)**		
Median (Min. – Max.)	-61.90(-100.0 – -33.33)	-50.0(-100.0 – 0.0)	298.0*	0.049*
Mean ± SD.	-67.77 ± 22.91	-55.44 ± 27.69		
Pain	**(n = 22)**	**(n = 18)**		
Median (Min. – Max.)	-80.0 (-100.0 – 0.0)	-66.67 (-100.0 – 0.0)	172.0	0.492
Mean ± SD.	-73.94 ± 31.09	-67.59 ± 31.43		
Total Score	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	-41.38(-53.85 – -15.38)	-40.91(-55.0 – -25.93)	412.0	0.574
Mean ± SD.	-38.75 ± 10.29	-40.72 ± 8.67		

Table 5 showed only a significant difference between the mean reduction score of pliability among groups regarding observer score of POSAS. **Table 6 and Table 7** did not show any difference of the total score of the observer scale of POSAS among groups of study.

Table (5) Comparison between the two studied groups according to observer scale

	POSAS (Observer scale)	Erbium (n = 30)	CO2 (n = 30)	U	p
Vascularity	**Pretreatment**				
	Median (Min. – Max.)	0.0 (0.0 – 8.0)	0.0 (0.0 – 8.0)	450.0	1.000
	Mean ± SD.	1.80 ± 2.55	1.80 ± 2.55		
	Post-treatment				
	Median (Min. – Max.)	0.0 (0.0 – 4.0)	0.0 (0.0 – 8.0)	382.0	0.265
	Mean ± SD.	0.80 ± 1.24	1.70 ± 2.43		
	p_1	**0.002***	**0.063**		
Pigmentation	**Pretreatment**				
	Median (Min. – Max.)	6.0 (1.0 – 9.0)	6.0 (1.0 – 9.0)	450.0	1.000
	Mean ± SD.	5.57 ± 2.01	5.57 ± 2.01		
	Post-treatment				
	Median (Min. – Max.)	6.0 (1.0 – 9.0)	5.0 (1.0 – 9.0)	396.0	0.415
	Mean ± SD.	5.47 ± 2.19	5.17 ± 2.03		
	p_1	**0.157**	**0.001***		
Pliability	**Pretreatment**				
	Median (Min. – Max.)	4.63 ± 2.04	4.63 ± 2.04	450.0	1.000
	Mean ± SD.	4.0 (1.50 – 8.0)	4.0 (1.50 – 8.0)		
	Post-treatment				
	Median (Min. – Max.)	3.0 (0.0 – 5.0)	2.0 (1.0 – 4.0)	316.0*	0.040*
	Mean ± SD.	2.73 ± 1.64	2.03 ± 0.92		
	p_1	**<0.001***	**<0.001***		
Thickness	**Pretreatment**				
	Median (Min. – Max.)	5.0 (2.0 – 7.0)	5.0 (2.0 – 7.0)	450.0	1.000
	Mean ± SD.	4.67 ± 1.77	4.67 ± 1.77		
	Post-treatment				
	Median (Min. – Max.)	5.0 (2.0 – 7.0)	3.0 (2.0 – 6.0)	326.0	0.057
	Mean ± SD.	4.07 ± 1.84	3.13 ± 1.38		
	p_1	**0.003***	**<0.001***		
Relief	**Pretreatment**				
	Median (Min. – Max.)	4.0 (2.0 – 7.0)	4.0 (2.0 – 8.0)	446.0	0.951
	Mean ± SD.	4.27 ± 1.46	4.33 ± 1.60		
	Post-treatment				
	Median (Min. – Max.)	3.0 (1.0 – 5.0)	2.0 (1.0 – 5.0)	422.0	0.664
	Mean ± SD.	2.93 ± 1.41	2.73 ± 1.14		
	p_1	**<0.001***	**<0.001***		

Table (6) Comparison between the two studied groups according to observer scale

Total Score Observer scale	Erbium (n = 30)	CO2 (n = 30)	t	p
Pretreatment				
Median (Min. – Max.)	21.0(11.0 – 30.0)	21.0(11.0 – 31.0)	0.043	0.966
Mean ± SD.	20.93 ± 5.96	21.0 ± 6.07		
Post treatment				
Median (Min. – Max.)	17.0(6.0 – 25.0)	14.0(6.50 – 21.0)	1.008	0.318
Mean ± SD.	16.0 ± 5.29	14.77 ± 4.11		
p_1	**<0.001***	**<0.001***		

Table (7) Comparison between the two studied groups according to % of change of observer scale

% Of change observer scale	Erbium	CO_2	U	p
Vascularity	**(n = 14)**	**(n = 14)**		
Median (Min. – Max.)	-50.0 (-100.0 – 0.0)	0.0 (-16.67 – 0.0)	18.0*	<0.001*
Mean ± SD	-55.71 ± 31.03	-4.76 ± 7.81		
Pigmentation	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	0.0 (-60.0 – 0.0)	0.0 (-40.0 – 0.0)	312.0*	0.006*
Mean ± SD	-4.0 ± 15.22	-8.01 ± 11.61		
Pliability	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	-40.0 (-100.0 – 0.0)	-50.0 (-75.0 – -33.33)	316.0*	0.042*
Mean ± SD	-45.25 ± 28.64	-53.76 ± 11.99		
Thickness	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	0.0 (-50.0 – 0.0)	-33.33 (-60.0 – 0.0)	238.0*	0.001*
Mean ± SD	-12.35 ± 19.31	-29.65 ± 20.63		
Relief	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	-33.33 (-66.67 – 0.0)	-33.33 (-66.67 – 0.0)	398.0	0.436
Mean ± SD	-33.14 ± 18.48	-36.40 ± 14.81		
Total Score	**(n = 30)**	**(n = 30)**		
Median (Min. – Max.)	-20.0 (-52.0 – -3.85)	-27.59 (-48.0 – -9.09)	314.0*	0.044*
Mean ± SD.	-24.08 ± 12.12	-28.87 ± 10.08		

Histopathological results: Normal skin has collagen fibers which are homogenously distributed, thick wavy well-organized bundles stained acidophilic with hematoxylin & eosin stain. These fibers are arranged in bundles that are loose in the papillary dermis and become thicker in the reticular dermis. Hematoxylin and eosin (H&E) stained sections of hypertrophic scars and keloids showed flattening of the rete ridges, hyperkeratosis, hypergranulosis, and thick and stretched collagen bundles throughout the dermis before the laser treatment. The dermal blood vessels were oriented vertically on the epidermis. A low-grade inflammation in the dermis was seen in the form of mononuclear cells around telangiectatic vessels. **Figure 1**

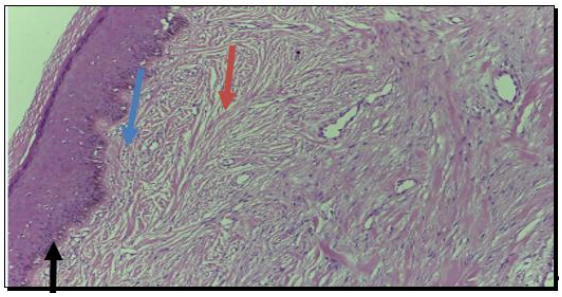

Fig. (1) Photomicrograph of skin section of a hypertrophic scar before treatment showing hyperkeratosis and flattening of rete ridges with diminished dermal papillae (black arrow). The dermis shows dense collagen fibers that run parallel to the skin surface (red arrow) with mononuclear cellular infiltrate (blue arrow) and many blood vessels. (H&E, 100)

After fractional Er:YAG laser treatment, increased thickness of epidermis was seen. There was replacement of the irregular collagen bands with organized parallel new collagen fibril and decrease in both the cellular infiltrate and vasculature **Figure 2 while** after fractional Co_2 laser the epidermal thickness was more prominent while the collagen bands were finer and more fibrillar **Figure 3.**

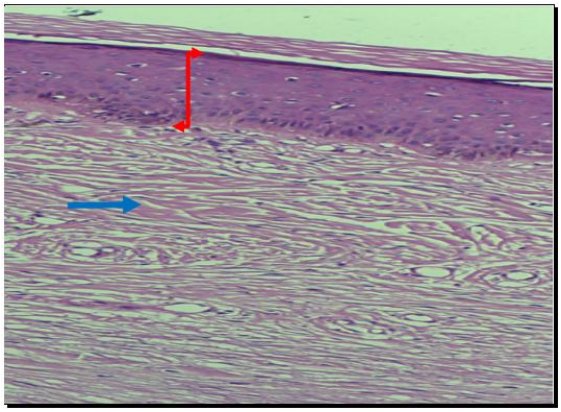

Fig. (2) Increased thickness of epidermis, The thickness of the epidermis was determined by measuring the distance between the ridge of stratum basal and the interface of stratum granulosum in µm (red arrow), restoration of the uneven collagen bands with parallel, structured new collagen fibrils (blue arrow) and a reduction in cellular infiltration and vasculature. after fractional Er:YAG laser

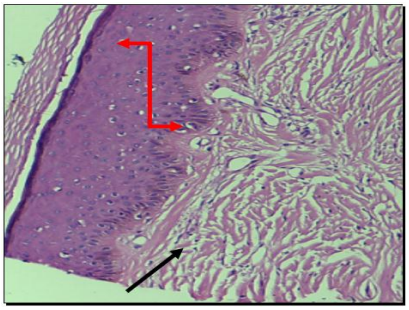

Fig. (3) After fractional Co_2 laser the epidermal thickness (red arrow) was more prominent while the collagen bands were finer and more fibrillar (black arrow)

No critical change was seen among groups of study concerning epidermal thickness or collagen score although there was marked increased in epidermal thickness 6 months after last laser treatment in both groups. **Table 8**

Table (8) Comparison between the two studied groups according to epidermal thickness and collagen score

		Erbium (n = 30)	CO2 (n = 30)	t	P
Epidermal thickness	**Pretreatment**				
	Median (Min. – Max.)	719.4 (637.0 – 1064.4)	722.0 (635.0 – 1055.70)	0.068	0.946
	Mean ± SD.	776.9 ± 135.3	779.3 ± 131.2		
	Post treatment				
	Median (Min. – Max.)	1872.0(1535.0 – 2952.8)	1877.4(1590.0 – 3017.9)	0.600	0.551
	Mean ± SD.	1957.9 ± 385.2	2017.4 ± 383.8		
	p_1	**<0.001***	**<0.001***		
Collagen score	**Pretreatment**				
	Median (Min. – Max.)	3.0 (2.0 – 5.0)	3.0 (2.0 – 5.0)	0.0	1.000
	Mean ± SD.	3.47 ± 0.90	3.47 ± 0.90		
	Post treatment				
	Median (Min. – Max.)	2.0 (1.0 – 5.0)	2.0 (1.0 – 4.0)	1.350	0.182
	Mean ± S.	2.73 ± 1.08	2.40 ± 0.81		
	p_1	**<0.001***	**<0.001***		

Immunohistochemistry (IHC) examination of CTGF expression: CTGF is mostly found in dermal fibroblasts and occasionally in epidermal basal cells. CTGF is selectively found in pathological fibrotic tissue, such as keloids and HTS, but not in normal tissue. IHC examination of skin biopsies for CTGF showed brown staining of positive cells, mostly found in the epidermis and dermis basal layers. CTGF positive cells/field significantly decreased in number 6 months following fractional Erbium YAG and CO_2 laser therapy ($P < 0.05$), decreasing by 50.49 ± 5.69% on average after Erbium While decreasing by 37.95 ± 9.03% on average after CO_2 laser **(Table 9; Figure 4-6)**. A significant change was seen among both groups regarding CTG factor expression.

Table (9) Comparison between the two studied groups according to CTG factor and % of CTGF

CTG factor	Erbium (n = 30)	CO2 (n = 30)	t	p
Pretreatment				
Median (Min. – Max.)	15.0 (10.0 – 25.0)	15.0 (10.0 – 25.0)	0.0	1.000
Mean ± SD.	16.60 ± 4.61	16.60 ± 4.61		
Post treatment				
Median (Min. – Max.)	8.0 (5.0 – 13.0)	10.0 (7.0 – 15.0)	3.376*	0.001*
Mean ± SD.	8.13 ± 2.13	10.07 ± 2.30		
p_1	**<0.001***	**<0.001***		
	Erbium	CO2	U	p
CTG factor (% of change) Median (Min. – Max.)	50.0 (40.0 – 60.0)	40.0 (25.0 – 52.94)	**100.0***	**<0.001***

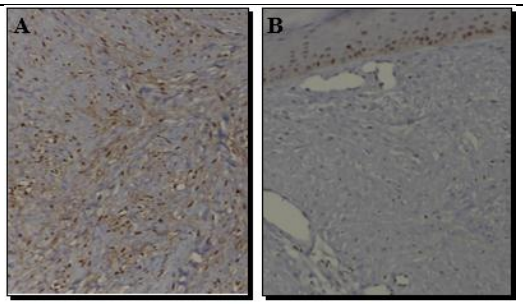

Fig. (4) CTGF immunohistochemical expression in hypertrophic scar prior to laser therapy. The expression was mostly cytoplasmic in dermal fibroblast (x400) performed using Anti-CTGF antibody - 6C-terminal ab135812 (Abcam PLC., Cambridge, UK) B): Immuno-histochemical expression of CTGF

in hypertrophic scar after Erbium YAG laser treatment. The intensity of expression was significantly lowered relative to the expression prior to therapy (x400)

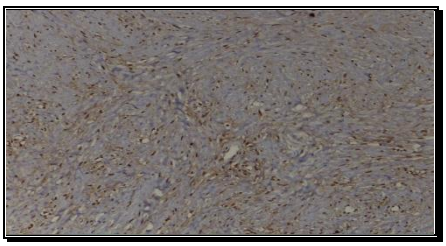

Fig. (5) CTGF immunohistochemical expression in hypertrophic scar prior to laser therapy. The expression was mostly cytoplasmic in dermal fibroblasts. (x400)

Fig. (6) CTGF immunohistochemical expression in hypertrophic scar after CO2 laser treatment. The expression was decreased relatively compared to baseline (x400)

Electron microscopic examination of Ultra-thin sections of skin biopsies

The following are the characteristics of an uncontrolled hypertrophic scar: The fibroblasts had a large, round or oval, infolded nucleus with one or several nucleoli. The cytoplasm was thick with electron-dense particles and was plentiful. The most prominent extracellular components of scar tissue were uneven, cross-sectioned and longitudinal collagen fibers. **Figure 7 and Figure 8**

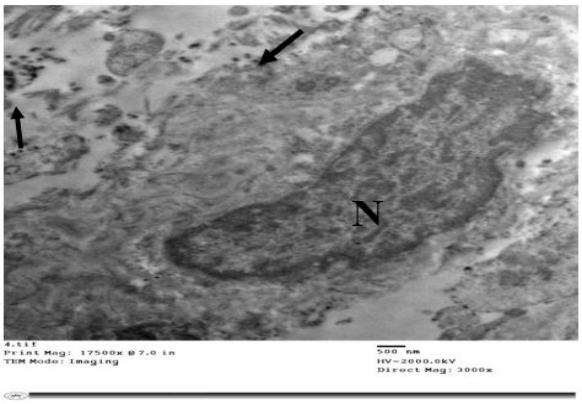

Fig. (7) A photograph of an uncontrolled hypertrophic scar exhibiting a fibroblast with a big oval cell nucleus (N) and nuclear infoldings. ribosomes and endoplasmic reticulum cisternae make up the electron dense substance in the cytoplasm.

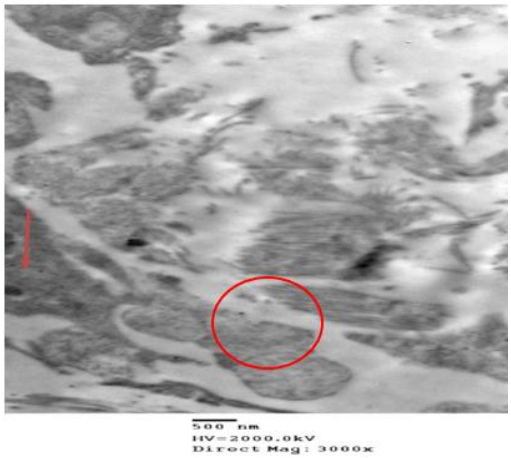

Fig. (8) Longitudinal (circle) and transverse (arrow) collagen fiber slices electron micrograph from an untreated scar.

Fibroblasts in skin biopsies taken 6 months after treatment with fractional Erbium YAG and CO_2 laser were not larger than scars that had not treated. The cell's nucleus had less euchromatin. There were extremely few organelles and intermediate filaments in the cytoplasm. **Figure 9**

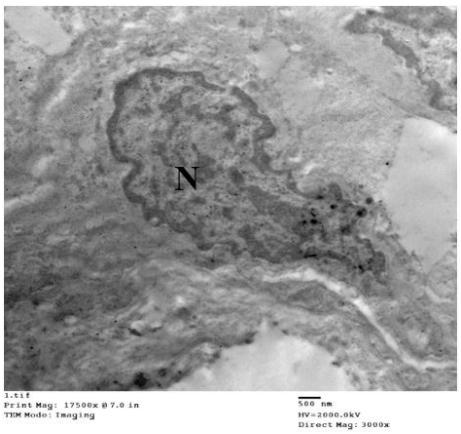

Fig. (9) Electron micrograph of fibroblast in hypertrophic scar 6 months after Erbium YAG laser treatment

The collagen fibers in biopsies taken 6 months after treatment with fractional Erbium YAG and CO_2 laser were mature, showing periodicity characteristics. In furthermore, the bulk of collagen fibres had a regular structure, running parallel to one another and creating bundles. **Figure 10**

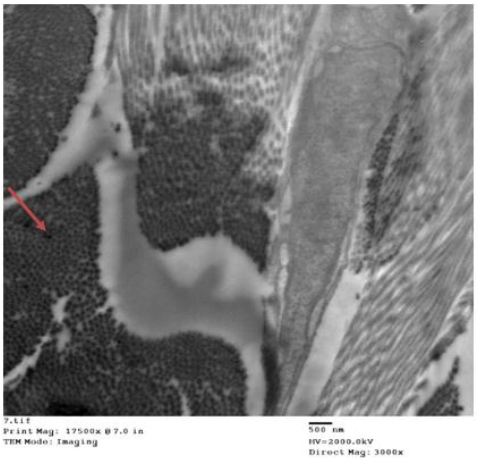

Fig. (10) Displaying Electron micrograph of parallel mature collagen fibers in hypertrophic scar 6 months after CO_2 laser treatment.

4. Discussion

Burn scars provide a significant clinical and cosmetic dermatological concern. furthermore, their substantial morbidity, the adverse effects and extended courses of various therapeutic methods for the treatment of burn scars impose an extra cost for patients **[9]**.

It is to be noted that in this latter study, scars were old, the average scar length was 4.62 years, with a minimum period of 0.167 years and a maximum duration of 20 years. The mean duration of scars in this research was 3 years, with a minimum length of 0.5 years and a maximum duration of 1.5 years.

Similar to Azzam et al. **(2016)**, There was no linkage between improvement in VSS measures and patient age (14).

As regards clinical assessment of the degree of improvement among the studied patients in this current study, a critical decrease in VSS was seen following fractional ablative Er:YAG and Co2 lasers treatment mainly due to improvement in vascularity, pliability and to a lesser extent in height with Er:YAG ,while after CO_2 improvement was significant in height and pliability only. Improvement in each variable of the POSAS proved that relief and pliability were the most significantly improved items after fractional ablative Er:YAG and CO_2. Besides that, thickness showed significant improvement after CO_2 fractional ablative laser treatments.

Clinical observation by Nicoletti et al. **(2012)**, in a study that evaluated fifty patients with moderate to severe keloids patients who had four CO2 laser treatments at 1- to 6-month intervals and were monitored for six months following the last treatment reported a decrease in scar thickness, height, and texture. In addition, several individuals exhibited improved skin pigmentation and suppleness in general look. Histopathological results mirrored the clinical recovery. Furthermore, the amount of new collagen deposition, dermal remodelling, and neo-vascularization identified histologically in this research associated closely with the improved clinical recovery. All these findings correlated to our results [15].

Our data comes parallel to results reported by El-Hoshy et al. **(2017)**, who treated twenty patients with burn scars by 3 fractional carbon dioxide laser sessions 4 to 8 weeks apart, following therapy, both the Vancouver Scar Scale and the Patient and Observer Scar Assessment Scale revealed a considerable decrease ($p<0.001$). Scar comfort and pliability enhanced the greatest, followed by vascularity [16].

Tawfic et al. (2020) enlisted thirty individuals with hypertrophic scars and keloids in his research. Each treatment area was assigned to four sessions treatment with fractional CO_2, 4–6 weeks apart. Clinical evaluation was performed using VSS and POSAS prior and one month after the final session. Hematoxylin and eosin, Masson's trichrome, and Orcein stains were utilized to assess the structure and arrangement of dermal collagen and elastic fibres. Both VSS and POSAS exhibited considerable enhancement after fractional CO2 therapy. In terms of look and pattern, collagen fibres exhibited substantial enhancement, although density exhibited little change. Significant densification of elastic fibres was seen following fractional CO2 treatment. With fractional CO2 laser, hypertrophic scars improved much more than with conventional lasers, our results concur with all those results [17].

Eleven participants received a total of three fractional ablative Er:YAG treatments at 4-week periods in Wulkan et al. **(2019)** study. This research demonstrated the safety and efficacy of scar therapy, with no adverse events recorded. Individual evaluations of dyschromia, atrophy/hyperplasia, vascularity, and texture all showed considerable enhancement. The average score for enhancement in overall look was 2.27 on a scale of 3.

Asfour et al. **(2017)** revealed statistically significant variations in vascularity, flexibility, height and pigmentation after treatment of 50 patients with post-burn scars with fractional ablative Er:YAG. These results go with our results except for pigmentation which showed no significant improvement either by VSS or POSAS [18].

Regarding the poor improvement of pigmentation in both studied groups, Azzam et al. **(2016)**, also showed no improvement in pigmentation with ablative fractional CO2 laser [14].

Two kinds of fractional ablative lasers exist, the 2,940 (Er:YAG) laser and the 10,600-nm (CO2) laser. To our knowledge, The general approach for hypertrophic burn scars is fractional ablative CO2 or erbium laser without distinction.

Relative to the CO2 laser, the Er:YAG laser's wavelength of 2,940 nm is roughly 15 times more efficiently absorbed by water. This decreases the amount of energy required for tissue destruction. In contrast, the additional

energy required for CO2 tissue burning leads to a thicker coagulation tissue zone [19].

A previous histology research found that various laser wavelengths cause distinct injury types. The coagulation zone is the primary distinction between the CO2 and Er:YAG laser damage patterns. The coagulation zone is a physical characteristic intrinsic to the laser wavelength. As CO2 lasers have substantially lower absorption coefficients than Er:YAG lasers, the CO2 requires more energy to remove excess epidermal and dermal tissue. Extra energy produces heat that promotes thermal collateral damage, culminating in the formation of a coagulation zone around the burning zone. Ablational fractional Er:YAG laser injuries have minimal or no coagulation zones [20].

According to clinical perspectives, our study is the only study aimed to compare clinical enhancement of scars utilizing ablative lasers fraction: Er:YAG and CO_2. Our current study proved that fractional ablative CO2 laser could be considered superior in improving scar height, pliability and relief while fractional ablative Er:YAG is superior as regards scar vascularity.

Histopathological architecture of burn scars showed significant changes after laser treatments. In current study, there were increased thickness of epidermis and irregular collagen bands arrangement with parallel organized new collagen fibril after fractional ablative Er:Yag. This is an indistinguishable result from those reported by Asfour et al. **(2017)** who confirmed epidermal hyperplasia; hyperkeratosis, flat ridges and papilomatosis. Asfour et al. **(2017)** also reported that Masson Trichrome stain showed compact, well-organized collagen bundles in the papillary dermis parallel to the epidermis [18].

The present work has showed that clinical enhancement of hypertrophic scars following CO2 laser friction treatment is reflected by histologic observations. Following treatment, the stratum corneum became thinner and the uneven dermal collagen bands were replaced by ordered parallel new collagen fibrils, bringing the skin closer to that of normal skin.

It came in agreement with Makboul et al. **(2014)**, who revealed that laser produces dermal collagen less dense and finer. They reported that after laser treatment, collagen remodeling occurred due to both collagenolysis and collagen synthesis [21].

Immunohistochemical previous studies in hypertrophic scar were limited and evaluated TGF-b1 expression only. This current study came to agreement with Yang et al. **(2012)** which is the only study to evaluate CTGF expression in keloids. Yang et al. **(2012)** has investigated CTGF expression orior to and following PDL, 595 nm therapy. According to their investigation, In the majority of instances, the number of positive cells dropped following therapy, while a small percentage of patients had an increase [22].

5. Conclusion

Fractional ablative lasers including both CO_2 and Er:YAG have combined impressive results of ablative lasers with lesser risk profile of nonablative lasers. Fractional ablative Er:YAG laser mainly improve vascularity and pliability of hypertrophic scars while Co2 improvement was significant in height and pliability. CTGF is selectively produced in pathological fibrotic tissue, such as keloids and HTS, but not in normal tissue. It is mostly produced in dermal fibroblasts and occasionally in epidermal basal cells, and its expression decreases dramatically following Fr Er:YAG treatment.

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