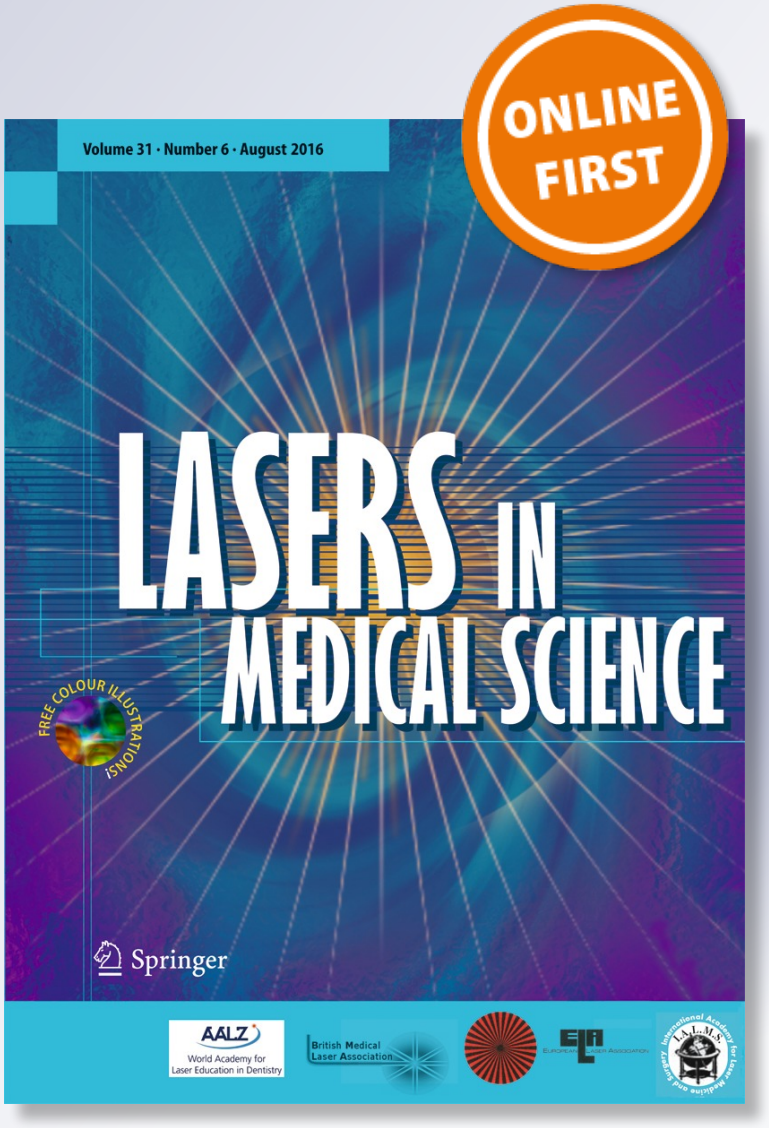
Fractional carbon dioxide laser versus low-dose UVA-1 phototherapy for treatment of localized scleroderma: a clinical and immunohistochemical randomized controlled study

S. M. Shalaby, M. Bosseila, M. M. Fawzy, D. M. Abdel Halim, S. S. Sayed & R. S. H. M. Allam

Lasers in Medical Science

ISSN 0268-8921

Lasers Med Sci
DOI 10.1007/s10103-016-2041-5



Springer

Lasers Med Sci
DOI 10.1007/s10103-016-2041-5

ORIGINAL ARTICLE

Fractional carbon dioxide laser versus low-dose UVA-1 phototherapy for treatment of localized scleroderma: a clinical and immunohistochemical randomized controlled study

S. M. Shalaby[1] · M. Bosseila[1] · M. M. Fawzy[1] · D. M. Abdel Halim[1] · S. S. Sayed[2] · R. S. H. M. Allam[3]

Received: 11 May 2016 / Accepted: 25 July 2016

Abstract Morphea is a rare fibrosing skin disorder that occurs as a result of abnormal homogenized collagen synthesis. Fractional ablative laser resurfacing has been used effectively in scar treatment via abnormal collagen degradation and induction of healthy collagen synthesis. Therefore, fractional ablative laser can provide an effective modality in treatment of morphea. The study aimed at evaluating the efficacy of fractional carbon dioxide laser as a new modality for the treatment of localized scleroderma and to compare its results with the well-established method of UVA-1 phototherapy. Seventeen patients with plaque and linear morphea were included in this parallel intra-individual comparative randomized controlled clinical trial. Each with two comparable morphea lesions that were randomly assigned to either 30 sessions of low-dose (30 J/cm^2) UVA-1 phototherapy (340–400 nm) or 3 sessions of fractional CO_2 laser (10,600 nm-power 25 W). The response to therapy was then evaluated clinically and histopathologically via validated scoring systems. Immunohistochemical analysis of TGF-ß1 and MMP1 was done. Patient satisfaction was also assessed. Wilcoxon signed rank test for paired (matched) samples and Spearman rank correlation equation were used as indicated. Comparing the two groups, there was an obvious improvement with fractional CO_2 laser that was superior to that of low-dose UVA-1 phototherapy. Statistically, there was a significant difference in the clinical scores ($p = 0.001$), collagen homogenization scores ($p = 0.012$), and patient satisfaction scores ($p = 0.001$). In conclusion, fractional carbon dioxide laser is a promising treatment modality for cases of localized morphea, with proved efficacy of this treatment on clinical and histopathological levels.

Keywords Fractional carbon dioxide laser · UVA-1 phototherapy · Localized scleroderma · Treatment · TGF-β1 · MMP1 · Randomized controlled trial

Background

Morphea or localized scleroderma is a rare inflammatory fibrosing disorder of the skin and underlying tissues which can cause significant morbidity and cosmetic disfigurement that may affect the patients' quality of life [1].

The treatment of morphea continued to be a strong challenge where several modalities of therapy are available with no clear consensus guidelines [2]. UVA-1 phototherapy (340–400 nm) is an effective well-established mode of treatment with a good safety profile. However, this treatment may not be feasible due to the need of multiple sessions, in addition to the unacceptable cosmetic risk of UVA-1-induced hyperpigmentation [3].

Fractional ablative lasers (FALs) were proven effective in the treatment of hypertrophic scars and keloids [4]. Since the hallmark of both scars and morphea is fibrosis, attention was

Clinical trial registration information: clinicaltrials.gov with an ID: DDCU-24684

✉ M. Bosseila
manal.bosseila@kasralainy.edu.eg

[1] Department of Dermatology, Faculty of Medicine, Cairo University, Cairo, Egypt

[2] Department of Histology, Faculty of Medicine, Cairo University, Cairo, Egypt

[3] Department of Ophthalmology, Faculty of Medicine, Cairo University, Cairo, Egypt

Published online: 10 August 2016

raised for the possible use of FALs as a treatment modality for morphea.

The rationale of the current study was to evaluate the efficacy of the fractional carbon dioxide laser as a new modality for treatment of morphea and to compare its results with the well-established method of UVA-1 phototherapy. We hypothesize that FAL therapy for localized morphea would have comparable results to UVA sessions using less number of visits to the dermatology outpatient clinic.

Patients and methods

The current study is a parallel intra-individual comparative randomized controlled clinical trial with allocation ratio 1:1.

Recruitment and enrollment

Twenty-five patients with localized morphea who consecutively presented over 1 year to outpatient clinics of Dermatology Department, Kasr Al-Aini Hospitals, Faculty of Medicine, Cairo University, were assessed for eligibility to be enrolled in the study. This sample size was based on the rarity of the disease, where the mean incidence of cases of morphea in the dermatology outpatient clinic, Cairo University along the past 5 years was 13 cases per year ± 2 patients. Inclusion criteria were patients with plaque and linear morphea (limb variant and en coup de sabre), either newly diagnosed or discontinuing any treatments for at least 3 months before the study. Patients with deep and systemic types of morphea, contraindications to phototherapy and/or laser therapy were excluded (Fig. 1). The study was approved

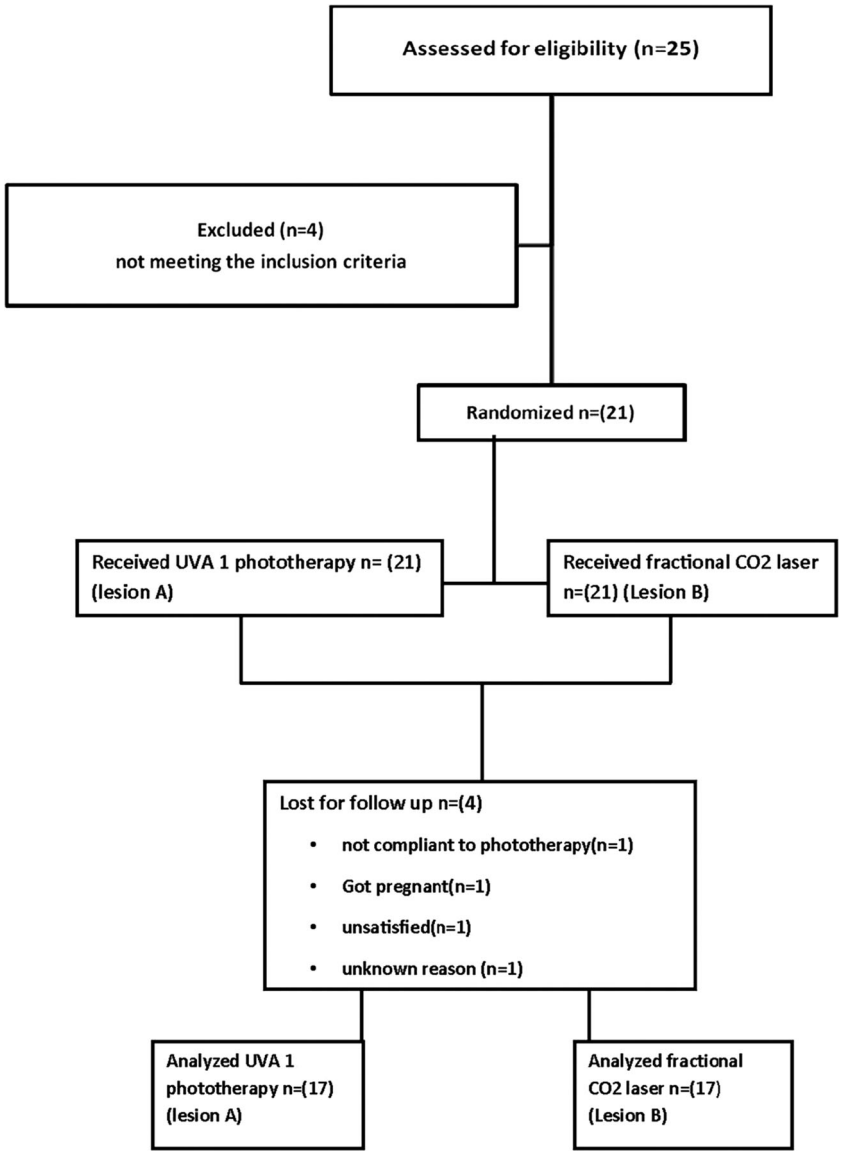


Fig. 1 Flow diagram of the intra-individual randomized controlled study

by the Dermatology Research Ethical Committee (Derma REC) at Kasr Al-Aini Hospital. Informed consents for participation and photography were signed by all patients. The study was registered on clinical trial.gov with an ID: NCT02002897.

Our primary outcome measure was to evaluate the efficacy of fractional ablative laser in treatment of morphea. Assessment was done as regards clinical improvement, histopathological and immunohistochemical analysis, and patient satisfaction scores. Results were compared to those of the standard treatment with low-dose UVA-1 phototherapy. Our secondary outcome measures were assessing the complications and the mechanism of action of fractional CO_2 laser on collagen remodeling.

Baseline assessment

Prior to the study, each patient was subjected to thorough history taking and clinical examination. The two most affected lesions with rather similar clinical scores were evaluated regarding: thickness, dermal atrophy, dyschromia, and erythema based on the scores adopted and modified from Localized Scleroderma Cutaneous Assessment Tool (LoSCAT) [5] (Table 1).

Evaluation

Clinical evaluation

Clinical evaluation was done before, on monthly basis, and at the end of study (EoS), following the same scoring system that was used in baseline assessment. Improvement was graded as follows: poor: no improvement, fair: <40 %, good: 40–59 %, very good: ≥60 % improvement. End of study (EoS) was at the last session in UVA-1-treated areas and 1 month after the last session in FAL-treated areas.

Table 1 Adopted localized scleroderma clinical scoring system (LoSCAT) [5]

Score (Unit)	0	1	2	3
Skin Thickness	Normal skin thickness/ freely mobile	Thickened / mobile	Moderate thickening / decreased ability to move the skin	Marked thickening / unable to pinch the skin
Dermal Atrophy	None / normal skin	Shiny	Visible vessels	Obvious dermal atrophy (cliff drop)
Erythema	None / normal skin	Mild	Moderate	Marked
Dyschromia	None / normal skin	Mild	Moderate	Marked

Arkachaisri et al. [5]

Patient satisfaction scores

Patient satisfaction scores were evaluated at the end point of the study regarding overall improvement, the feasibility of therapy, and side effects according to a standardized patient satisfaction score [6].

Histopathological evaluation

Three-millimeter deep punch biopsies were obtained before and after the treatment period for each lesion. The post-treatment biopsies were obtained at the study end point (after the last session in UVA-1-treated areas and 1 month after the last session in FAL-treated areas). Biopsies were fixed and stained with hematoxylin and eosin (H&E) for routine histopathological evaluation and anti-TGF-ß1 antibodies and anti-MMP1 antibodies immunohistochemically.

The H&E stained sections were examined under light microscopy by a certified dermatopathologist who was blinded to the modes of therapy. The extent of collagen homogenization in papillary, superficial reticular, and deep reticular dermis was assessed semi-quantitatively according to the scoring system suggested by Verrecchia et al. [7].

The perivascular infiltrate was assessed as follows: 0 indicates no infiltrate, 1 indicates mild infiltrate, and 2 indicates tight cuffing of the blood vessels.

Immunohistochemical evaluation

Sections (5-μm thick) were immunostained for detection of TGF-β1 (US Biological, Massachusetts, USA) (Cat.# 029905) and MMP-1 (Lab Vision, Co, Fremont, USA) (Cat.#RB-1536-P0). Staining of formalin-fixed tissues required boiling of tissue sections in 10 mM/L citrate buffer, pH 6.0 (Labvision/NeoMarkers, Fremont, CA, USA) for 10–20 min followed by cooling at room temperature for 20 min. Antigen visualization was performed using Super SensitiveTM Link-Label Detection Systems Concentrated Format (BioGenex, Fremont, CA, USA), avidin–biotin complex technique, and 3,30-diaminobenzidine tetrahydrochloride (DAB chromogen) (BioGenex, Fremont, CA, USA). Mayer's hematoxylin was used for counter staining [8].

Quantitative morphometric studies of TGF-ß1 and MMP1 immunohistochemistry of all skin sections were performed using the image analyzer (Leica Qwin 500 LTD image analysis computer system, UK). The pattern of expression and the degree of positivity were recorded. The quantitative

morphometric measurements were recorded for both TGF-ß1 and MMP1 in 5 randomly selected, non-overlapping high power fields (HPF) using ×40 objective lens. The mean area percent of positive immunoreaction was recorded using binary mode. Positive immune reaction appeared as brown deposits, in a cytoplasmic pattern of staining [9, 10].

Evaluation by ultrasound biomicroscopy

Dermal thickness in millimeters was measured by ultrasound biomicroscopy (UBM) for a sample of nine patients only (due to difficult access to the skin lesion of the rest of patients as the examination arm is designed for ophthalmologic examination with limited mobility). The used machine was paradigm ultrasound biomicroscopy plus Model P45 using very high frequency ultrasound (50 MHz).

Treatment interventions

The two most affected lesions with relative similar clinical scores were randomized to the treatment methods. Randomization was carried out using the sealed envelope method where the patients drew lots between sealed envelopes, contact cards with treatment codes either UVA-1 phototherapy for lesion A and fractional CO_2 laser therapy for lesion B. The first author generated the random allocation and enrolled the participants, whereas clinical assessment was done by the fourth author who was blinded to the used intervention.

UVA-1 treatment

Lesion A received a total of 30 sessions of low-dose UVA-1 phototherapy, at a rate of 3 sessions per week over 10 weeks. A UVA-1 phototherapy hand lamp unit (Waldmann, UV 109 A, Germany) was used with a radiation spectrum ranging from 350 to 400 nm with a maximum peak at 370 nm. The dose per session was 30 J/cm^2.

Fractional CO_2 laser treatment

Lesion B received three laser sessions, separated by 1-month intervals, using fractional CO_2 laser (DEKA, SmartXide DOT, Italy). Treatment parameters were as follows: Power 25 W stack 2, dwelling time 500 msec, spacing 500 μm.

Statistical methods

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Wilcoxon signed rank test for paired (matched) samples. Correlation between various variables was done using Spearman rank correlation equation. p values less than 0.05 were considered statistically significant, and p values less than 0.001 were considered statistically highly significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) release 15 for Microsoft Windows (2006).

Results

Demographic and clinical data of the 17 patients are summarized in Table 2. A flow diagram for the study is shown in Fig. 1. The study lasted for about 1 year.

Clinical evaluation

Both studied groups of lesions showed clinical improvement. The UVA-1 group showed poor response in 1 patient (6 %), fair response in 10 patients (59 %), and good response in 6 patients (29 %). The fractional CO_2 laser-treated group showed fair response in 1 case (6 %), good response in 9 cases (53 %), and very good response in 7 cases (41 %).

In comparing the 2 groups, the fractional CO_2 laser showed better results, evident by the improved clinical scores after treatment in a highly significant manner ($p = 0.001$) (Table 3, Fig. 2). Patient satisfaction scores were significantly higher in the fractional CO_2 laser group compared to the UVA-1 group ($p = 0.001$) (Table 3). The main side effects encountered with both treatment modalities are summarized in Table 4.

Histopathological assessment

Both study groups showed an improvement of collagen homogenization and inflammatory infiltrate. However, the fractional CO_2 laser group exhibited better improvement of collagen homogenization scores which was statistically significant ($p = 0.012$) (Table 3, Fig. 3). On the other hand, the inflammatory infiltrate scores decrease was higher in the UVA-1-treated group (scores decreased from 23 to 14), compared to less decrease in the fractional CO_2 laser group (scores decreased from 23 to 19).

Immunohistochemical results

The levels of TGF–ß1 in the epidermis were undetectable in all sections of the study. In the dermis, positive brown staining was detected in endothelial cells of blood vessels and inflammatory cells (mainly fibroblasts). Both UVA-1 and fractional CO_2 laser-treated lesions showed a statistically significant decrease in the mean TGF-ß1 staining after treatment ($p = 0.001$), indicating the improvement

Table 2 Summary of demographic and clinical data of patients completing the study ($n = 17$)

Item		Value	Mean ± SD or percentage %
Age in years	Min	7	25.6 ± 11.06
	Max	47	
Sex	Males	2	12 %
	Females	15	88 %
Skin type	III	10	59 %
	IV	7	41 %
Type of morphea	Plaque	12	70 %
	Linear	3	18 %
	En coup de sabre	2	12 %
Stage of disease	Active	7	41 %
	Indurated	9	53 %
	Atrophic	1	6 %
Duration of diseases in months	Min	6	23.6 ± 29.3
	Max	96	
Previous therapy	Tried	12	71 %
	Not	5	29 %

SD standard deviation

of the fibrotic state (Fig. 4). However, the difference between both groups was not statistically significant ($p = 0.795$) (Table 3).

As for MMP1, positive brown staining was detected mainly in extracellular matrix, keratinocytes, inflammatory cells (mainly fibroblasts), endothelial cells of blood vessels, and adnexa. Both UVA-1 and fractional CO_2-treated lesions showed increase in the mean MMP1 staining after treatment compared to before treatment, which was statistically highly significant ($p = 0.000$) indicating collagen degradation after treatment (Fig. 5). In comparing the two groups, the difference of the mean increase of MMP1 staining was not statistically significant ($p = 0.868$) (Table 3).

Ultrasound assessment of dermal thickness

A statistically significant decrease in dermal thickness after treatment was noted in both modalities compared to before treatment ($p = 0.015$ and $p = 0.008$ for UVA-1 and fractional CO_2 laser, respectively). Comparing the two groups, the fractional CO_2

Table 3 Summary of clinical, histopathological, immunohistochemical ($n = 17$), and UBM ($n = 9$) results

Evaluation method	Time of Evaluation	UVA-1 phototherapy Mean ± SD	Fractional CO_2 laser Mean ± SD	*p* value
Clinical score (unit) (LoSCAT)	Before	6.24 ± 2.412	6.53 ± 2.322	0.001*
	EoS	4.24 ± 1.921	2.65 ± 1.73	
Patient satisfaction score after treatment (unit)	EoS	1.12 ± 0.06	2.24 ± 0.664	0.001*
Collagen homogenization scores (unit)	Before	7.53 ± 1.94	7.53 ± 1.94	0.012*
	EoS	5.41 ± 2.45	3.71 ± 2.59	
TGF-ß1 (Mean area %)	Before	2.52 ± 0.93	2.97 ± 0.83	0.795
	EoS	1.40 ± 0.82	1.70 ± 0.96	
MMP1 (Mean area %)	Before	15.41 ± 4.07	15.41 ± 4.48	0.868
	EoS	32.88 ± 8.53	33.17 ± 9.05	
UBM dermal thickness (mm)	Before	1.44 ± 0.29	1.42 ± 0.29	0.017*
	EoS	1.06 ± 0.18	0.91 ± 0.2	

*$P \leq 0.05$ (significant)

S.D standard deviation, *LoSCAT* localized scleroderma cutaneous assessment tool, *EoS* end of study, *TGF-ß1* transforming growth factor ß1, *MMP1* matrix metalloproteinase 1, *UBM* ultrasound biomicroscopy

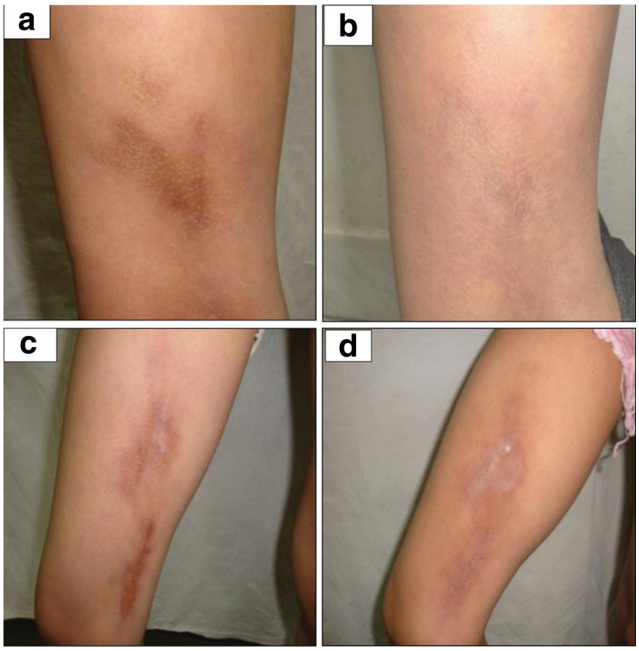


Fig. 2 Patient number 10:10 years old female with **a** plaque morphea lesion on the left thigh before laser treatment. **b** After fractional CO_2 laser treatment, showing excellent response with almost normalization of the skin, improvement as regards softening and dyschromia (score pretreatment: 9, score post-treatment: 1). **c** Linear morphea lesion on the right thigh before treatment. **d** After UVA-1 phototherapy (score pretreatment: 9, score post-treatment: 4). Note the post-UVA-1 hyperpigmentation, giving impression of worsening of the condition in spite of evident softening by clinical evaluation

laser group showed better decreased dermal thickness than the UVA-1 group, which was statistically significant ($p = 0.017$) (Table 3, Fig. 6).

Discussion

The results of the current study proved that fractional ablative carbon dioxide laser is a promising treatment modality for cases of morphea, evident by the highly significant improvement in both the clinical scores and histopathological scores.

Table 4 Side effects encountered in both groups ($n = 17$)

Side effect	UVA-1 *N* (%)	FAL *N* (%)
Hyperpigmentation	4 (24 %)	1 (6 %)
Non-compliance	1 (%)	0 (0 %)
Mild to moderate pain	0 (0 %)	17 (100 %)
Marked pain	0 (0 %)	10 (59 %)
Persistent erythema	0 (0 %)	1 (6 %)
Itching in 1st 24 h	0 (0 %)	8 (47 %)

FAL fractional ablative laser

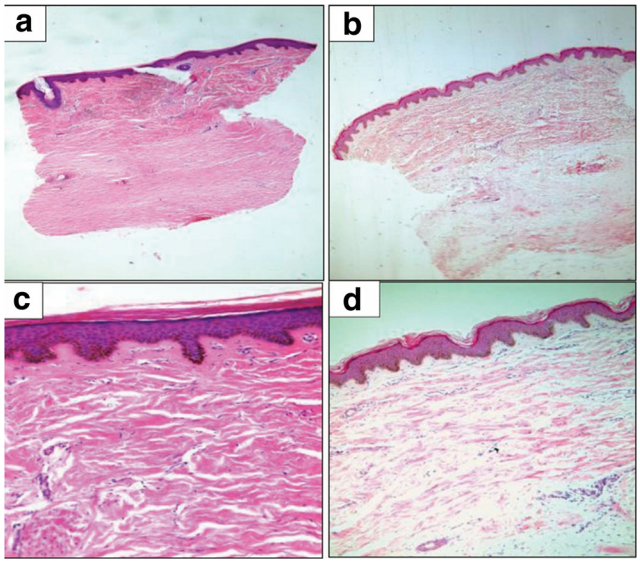


Fig. 3 Photomicrographs of skin sections obtained from patient number 8 who achieved marked clinical improvement. **a** Pretreatment biopsy (×40) square-shaped biopsy with marked homogenization of collagen. **b** Post-fractional CO_2 laser treatment biopsy (×40), showing thinning of the collagen fibers. **c** Pretreatment biopsy (×40) showing thickened homogenized collagen. **d** Post-UVA-1 phototherapy biopsy (×40), showing thinning of the collagen fibers

The improved clinical scores of FAL-treated lesions are in agreement with the results of the two previous case reports [11, 12].

In the light of those case reports, as well as studies using FALs in other fibrotic conditions such as burn scars [4], a high fluence and multiple stacking were used in the current study. In addition, the Smart Pulse technology that was used in the current study has a unique advantage in targeting the deeper tissues, where the first part of the pulse (10 % of the entire pulse) consists of high peak power pulse allowing for rapid ablation of the epidermis and superficial dermis, while the second part of the pulse with low peak power allowing for targeted heating of the deeper tissues and subsequent collagen remodeling [13]. The mode of action of FALs in the treatment of morphea is suggested to be through an *immediate mechanical effect* through the induced microscopic treatment zones (MTZs) which remove some of the homogenized or fibrotic tissue releasing the skin tightness, and a rather more *delayed sustained effect* of dynamic wound healing with induction of collagen remodeling [4, 11] as proven by in vivo imaging technique [14].

In the present study, a significant decrease in the profibrotic marker TGF-ß1 level was detected after FAL treatment. It is reported that after an initial rise of TGF-ß1 in the first 3 days after FAL treatment, gradual drop of TGF-ß1 follows for 30 days (time of assessment in the current study), suggesting a physiological controlled wound healing process [15, 16]. The significant rise of the MMP1 levels after laser

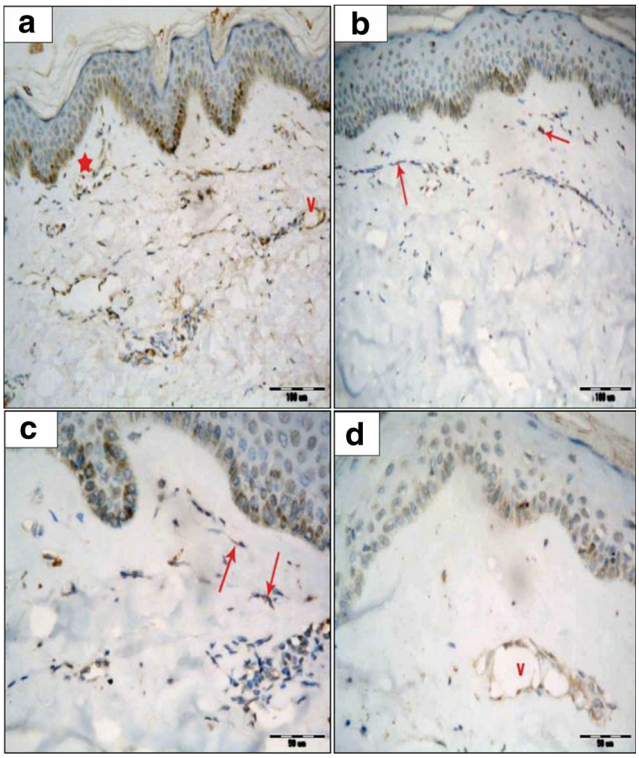


Fig. 4 Photomicrographs of skin sections obtained from patient number 10 who obtained very good clinical response. **a** Before treatment showing positive TGF-ß1 staining localized in the spindle-shaped fibroblasts, perivascular infiltrate (*star*) and endothelial lining of blood vessels. **b** After fractional CO_2 laser treatment with decrease in the area of positive staining limited to few fibroblasts (*arrow*) (TGF-ß1 immunohistochemistry, ×200). **c** Before treatment showing positive TGF-ß1 staining localized in the spindle-shaped fibroblasts (*arrows*) perivascular infiltrate and endothelial lining of blood vessels. **d** After UVA-1 treatment with decrease in the area of positive staining limited to endothelial lining of blood vessel (*v*) (TGF-ß1 immunohistochemistry, ×400)

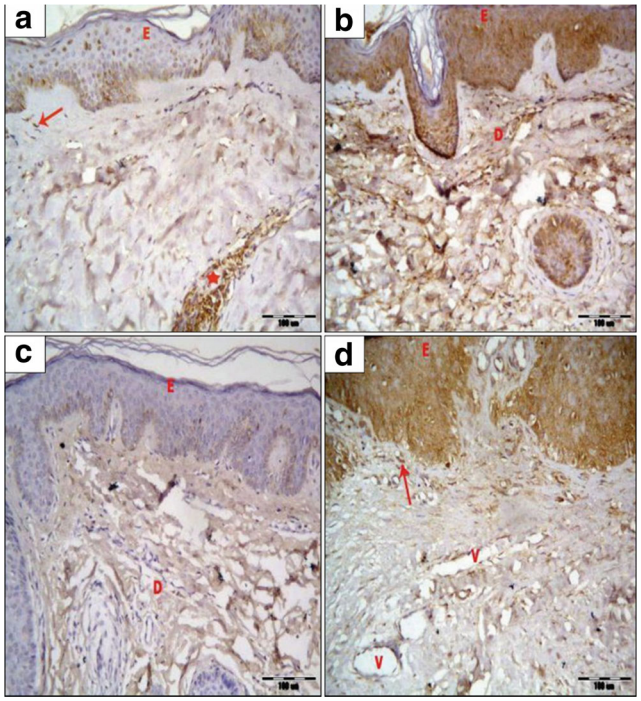


Fig. 5 Photomicrographs of sections obtained from patient number 10 who showed good clinical response. **a** Before treatment showing mild positive MMP1 staining localized in some epidermal keratinocytes, fibroblasts (*arrow*), extracellular matrix and endothelial lining of blood vessels (*star*). **b** After fractional CO_2 laser treatment with marked increase in the area of positive staining localizing to the epidermis (*E*), extracellular matrix (*D*), fibroblasts, and endothelial lining of blood vessels. **c** Before treatment showing mild positive MMP1 staining localized in extracellular matrix mainly (*D*). **d** After UVA-1 phototherapy with marked increase in the area of positive staining localizing to the epidermis (*E*), extracellular matrix, fibroblasts (*arrows*), and endothelial lining of blood vessels (*v*) (MMP1 immunohistochemistry, ×200)

treatment was also reported previously [15–17] to lead to degradation of the abnormal homogenized collagen bundles and allowing its replacement with new normal collagen.

In the current study, FAL showed more significant improvement clinically, histopathologically, and by ultrasound which reflects decreased collagen density and homogenization [18].

The superior response of FALs compared to the low-dose UVA-1 phototherapy may be attributed to several factors. First, FALs exert more diverse actions on collagen remodeling. The main pathway of collagen degradation in UVA-1-treated lesions was only increased collagenase (MMP1) as reported beforehand [19, 20]. Molecular studies [16, 17] demonstrated that the efficacy of FALs was attributed to rise of multiple MMPs including MMP 1,3,9 and 13.

Second, heat shock proteins (HSPs) are up-regulated in response to damage, resulting from laser-induced coagulation [21]. HSP72 was significantly elevated around the MTZs in the early phase post-FALs treatment, which resulted in activation of epidermal stem cells and cells within the dermis inducing rapid healing and initiating collagen remodeling. Later on, HSP47 showed sustained up-regulation in the dermis in a diffuse pattern for 3 months post-FALs treatment emphasizing the activation of fibroblasts in and around the MTZs allowing long-term collagen dermal remodeling [22]. This action was the main rationale suggested for improvement of burn scars treated with FALs [23].

Third, the ablation of MTZs itself removes some of the homogenized tissue allowing early improvement of the skin texture with release of the morphea-induced contracture before the actual start of the wound healing process and collagen remodeling [11].

Lastly, an important benefit of FALs over UVA-1 is the much lower incidence of post-inflammatory hyperpigmentation which worsens the cosmetic appearance of lesions, and it may act as a hindering factor to anti-inflammatory and anti-fibrotic effect of further UVA-1 sessions, especially in darker skin types [24]. In agreement with our results, the effectiveness of low-dose UVA-1 phototherapy in treatment of

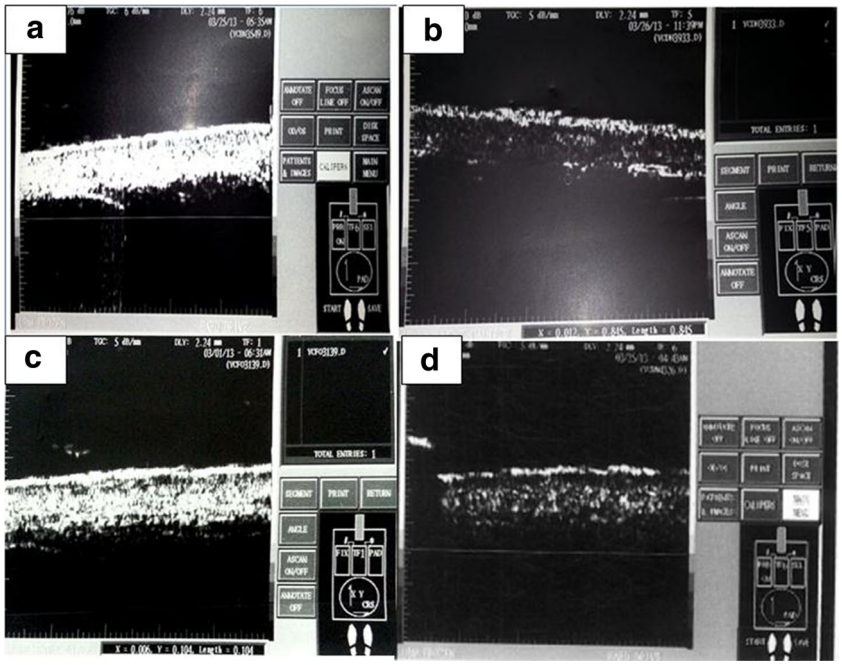


Fig. 6 Ultrasound biomicroscopy of morphea lesions of patient number 10 who showed clinical improvement. **a** Before treatment showing thickened dermis. **b** After fractional CO_2 laser showing marked decrease in dermal thickness indicative of the decrease collagen homogenization. **c** Before treatment showing thickened dermis. **d** After UVA-1 phototherapy showing marked decrease in dermal thickness indicative of the decrease collagen homogenization

morphea was proven in several studies [25–27]. In collating to medium-dose UVA-1, clinical improvement was comparable [28]. On the other hand, a recent study states that medium- and high-dose regimens outperform low-dose UVA-1 in dermatoses beyond localized sclerosis, namely in 16 systemic sclerosis patients [29].

The current study was superior to previous studies, in being the first (to the best of our knowledge) to assess the efficacy of low-dose UVA-1 phototherapy in plaque and linear morphea treatment in darker skin types (Fitzpatrick III and IV).

The main privilege that can be attributed to the UVA-1 phototherapy over FAL is its unique apoptotic effect on the inflammatory T cells [30], mediating a strong anti-inflammatory response in cases of active morphea. This was evident histopathologically by a decrease in the inflammatory cell scores in the UVA-1 group more than the fractional carbon dioxide laser group.

Patients showed significantly higher patient satisfaction scores in the fractional carbon dioxide laser group. The dyschromia noted after UVA-1 treatment was the main concern as well as the feasibility of the treatment modality. This was in accordance with Gambichler et al. [3] who described hyperpigmentation as a side effect.

The main reported side effect of fractional CO_2 laser was pain during the sessions especially in children, as formerly reported [31]. Proper use of topical anesthetic and cooling during sessions helps minimize the pain.

In conclusion, the current study proved that fractional carbon dioxide laser is a promising treatment modality for cases of plaque and linear morphea. It showed superiority to low-dose UVA-1 phototherapy.

Limitations of the study

FAL therapy was compared to low-dose UVA1 and not to medium-dose UVA1 therapy.

Compliance with ethical standards

Conflict of interest None declared.

Funding sources Cairo University (in partial).

References

1. Fett N (2013) Scleroderma: nomenclature, etiology, pathogenesis, prognosis, and treatments: facts and controversies. Clin Dermatol 31: 432–437
2. Zwischenberger BA, Jacobe HT (2011) A systematic review of morphea treatments and therapeutic algorithm. J Am Acad Dermatol 65:925–941
3. Gambichler T, Terras S, Kreuter A (2013) Treatment regimens, protocols, dosage, and indications for UVA1 phototherapy: facts and controversies. Clin Dermatol 31:438–454
4. Waibel J, Wulkan AJ, Lupo M, Beer K, Anderson RR (2012) Treatment of burn scars with the 1,550 nm nonablative fractional Erbium Laser. Lasers Surg Med 44:441–446
5. Arkachaisri T, Vilaiyuk S, Torok KS, Medsger TA Jr (2010) Development and initial validation of the localized scleroderma skin damage index and physician global assessment of disease damage: a proof-of-concept study. Rheumatology 49:373–381
6. Leheta T, El Garem Y, Hegazy R, Abdel Hay RM, Abdel Halim D (2013) Non-ablative 1540 fractional laser: how far could it help injection lipolysis and dermal fillers in lower-face rejuvenation? A randomized controlled trial. J Cosmet Laser Ther 15:13–20

7. Verrecchia F, Laboureau J, Verola O et al (2007) Skin involvement in scleroderma—where histological and clinical scores meet. Rheumatology (Oxford) 46:833–841
8. Birkedal-Hansen B, Moore WG, Taylor RE, Bhown AS, Birkedal-Hansen H (1988) Monoclonal antibodies to human fibroblast procollagenase. Inhibition of enzymatic activity, affinity purification of the enzyme, and evidence for clustering of epitopes in the NH2-terminal end of the activated enzyme. Biochemistry 27:6751–6758
9. Windsor LJ, Birkedal-Hansen H, Birkedal-Hansen B, Engler JA (1991) An internal cysteine plays a role in the maintenance of the latency of human fibroblast collagenase. Biochemistry 30:641–647
10. Roberts AB, Sporn MB (1990) The transforming growth factor-ß. In: Roberts AB (ed) Handbook of experimental pharmacology (Sporn MB. Springer Verlag, Heidelberg, pp 419–472
11. Kineston D, Kwan JM, Uebelhoer NS, Shumaker PR (2011) Use of a fractional ablative 10.6-μm carbon dioxide laser in the treatment of a morphea-related contracture. Arch Dermatol 147:1148–1150
12. Kozarev J (2012) Laser treatment of scarring skin disorders: focus on morphea. Laser in medicine 1:1–9
13. Le Pillouer-Prost A, Zerbinati N (2008) Fractional laser skin resurfacing with SmartXide DOT. Initial results. DEKA s.r.l 1–4.
14. Cicchi R, Kapsokalyvas D, Troiano M et al (2014) In vivo non-invasive monitoring of collagen remodelling by two-photon microscopy after micro-ablative fractional laser resurfacing. Biophotonics 7:914–925
15. Prignano F, Campolmi P, Bonan P et al (2009) Fractional CO2 laser: a novel therapeutic device upon photobiomodulation of tissue remodeling and cytokine pathway of tissue repair. Dermatol Ther 22:S8–S15
16. Reilly MJ, Cohen M, Hokugo A, Keller GS (2010) Molecular effects of fractional carbon dioxide laser resurfacing on photodamaged human skin. Arch Facial Plast Surg 12:321–325
17. Qu L, Liu A, Zhou L et al (2012) Clinical and molecular effects on mature burn scars after treatment with a fractional CO2 laser. Lasers Surg Med 44:517–524
18. Andres C, Kollmar A, Mempel M, Hein R, Ring J, Eberlein B (2010) Successful ultraviolet A1 phototherapy in the treatment of localized scleroderma: a retrospective and prospective study. Br J Dermatol 162:445–447
19. Fisher GJ, Kang S (2002) Phototherapy for scleroderma: biologic rationale, results, and promise. Curr Opin Rheumatol 14:723–726
20. El-Mofty M, Mostafa W, El-Darouty M et al (2004) Different low doses of broad-band UVA in the treatment of morphea and systemic sclerosis. Photodermatol Photoimmunol Photomed 20:148–156
21. Helbig D, Paasch U (2011) Molecular changes during skin aging and wound healing after fractional ablative photothermolysis. Skin Res Technol 17:119–128
22. Hantash BM, Bedi VP, Chan KF, Zachary CB (2007) *Ex vivo* histological characterization of a novel ablative fractional resurfacing device. Lasers Surg Med 39:87–95
23. Waibel J, Beer K (2009) Ablative fractional laser resurfacing for the treatment of a third-degree burn. J Drugs Dermatol 8:294–297
24. Wang F, Garza LA, Cho S et al (2008) Effect of increased pigmentation on the antifibrotic response of human skin to UV-A1 phototherapy. Arch Dermatol 144:851–858
25. Gruss CJ, Von Kobyletzki G, Behrens-Williams SC et al (2001) Effects of low dose ultraviolet A- 1 phototherapy on morphea. Photodermatol Photoimmunol Photomed 17:149–155
26. Pereira N, Santiago F, Oliveira H, Figueiredo A (2012) Low-dose UVA-1 phototherapy for scleroderma: what benefit can we expect? J Eur Acad Dermatol Venereol 26:619–626
27. De Rie MA, Enomoto DN, de Vries HJ, Bos JD (2003) Evaluation of medium-dose UVA1 phototherapy in localized scleroderma with the cutometer and fast Fourier transform method. Dermatology 207: 298–301
28. Sator PG, Radakovic S, Schulmeister K, Hönigsmann H, Tanew A (2009) Medium-dose is more effective than low-dose ultraviolet A1 phototherapy for localized scleroderma as shown by 20-MHz ultrasound assessment. J Am Acad Dermatol 60:786–791
29. Connolly KL, Griffith JL, McEvoy M, Lim HW (2015) Ultraviolet A1 phototherapy beyond morphea: experience in 83 patients. Photodermatol Photoimmunol Photomed 31:289–295
30. York NR, Jacobe HT (2010) UVA-1 phototherapy: a review of mechanism and therapeutic application. Int J Dermatol 49:623–630
31. Goel A, Krupashankar DS, Aurangabadkar S, Nischal KC, Omprakash HM, Mysore V (2011) Fractional lasers in dermatology—current status and recommendations. Indian J Dermatol Venereol Leprol 77:369–379