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ORIGINAL ARTICLE

Persistent Pixel Stamping Marks: a novel complication of fractional CO_2 laser in scar treatment

Samia M. Esmat[1] · Mona R. E. Abdel-Halim[1] · Heba I. Gawdat[1] · Marwa Kamel[1] · Amany ElRamly[1] · Kholoud Helmy[1] · Soheir M. Esmat[2]

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Abstract

Fractional CO_2 laser rejuvenation of scars offers a high safety profile. Laser marks usually disappear clinically within 1 week. The authors observed occasional persistence of the laser marks on the scar surface. The purpose of this study is to report the incidence and to describe the clinical, dermoscopic, and histological features of a novel observed complication of fractional CO_2 laser scar rejuvenation "Persistent Pixel Stamping Marks (PPSM)".One hundred seventy-one cases were consecutively recruited from patients assigned for fractional CO_2 laser scar rejuvenation. Patients who developed the phenomenon 1 month post laser session were recorded and subjected to clinical photography, dermoscopy, and optical coherence tomography (OCT) as well as a 4-mm punch biopsy from pixelated scars. The evolution of PPSM was followed up for 6 months. PPSM developed in 16 patients (9.4%), 15 of which were post burn hypertrophic scars. PPSM was significantly related to darker skin type, darker scar color, and longer scar duration. Histopathological findings included characteristic holes in stratum corneum and superficial dermis, thick collagen bundles perpendicular to the skin surface with loss of elastic tissue, focal interface changes, and triangular focus of fibroblastic proliferation. The marks disappeared in 5 and lasted in 11 patients. Their longevity was significantly related to longer dwell times and lower densities. PPSM represent miniature scarring at the sites of the microscopic thermal zones or a sign of their delayed healing. They tend to follow fractional CO_2 laser resurfacing of hyperpigmented, long-standing burn scars. Longer dwell times and lower densities make them last longer.

Keywords Persistent Pixel Stamping Marks · Fractional CO_2 laser · Scars · Complication

Introduction

Fractional ablative lasers have the advantage of a higher safety profile compared to traditional ablative lasers [1]. Fractional CO_2 laser has been used with great success in the treatment of various types of scars including post acne, surgical, traumatic, and burn scars with improvement in scar color, texture, and contractures [2–11]. Moreover, it has shown considerable efficacy in ameliorating facial rhytides and photodamaged skin [12].

Following fractional CO_2 laser resurfacing, a molecular cascade occurs involving various changes in the levels of tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), transforming growth factor-β (TGF-β), heat shock proteins (HSPs), and matrix metalloproteinases (MMPs) from 2 days post treatment up to 3 months that ultimately leads to wound healing, collagen remodeling, and scar regression [13].

Fractional ablative laser leads to immediate ablation of both the epidermis and dermis. Throughout the 48 h post treatment, the zone of ablation is completely taken over by invaginating epidermal cells. The microscopic ablative zone (MAZ) surrounded the invaginated epidermal tissue and microscopic epidermal necrotic debris (MEND) is found at the level of the stratum corneum. By the seventh day, MEND exfoliation is evident with residual material at the most superficial aspect of the stratum corneum. There is increase in the number of dermal spindle cells, demarking the continued presence of fibroblast activity. One month post treatment, the MEND is replaced by normal stratum corneum and is no longer detectable [14].

During the past 10 years, rejuvenation of scars by fractional CO_2 laser has been performed for thousands of cases in the

✉ Heba I. Gawdat
heba.gawdat@yahoo.com

[1] Dermatology Department, Faculty of Medicine, Cairo University, 59 Street 104, Maadi Gardens, Cairo 11431, Egypt

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

Laser Unit, Dermatology Department, Cairo University. The authors noticed that complete clinical healing (complete disappearance of laser beam insult indicated by falling of the crust leaving no residual erythema) could be achieved in almost all patients within 7 days post treatment. However, occasionally, a peculiar phenomenon developed in which unusual persistent marks developed on the laser-treated areas (Fig.1). These marks were observed when the patient came for the second treatment session, which was 4 weeks or more after the first session. Since these persistent marks followed the same pixel pattern of each laser stamp, the authors suggested naming them "Persistent Pixel Stamping Marks" (PPSM).

The aim of this work was to study this phenomenon and find out the possible predisposing factors that lead to its development as well as watching their evolution along the regular monthly sessions, in a trial to highlight the behavior of such newly reported finding.

Patients and methods

Following approval by the Dermatology Department Research Ethical Committee and signing informed consents and assents of the parents of patients less than 18 years old, this observational cohort study was conducted.

One hundred seventy-one consecutive patients with cutaneous scars, who were examined in our Laser Unit, Dermatology Department, Cairo University, from October 2016 to January 2017 and were eligible for fractional CO_2 laser resurfacing, were enrolled in the current study. Patients with a history of previous laser therapy were excluded.

Fitzpatrick skin type, anatomical location of the scar, duration of the scar, culprit insult, and previous treatment trials were recorded for each patient. In addition, the following scar scores were calculated at baseline according to the type of scar: Vancouver Scar Scale (VSS) [15] and Patient and Observer Scar Assessment Scale (POSAS) [16, 17] for hypertrophic traumatic and surgical scars, as well as post burn scars. Global Acne Scarring Classification [18], Investigator's Global Assessment for Acne Vulgaris, and Acne Scar Assessment Scale [19] were calculated for post acne scars.

Fig. 1 PPSM in a scar following fractional CO_2 laser resurfacing affecting more the darker area of the scar

All patients underwent monthly treatment sessions using fractional CO_2 laser (DEKA SmartXide DOT, column diameter is 120 μm ablative and 350 μm stimulative, Italy). Laser parameters were individualized according to the treatment guidelines of the laser unit depending on scar type and Fitzpatrick skin type (Table 1). All patients were assessed 1 month after the first laser treatment for the development of PPSM. However, they were instructed to seek consultation before that period (1 month), in case any undesirable effect occurred such as prolonged erythema (erythema lasting more than 3 days), pain (mild, moderate, severe), swelling, discharge, or other signs denoting infection. In addition, hyper and or/hypopigmentation were recorded.

Evaluation of PPSM

Any developed PPSM were digitally photographed using a digital camera (Sony DSC-W530, 14 M pixel) with fixed distance and illumination. They were also subjected to dermoscopic examination using a DermLite III pro® dermoscope (3Gen, San Juan Capistrano, CA, USA). In addition, images by optical coherence tomography (OCT) (RTVue premier, Optovue Medical Industries, Fremont, CA, USA) were taken to provide scan range with a depth of 2–2.3 mm, scan beam of wave length $\lambda = 840 \pm 10$ nm, external image of 13 mm × 9 mm, depth resolution in tissue of 5.0 μm, transverse resolution of 8 μm, and with working distance of 22 mm.

Pre-treatment biopsies (4 mm punch) were taken from 20 randomly selected hypertrophic scars. In addition, biopsies were also retrieved from scars developing PPSM 1 month after the first session (only from patients 18 years or above, according to our Ethical Committee guidelines).

Biopsies were fixed in 10% neutral-buffered formalin, processed, and embedded in paraffin blocks. Blocks were serially sectioned. Sections from same levels were stained with H&E [20] for routine examination, with Masson's trichrome (MT) for histochemical staining of collagen fibers, and with Orcein stain for histochemical staining of elastic fibers [21]. For each case, a slide was also stained with Masson Fontana stain for histochemical assessment of melanin pigment within the basal cell layer [22]. All slides were examined using a Zeiss, Primostar light microscope (Zeiss, Germany), and assessed for any feature that might represent a residual microthermal zone (MTZ) (regularly alternating changes in the epidermis, melanin pigmentation, collagen, or elastic fibers) down to the superficial reticular dermis.

Table 1 Laser parameters for various types of scars

Type of scar	Number	Parameters			
		Power	Dwell time	Spacing/density (%)	Stacking
Post acne scars	11	15–20j	600–800 μsec	600–800 μm (10.7–7.3%)	1–2
Atrophic scars	60	15–20j	600–800 μsec	600–800 μm (10.7–7.3%)	1–2
Hypertrophic post traumatic scars	44	15–20j	800–1000 μsec	300–400 μm (22.8–17%)	2–3
Post burn scars	56	15–25j	800–1200 μsec	700–1000 μm (8.7–5.3%)	2–3

Photomicrographs depicting the various histopathological and histochemical findings were obtained with an integrated camera. All photomicrographs presented are according to their original magnification. Any possible positive findings were compared with the pre-treatment biopsies.

Follow-up of patients with PPSM

Patients who developed PPSM were followed up for 6 months, during which they continued receiving their monthly laser sessions, to observe the behavior of such marks under regular treatment protocol for scar resurfacing.

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 the Mann Whitney *U* test for independent samples. For comparing categorical data, chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is less than 5. *p* values less than 0.05 were considered statistically 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

Sixteen out of the 171 recruited patients (9.4%) developed PPSM following the first fractional CO_2 laser session. They included 15 patients with post burn scars (93.7%) and 1 patient with hypertrophic post traumatic scar (6.3%). PPSM involved the whole scar in 10 cases (62.5%), while in 6 cases (37.5%), they involved only parts of the scar. The study group included 89 females (52%) and 82 males (48%). Their ages

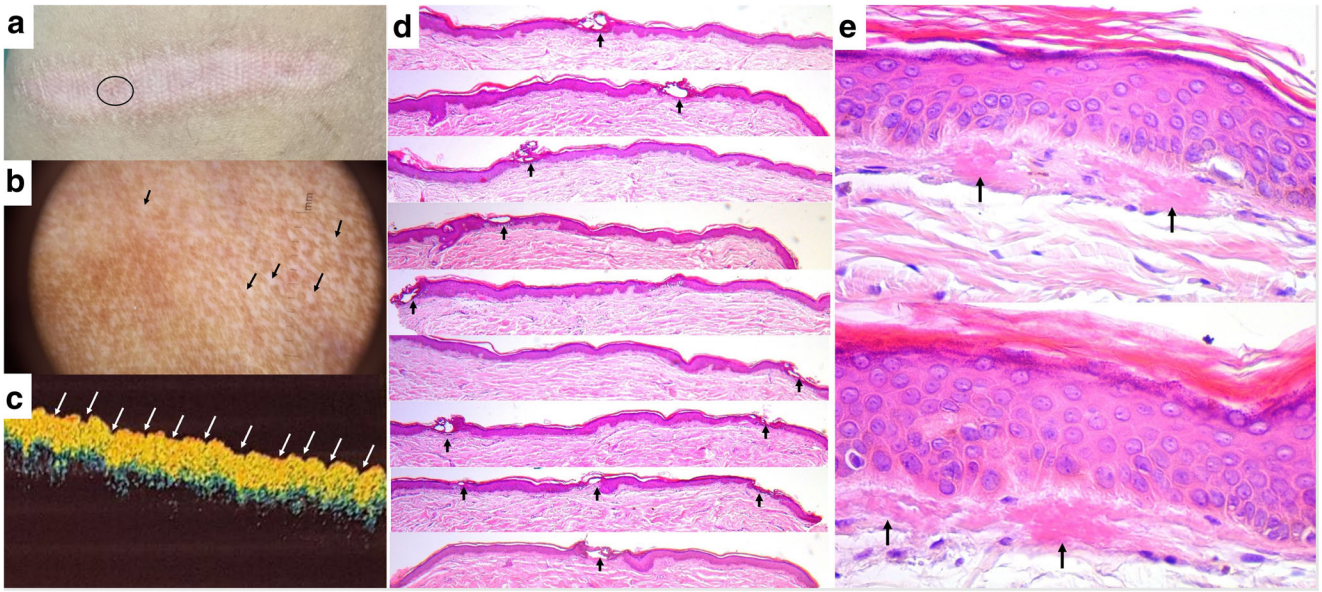

Fig. 2 **a** PPSM in a scar following fractional CO_2 laser resurfacing. **b** Dermoscopic features of the PPSM (encircled area in Fig. 2a): absence of follicular openings with pseudo-follicular white dots (black arrows). **c** Appearance of the PPSM by OCT as irregular hyper-reflective superficial layers with diffuse multiple dimples (white arrows). **d** Photomicrographs of a biopsy from the PPSM showing peculiar holes in the stratum corneum and epidermis (residual MTZs) at different locations in 9 consecutive sectioning levels with focal interface changes beneath them (black arrows) (H&E, × 100). **e** Photomicrographs of the same biopsy from the PPSM showing focally thickened collagen bundles beneath the epidermis detected in two consecutive sectioning levels (black arrows) (H&E, × 400)

ranged from10–55 years, mean ± SD age 21.50 ± 9.06. Sixty-two (36.3%) of the recruited patients were under 18 years old, and in accordance with our Ethical Committee guidelines, they were not biopsied.

Description of the PPSM

Clinical appearance

The PPSM appeared as non-palpable minute depressions that sometimes appeared paler than the surrounding area. These depressions followed the same pixel pattern of the laser stamp (Fig. 2a).

Dermoscopic features

Dermoscopic examination of the PPSM revealed the absence of follicular openings with the presence of white scar-like patches with pseudo-follicular white dots. There were regularly arranged crypts forming a rhomboid pattern corresponding to the minute persistent cutaneous depressions (Fig. 2b).

Optical coherence tomography results

Examination of PPSM with OCT revealed excessive irregular hyper-reflective superficial layers with diffuse multiple dimples compared to normal skin and non-pixelated scars (Fig. 2c).

Histopathological features

Only 14 out of the 16 patients who developed PPSM were eligible for a biopsy from the pixelated marks. Improvement of collagen arrangement and reappearance of elastic fibers was observed in post treatment sections compared to the pre-treatment ones. Histopathological features mostly representing residual MTZs were seen only in 5 cases

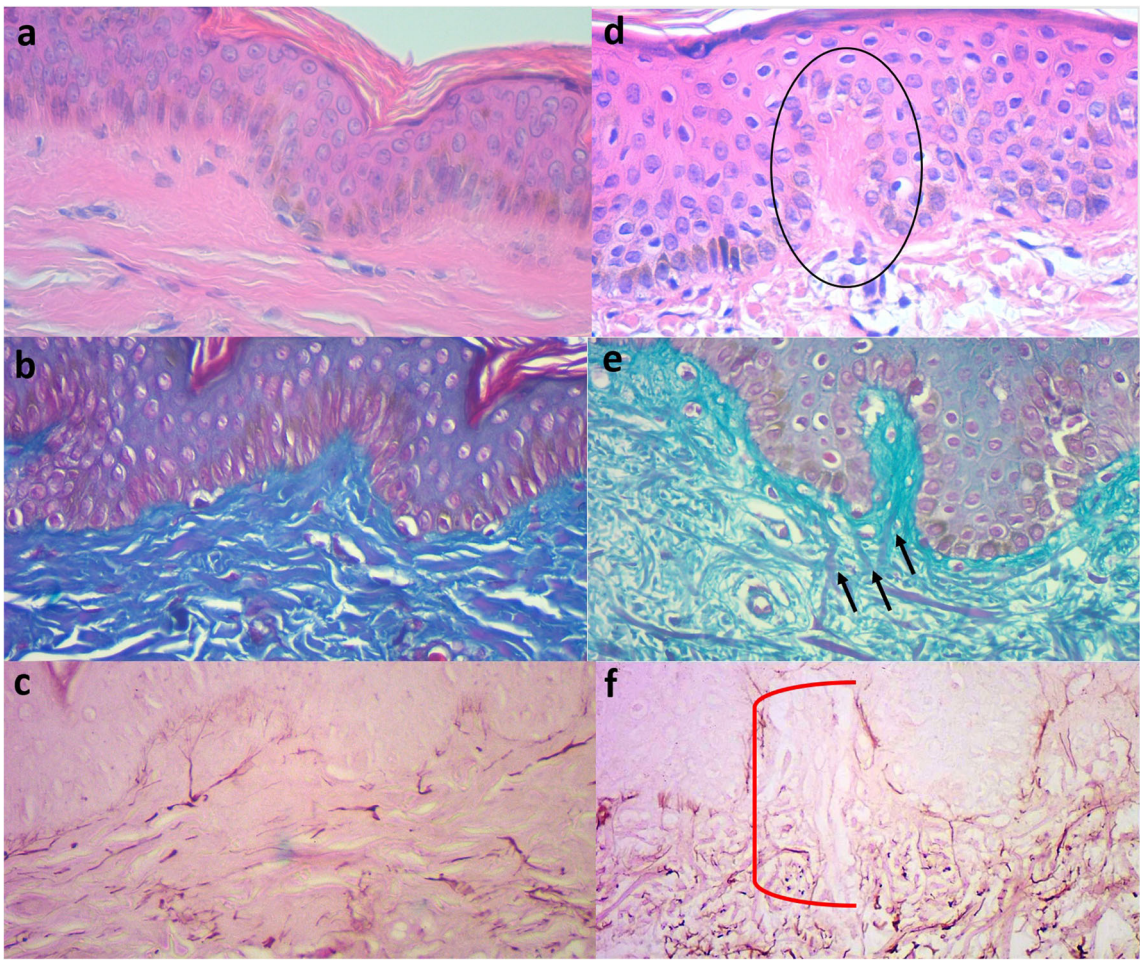

Fig. 3 Photomicrographs from another case that developed PPSM. **a**–**c** Pre-treatment biopsy showing thick collagen bundles in the superficial dermis with reduced elastic fibers (**a** H&E, × 400; **b** Masson trichrome stain, × 400; and **c** Orcein stain, × 400). **d**–**f** Biopsy from post treatment PPSM showing improvement of collagen quality in the superficial dermis with residual focally thick collagen bundles perpendicular to the epidermis (black circle and black arrows) and increased density of elastic fibers in the superficial dermis with residual area of loss of elastic fibers perpendicular to the epidermis (red bracket) (**d** H&E, × 400; **e** Masson trichrome stain, × 400; and **f** Orcein stain, × 400)

(35.7%). These included two epidermal findings and three dermal findings. Epidermal findings included peculiar holes in the stratum corneum and epidermis recurring at different locations in sequential serial levels with focal interface changes beneath them in two cases (Fig. 2d). Dermal findings included focally thickened areas of collagen beneath the epidermis in one case (Fig. 2e) and focally thick collagen bundles oriented perpendicular to the epidermis with loss of elastic fibers in two cases (Figs. 3 and 4**)**. These epidermal and dermal findings were not found in any of the pre-treatment biopsies available **(**Figs. 3 and 5**)**.

Factors related to the development of the PPSM

Patients' age and gender did not affect the development of PPSM, patients with skin types III and V were significantly more prone to develop them ($p < 0.001$) (Table 2). Hypertrophic post burn scars were most commonly affected followed by hypertrophic post traumatic scars ($p < 0.001$) (Table 3). Among other scar-related factors, longer scar duration as well as scars with a darker color at baseline was more significantly associated with the development of PPSM ($p = 0.0008$, $p = 0.004$,

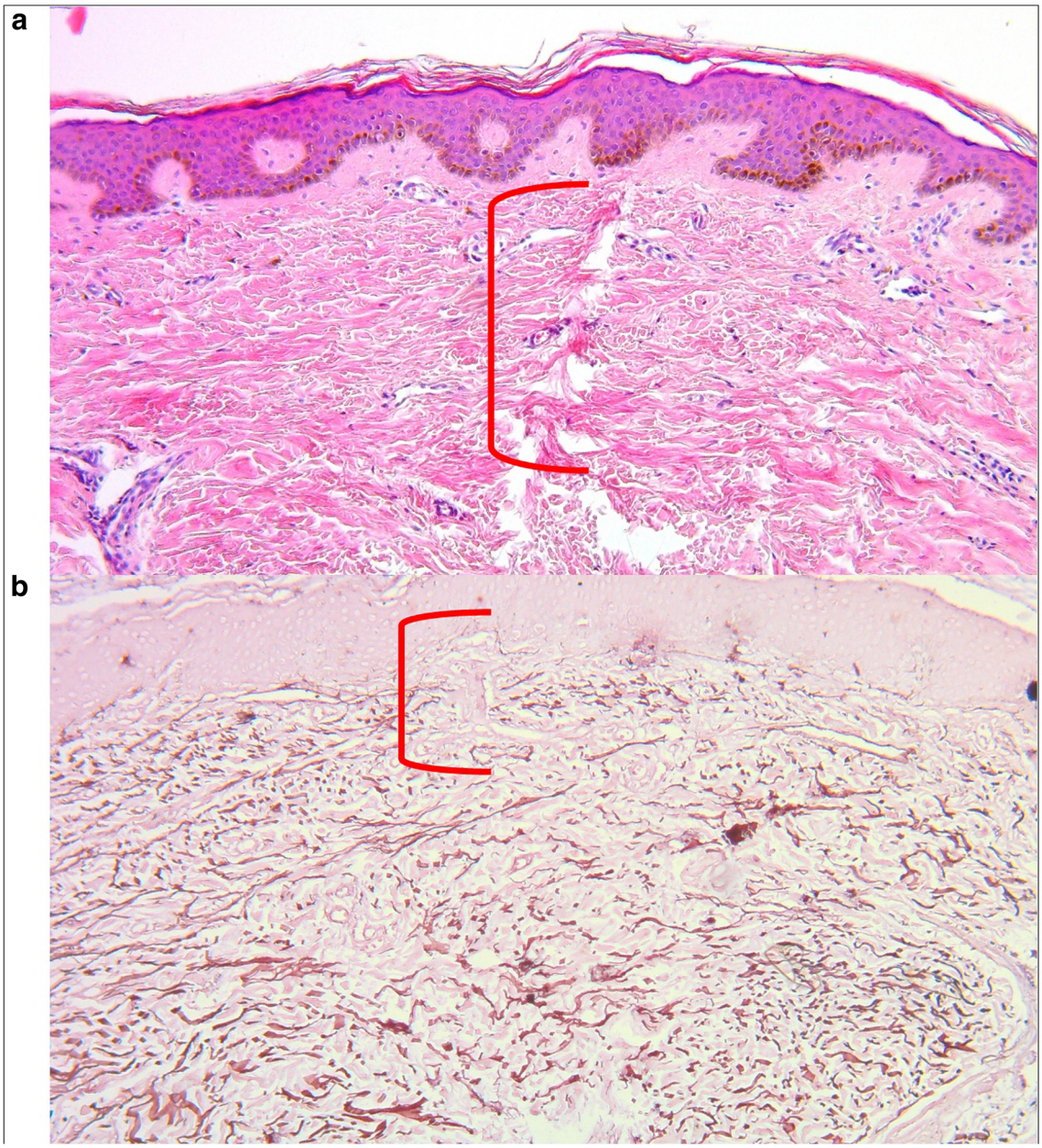

Fig. 4 Photomicrographs of another biopsy of PPSM showing focally thick collagen bundles perpendicular to the epidermis and showing partial loss of elastic fibers (red brackets) (**a** H&E, × 100; and **b** Orcein stain, × 100)

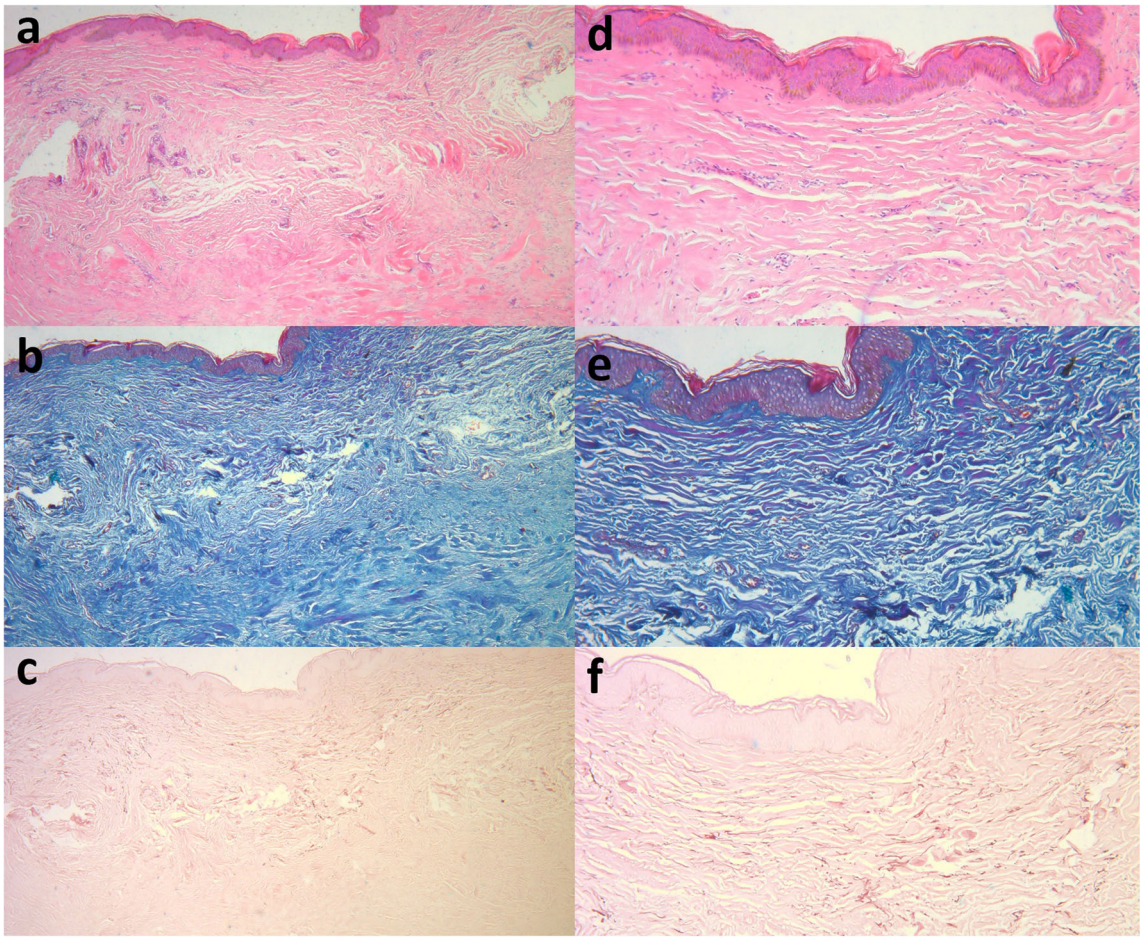

Fig. 5 Photomicrographs of a pre-treatment biopsy of a scar. Note the absence of any of the epidermal or dermal findings described in the biopsies taken from the PPSM. **a** H&E × 40, **b** Masson trichrome stain × 40, **c** Orcein stain × 40, **d** H&E × 100, **e** Masson trichrome stain × 100, and **f** Orcein stain × 100)

respectively) (Table 4). Laser parameters and laser-related side effects did not affect the induction of PPSM. Moreover, the longer the patient's downtime (time required for complete clinical healing denoted by falling of the crust), the more was the possibility to develop these marks ($p < 0.001$) (Table 5).

Follow-up of cases with PPSM and factors affecting its longevity

During the 6 months follow-up period, PPSM disappeared in 5 (31.3%) patients (after 1 month in one patient, 2 months in three patients, and 5 months in one patient), while 11 patients

Table 2 Effect of patients' age, gender, and Fitzpatrick's skin type on the development of PPSM

Variable	Patients who developed PPSM ($n = 16$)	Patients who did not develop PPSM ($n = 155$)	*P* value
Age	10–38 23.06 ± 7.870	10–55 21.34 ± 9.176	0.264
Gender	Females 9 (56.3%) Males 7 (43.8%)	Females 80 (51.6%) Males 75 (48.4%)	0.884
Fitzpatrick's skin type	III 5 (31.3%) IV 7 (43.8%) V 4 (25.0%)	III 40 (25.8%) IV 111 (71.6%) V 4 (2.6%)	< 0.001

Data are expressed as range and mean ± SD or number (%)

P value is significant if < 0.05

Table 3 Effect of scar-related variables on the development of PPSM

Variable		Patients who developed PPSM ($n = 16$)	Patients who did not develop PPSM ($n = 155$)	P value
Scar duration (months)		12–288 120.75 ± 88.450	2–360 67.63 ± 68.430	0.008
Scar anatomical location	Face	5 (31.3%)	94 (60.5%)	0.128
	Neck	1 (6.3%)	2 (1.3%)	
	Trunk	5 (31.3%)	13 (8.3%)	
	Upper limbs	5 (31.3%)	32 (20.6%)	
	Lower limbs	0 (0%)	14 (9%)	
Scar type	Post acne	0 (0%)	11 (7.1%)	< 0.001
	Post chicken pox	0 (0%)	2 (1.3%)	
	Post surgical atrophic	0 (0%)	3 (1.9%)	
	Post surgical hypertrophic	0 (0%)	7 (4.5%)	
	Post traumatic atrophic	0 (0%)	55 (35.4%)	
	Post traumatic hypertrophic	1 (6.3%)	36 (23.2%)	
	Post burn hypertrophic	15 (93.7%)	41 (26.5%)	
Previous scar treatment	Intralesional steroids	1 (6.3%)	13 (8.3%)	0.748
	Oral isotretinoin	0 (0%)	1 (0.6%)	
	Surgical grafting	1 (6.3%)	1 (0.6%)	
	Systemic antibiotics and topical retinoids	0 (0%)	5 (3.2%)	
	Topical retinoids	0 (0%)	1 (0.6%)	
	Topical retinoids and topical antibiotics	0 (0%)	3 (1.9%)	
	None	14 (87.5%)	131 (84.5%)	

Data are expressed as range and mean ± SD or number (%)

P value is significant if < 0.05

had their PPSM throughout the follow-up period. When compared clinically, no statistically significant differences were detected as regards scar, and patient-related factors. As regards laser parameters, shorter dwell times ($p = 0.028$) and higher densities ($p = 0.004$) were significantly associated with faster disappearance of the marks **(**Table 6**).**

Correlation with histological findings (1 month post laser therapy) revealed that cases showing only epidermal changes and/or triangular area of fibroblast proliferation PPSM disappeared before 6 months. While cases showing focally thickened areas of collagen, PPSM lasted more than 6 months.

Discussion

The current study is the first to report the occurrence of PPSM as a complication that may follow the treatment of scars using fractional ablative CO_2 laser.

Clinically, PPSM appeared like minute pale depressions that cannot be palpated. They follow the same pattern of the laser stamp on the treated scar and persist more than 4 weeks. Sixteen (9.4%) of our patient series developed this phenomenon, 15 of which had post burn scars and one had post traumatic hypertrophic scar, while none of the other types of scars included in the study developed these marks. We could therefore consider this phenomenon of PPSM mainly related to burn scars. Goel and Shrivastava described burn scars to be unfavorable compared to surgical and dermatologic scars [23]. Burn injuries cause macerated tissue, irregular borders, high tension, and tissue loss involving disruption of the epidermis, as well as increased risk of infection. Subsequently, the necrotized traumatized skin edges, especially if complicated by infection, will end in severe atrophic or hypertrophic scars with an extremely poor cosmetic outcome [24]. The poor nature of the scar or maybe a peculiar behavior of the fibroblasts might be behind the development of the PPSM almost exclusively in burn scars. This assumption could also be supported by our finding that PPSM were more prone to develop in hypertrophic rather than atrophic scars. This observation suggests that the abnormal collagen and elastic tissues of hypertrophic scars might be behind the occurrence of this phenomenon. In addition, PPSM were not observed in the normal skin surrounding the scar or in post acne scars where most of the skin in between is normal.

Table 4 Effect of VSS and POSAS parameters on the development of PPSM

Variable		Patients who developed PPSM ($n = 16$)	Patients who did not develop PPSM ($n = 84$)	P value
VSS (pre-treatment)	VSS score	3–10 6.63 ± 1.708	3–13 7.44 ± 2.320	0.218
	Pigmentation score 0 in VSS Pigmentation score 1 in VSS	0 (0%) 0 (0%)	21 (25%) 16 (19%)	0.004
	Pigmentation score 2 in VSS	16 (100.0%)	47 (56%)	
	Pliability score 1 in VSS Pliability score 2 in VSS	3 (18.8%) 2 (12.5%)	17 (20.2%) 28 (33.3%)	0.328
	Pliability score 3 in VSS	10 (62.5%)	34 (40.5%)	
	Pliability score 4 in VSS	1 (6.3%)	5 (6%)	
POSAS (pre-treatment)	Observer total scar assessment scale (pre-treatment)	9–42 24.19 ± 7.148	13–44 26.05 ± 7.033	0.283
	Patient total scar assessment scale (pre-treatment)	20–45 37.00 ± 6.772	18–56 36.35 ± 7.669	0.651
	Patient score for scar color in relation to normal skin (pre-treatment)	4–10 8.81 ± 1.759	2–10 7.98 ± 1.994	0.121
	Patient score for scar stiffness in relation to normal skin (pre-treatment)	2–10 7.13 ± 2.446	2–10 6.94 ± 2.073	0.752

Data are expressed as range and mean ± SD or number (%)

P value is significant if < 0.05

Although the parameters used in different types of scars were not the same, yet we could not consider this variation to be behind the higher incidence of PPSM in burn scars, as there was no significant relationship between the laser parameters used and the induction of these marks. Furthermore, PPSM developed in 15 cases only and not all patients with hypertrophic burn scars despite using the same parameters.

Ten patients had PPSM following laser treatment in the whole scar area, while six patients had these marks only in some parts of the scar, owing to the fact that burn scars are usually not uniform in color and consistency. The development of PPSM was significantly higher in scars with a darker color at baseline. This finding is probably due to the pale color of these marks that make them more

Table 5 Effect of laser parameters and laser related side effects on the development of PPSM

Variable		Patients who developed PPSM ($n = 16$)	Patients who did not develop PPSM ($n = 155$)	P value
Power (watt)		15–18 17.19 ± 1.047	12–18 16.81 ± 1.217	0.219
Dwell time (microseconds)		600–1000 762.50 ± 108.781	300–1000 726.45 ± 108.758	0.318
Spacing (micrometer-μm)		300–800 643.75 ± 141.274	300–900 644.84 ± 117.492	0.857
Stacks		1–2 1.94 ± 0.250	1–3 2 ± 0.426	0.567
Down time (days)		7–28 8.85 ± 4.205	3–20 8.09 ± 3.147	< 0.001
Laser-related side effects	Hyperpigmentation Hypopigmentation	0 (0%) 0 (0%)	3 (1.9%) 1 (0.6%)	0.887
	Persistent erythema	0 (0%)	2 (1.3%)	
	None	16 (100%)	149 (96.1%)	

Data are expressed as range and mean ± SD or number (%)

P value is significant if < 0.05

Table 6 Effect of different variables on the disappearance of PPSM during the follow-up period

Variable		Patients who had PPSM till the end of follow-up period ($n = 11$)	Patients in whom PPSM disappeared during the follow-up period ($n = 5$)	*P* value
Age		15–38 23.36 ± 7.325	10–37 22.40 ± 9.864	0.733
Gender		Females 5 (45.5%) Males 6 (54.5%)	Females 4 (80.0%) Males 1 (20.0%)	0.197
Fitzpatrick's skin type		III 3 (27.3%) IV 5 (45.5%) V 3 (27.3%)	III 2 (40.0%) IV 2 (40.0%) V 1 (20.0%)	0.827
Scar duration (months)		12–288 138.55 ± 94.999	24–156 81.60 ± 63.157	0.233
Scar anatomical location	Face Neck	2 (18.2%) 5 (45.5%)	1 (20%) 0 (0%)	0.057
	Trunk	1 (9.1%)	0 (0%)	
	Upper limbs	2 (18.2%)	0 (0%)	
	Lower limbs	1 (9.1%)	4 (80%)	
Scar type	Post traumatic hypertrophic Post burn hypertrophic	0 (0%) 11 (100%)	1 (20.0%) 4 (80.0%)	0.126
Previous scar treatment	Intralesional steroids Surgical grafting	1 (9.1%) 1 (9.1%)	0 (0%) 0 (0%)	0.595
	None	9 (81.8)	5 (100%)	
Extent of PPSM within the scar	Involving part of the scar Involving whole of the scar	5 (45.5%) 6 (54.5%)	1 (20%) 4 (80%)	0.264
VSS		3–8 4.86 ± 1.773	2–4 3 ± 1	0.693
Observer total scar assessment scale		10–23 18 ± 4.583	7–17 11.20 ± 4.207	0.558
Patient total scar assessment		20–38 28.43 ± 5.503	15–30 20.60 ± 5.857	0.845
Power (watt)		15–18 17.27 ± 1.104	16–18 17 ± 1	0.528
Dwell time (microseconds)		600–1000 800 ± 100	600–800 680 ± 83.666	0.028
Spacing (micrometer-μm)		500–800 709.09 ± 83.121	300–600 500 ± 141.421	0.004
Stacks		1–2 1.91 ± 0.302	2 2 ± 0	0.500

Data are expressed as range and mean ± SD or number (%)

P value is significant if < 0.05

apparent against the darker surrounding background of the scar.

There was a statistically significant relationship between the duration of the scar and the development of PPSM following laser treatment. It seems that the older the age of the scar, the harder it is for the fibroblasts or maybe keratinocytes to heal normally, even the minute injuries caused by the microthermal zones (MTZs) of fractional CO_2 laser. This finding highlights the value of early intervention by fractional CO_2 laser in burn scar treatment.

Patients with longer downtime after laser treatment had a significantly higher incidence of PPSM. Accordingly, the delayed falling off of the crusts was probably accompanied by delayed or improper healing of the MTZ.

Optical coherence tomography examination demonstrated PPSM as minute superficial hyper-reflective dimples compared to normal skin and non-pixelated scars. This finding indicated the dots clinically seen are actual depressions. Dermoscopic examination revealed the absence of follicular openings with white scar-like patches and pseudo-follicular white dots. There were regularly arranged crypts forming a rhomboid pattern corresponding to the minute persistent cutaneous depressions supporting the assumption of PPSM being minute superficial scars [25] corresponding to the sites of the MTZs.

Among the 16 cases who developed PPSM, only five showed specific histopathological features as demonstrated by nine serial sections of each specimen. The most characteristic were holes in the epidermis recurring at different sites in sequential different levels of two biopsies. These holes had histopathological features of MTZ [26]. We believe that these are residual MTZs that did not re-epithelialize as usual within 48 h. The healing was delayed, and the holes persisted for 1 month after the laser session. These holes were associated with interface changes along the dermoepidermal junction (DEJ) in the form of vacuolar degeneration and necrotic keratinocytes, indicating residual damage at the DEJ. One case showed a peculiar single triangular focus of fibroblastic proliferation beneath the epidermis in one level indicating the presence of another temporary feature of the MTZ lasting longer than usual. On the other hand, two cases showed histological features of minute scarring at the site of the MTZs. One showed areas of thickened collagen in the superficial dermis, the other showed thick perpendicular collagen bundles in the papillary dermis with loss of elastic fibers as verified by Orcein stain [14].

An interesting finding that compared to pre-treatment sections, those minute scar-like structures appeared de novo beneath the epidermis of the pixelated scar in contrast to the surrounding dermis showing signs of rejuvenation. This suggests PPSM probably do not interfere with rejuvenation.

The persistent stamping pattern is mostly not related to altered pigmentation as Masson Fontana stain did not reveal any pigment distribution abnormality along the basal cell layer. This suggests that the pallor of the PPSM is probably due to abnormal dermal texture rather than real hypopigmentation.

The failure to detect specific histopathological findings of the marks in 9 patients (64.3%) might be explained by their minute size (beam diameter is 120 μm) and their small number, as well as wide inter-dot space (800 μm) used in burn scars. Thus, MTZs are expected to be range from 16-18 per specimen. Accordingly, the multiple serial sections done could not prevent missing some pathological defects.

At the end of the follow-up period (6 months), 11 patients still had PPSM, while the marks disappeared in the remaining five patients. Although none of the laser parameters were found to affect the development of PPSM, yet, longer dwell times and higher densities significantly increased the longevity of such marks. The notion of whether continuing laser sessions or not would affect the duration and or disappearance of such marks warrants further studies.

Intriguingly, patients whose biopsies showed only epidermal changes and/or triangular areas of fibroblast proliferation experienced disappearance of PPSM, while those who showed thickened bands of collagen at the dermis, their marks persisted. This might raise the possibility that some of the patients presenting with PPSM have only epidermal changes reflecting delayed healing of the MTZ while others present with superficial scarring in such sites. PPSM being reversible in almost one-third of the cases make it questionable if this phenomenon would or not require treatment. Longer follow-up periods are needed to further elucidate such observations.

Persistent Pixel Stamping Marks (PSSM) represent a novel reported phenomenon that can follow fractional CO_2 laser treatment of scars. They indicate either delayed healing or microscopic scarring at the site of MTZs. These marks can be expected in long-standing, hypertrophic post burn scars, especially darker ones. Shorter dwell times and higher densities hasten their disappearance.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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