The efficacy of combining fractional CO_2 laser and tacrolimus ointment in the treatment of vitiligo

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Background
Treatment of vitiligo is very challenging. Topical tacrolimus is one of the topical modalities reported to be successful. No single treatment, however, gives on its own satisfactory results, and combination treatments offer better outcomes. Fractional CO_2 laser (FCO_2) has been recently studied as an adjuvant treatment with other modalities in vitiligo.
Objective
To evaluate the value of adding FCO_2 laser before tacrolimus ointment in vitiligo.
Patients and methods
This is a randomized controlled prospective clinical trial including 27 patients with stable vitiligo with a total of 152 lesions. Lesions on one side of the body received one session of FCO_2 every month for 3 months in addition to tacrolimus ointment twice daily for 3 months, whereas lesions on the other side of the body received tacrolimus ointment only.
Results
Lesions treated with the combination modality showed improvement in 17 (22.36%) lesions, with a mean reduction in Vitiligo Area Scoring Index (VASI) of 10.05%, whereas lesions treated with tacrolimus only showed improvement in 13 (17.1%) lesions, with a mean reduction in VASI of 5.535%, yet these differences were not statistically significant (P=0.46 and 0.24, respectively). Acral lesions showed significantly more reduction in VASI when treated with combination therapy than with tacrolimus only (P=0.034). Lesions with shorter duration showed significant more reduction in VASI scores when treated with the combination (P=0.024).
Conclusion
Treatment of stable vitiligo with FCO_2 before tacrolimus yields mild improvement, statistically not significantly better than tacrolimus only except in acral areas. Lesions of shorter duration show significantly better response to the combination treatment than to tacrolimus only.

Keywords:
combination treatment, fractional CO_2 laser, tacrolimus ointment, vitiligo

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Introduction

Despite continuous efforts, vitiligo remains to pose a clinical challenge in many cases. Throughout decades, a wide range of medical, surgical, and physical modalities have been used to manage vitiligo, yet still novel efficacious treatment modalities are needed to improve the therapeutic outcomes. Combination therapies have been introduced to reach better results and allow decreasing the doses of treatments needed, thereby reducing the potential adverse effects that may occur [1].

Topical tacrolimus in vitiligo has a rapid onset of action and sustained therapeutic effects with an efficacy similar to that of moderate to potent topical corticosteroids [2].

Tacrolimus exerts its action on vitiligo by down-regulating antigen-specific T-cell activities and associated proinflammatory cytokine production such as interleukin 2 (IL-2), IL-3, IL-4, IL-5, interferon γ (IFN-ϒ), tumor necrosis factor-α (TNFα), and granulocyte stimulating factors [3,4]. It increases IL-10 expression in vitiligo lesions, and thereby inhibits melanocyte destruction triggered by unchecked Th1 pathways [3]. It has a stimulatory effect on melanocyte proliferation [5] and increases the level of stem cell factor (SCF) and matrix metalloproteinase 9 (MMP 9) activity in keratinocytes [6]. Moreover, tacrolimus increases tyrosinase activity and subsequently melanin biosynthesis. It also promotes cell migration [7] and increases the expression of Syndecan-2 that enhances melanocyte migration [8]. It increases the

stability of intracellular tyrosinase through increasing cellular pH. Additionally, the tacrolimus/FK-BP complex increases melanosome secretion, uptake, and transfer to keratinocytes [9].

Fractional CO_2 (FCO_2) laser has been shown to improve vitiligo lesions both by itself [10] and when followed by sun exposure [11], narrowband UVB (UVB) phototherapy [12], or topical 5 fluorouracil (5FU) application [13].

It is suggested that FCO_2 may exert its action through several mechanisms. Several investigators proposed that ablative therapies such as FCO_2 may enhance drug and UV penetration into the skin [14]. It was also suggested that the mechanical trauma inflicted by such therapies may promote the proliferation and migration of melanoblasts from edges of the lesions toward the depigmented center, as well as induce stem cell differentiation in the dermis of the lesions by cytokine-mediated inflammatory cascade activation [15,16]. Additionally, FCO_2 laser stimulates the production of different cytokines like transforming growth factor β1 [17], which is decreased in some patients with vitiligo [18]. It also causes a rise in MMP 2 and 9 [6] which enhance the migration of melanoblasts [19,20] and have shown altered levels in vitiligo lesions [21].

The aim of this work was to assess and compare the efficacy and safety of the use of FCO_2 laser followed by tacrolimus ointment application versus tacrolimus ointment alone in the treatment of stable vitiligo lesions not responding to regular forms of therapy, whether topical creams, systemic treatment, or phototherapy. Patients with stable vitiligo were selected to avoid koebnerization, which may occur upon trauma to the skin by the FCO_2 in patients with active vitiligo.

Patients and methods

This controlled prospective clinical trial was performed at the Department of Dermatology, Kasr El Aini Hospital, Cairo University, over a period of 12 months (April 2015–April 2016). Approval of the Dermatology Research Ethical Committee (Derma REC) of our hospital and written informed consents from all participants were obtained. A total of 27 patients with stable, bilateral, and somewhat symmetrical vitiligo lesions were included in the study (stability being defined as no eruption of fresh lesions and no extension of pre-existing lesions for a period of 6 months) [22]. Patients were off any topical or systemic treatment for at least 3 months. Patients with any concomitant dermatologic disease that may affect the study result (e.g. eczema, psoriasis, uricaria, etc.) were excluded. All patients were subjected to detailed history taking and dermatological examination. Vitiligo Area Scoring Index (VASI) score was calculated for each lesion to be treated [23]. One hand unit (palm+volar surface of all digits) was used to determine the baseline percentage of vitiligo involvement in each body region. The extent of depigmentation was expressed as follows:

10%=specks of depigmentation present.
25%=pigmented area exceeds depigmented area.
50%=pigmented and depigmented areas are equal.
75%=depigmented area exceeds pigmented area.
90%=specks of pigment present.
100%=no pigment present

Digital photographs were taken of all the areas selected for treatment in each patient before treatment.

Vitiligo lesions on one side of the body received 3 monthly sessions of FCO_2 laser [DEKA, SmartXide DOT (Dermal Optical Thermolysis), Italy fractional Carbon Dioxide Laser], in addition to topical tacrolimus 0.03% ointment [Cipla Ltd, Mumbai, India; packed by: SPI (SIGMA); Al Andalous Medical Company, Egypt] on the lesions twice daily for a period of 3 months. The laser parameters used were 15 W, 500 μs pulse duration, 500 μm spacing, and 1 stack. Topical lidocaine 5% was applied on the treatment area before the laser treatment to avoid pain and discomfort. No topical treatment other than the tacrolimus ointment routine was used directly after the laser treatment.

The other side of the body was used as a control, as symmetrical vitiligo lesions were treated with only topical tacrolimus 0.03% ointment twice daily for 3 months.

After the treatment period was completed (1 month after the last FCO_2 session), VASI score was done again for each lesion to evaluate the efficacy of the treatment and compare between both sides. Based on the digital photographs taken before treatment, evaluation also included counting the number of lesions that disappeared, the number of lesions that showed some improvement and the number of lesions that did not show any improvement after treatment. The difference in response between the different locations on the body was evaluated as well as the

factors that may affect the response to treatment such as age, sex, duration of the disease, disease extent and severity, and sun exposure. The patients were followed up for 3 months after treatment to evaluate any change in the treated area results.

Statistical methods

Data were statistically described in terms of mean, SD, median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student's *t*-test for independent samples. For comparing categorical data, χ^2-test was performed. Exact test was used instead when the expected frequency is less than 5. *P* values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program SPSS (Statistical Package for the Social Sciences; SPSS Inc., Chicago, Illinois, USA) release 15 for Microsoft Windows (2006).

Results

This clinical trial included 27 Egyptian patients, comprising 11 (40.7%) males and 16 (59.3%) females with stable vitiligo. Details of the patients, demographic, and clinical data are mentioned in Table 1.

The repigmentation patterns found in the treated vitiligo lesions were mainly in the form of tanning and pigmentation from the periphery with narrowing of the lesions, and a few lesions showed perifollicular pigmentation (Fig. 1).

Improvement of the lesions treated with combined FCO_2 laser and tacrolimus was evaluated and showed improvement in 17 (22.36%) lesions. The mean percentage of reduction in VASI score was 10.05%. The lesions treated with tacrolimus only showed improvement in 13 (17.1%) lesions, and the mean percentage of reduction in VASI score was 5.535%.

There were no statistically significant differences between the two groups regarding the number of lesions that improved or the percentage of reduction in VASI scores (*P*=0.46 and 0.24, respectively).

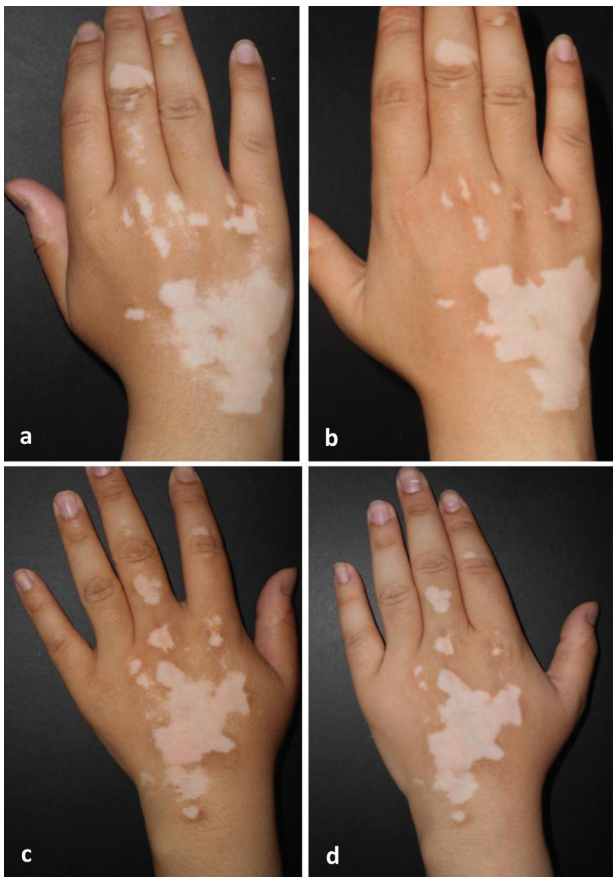
Among the lesions treated with combined FCO_2 and tacrolimus, the highest improvement regarding the number of lesions that improved after treatment was noted in the head and neck regions followed by the upper limbs, trunk, and then acral lesions. No improvement was noted in the lower limb lesions. No statistically significant differences were noted

Table 1 Demographic and clinical data of the patients

Characteristics	Cases (*n*=27)
Age (mean±SD) (years)	31.96±13.7
Sex [*n* (%)]	
Male	11 (40.7)
Female	16 (59.3)
Age of onset (mean±SD) (years)	23.8±12.9
Duration (mean±SD) (years)	6.29±5.1
Distribution of lesions (*n*=152)	
Head and neck	46
Acral	52
Upper limb	36
Trunk	8
Lower limb	10
VASI (mean±SD)	20.3±10.1
Smokers [*n* (%)]	8 (29.6)
Positive family history [*n* (%)]	3 (11.1)

VASI, Vitiligo Area Scoring Index.

Figure 1



(a) Lesions on the dorsum of hand before treatment with combined tacrolimus and FCO2; (b) same lesions showing repigmentation starting from the periphery with narrowing of lesions after 3 months of treatment with combined FCO2 and tacrolimus (0.03%) ointment; (c) lesions on the dorsum of the other hand of the same patient before treatment with tacrolimus only; (d) same lesions showing repigmentation from the periphery after 3 months of treatment with tacrolimus (0.03%) ointment only.

Table 2 Improvement of vitiligo lesions in different sites according to the number of lesions improved and percentage reduction in Vitiligo Area Scoring Index scores

Number and distribution of lesions in each group	Number of lesions improved [*n* (%)]		*P* value	Mean% of reduction in VASI (%)		*P* value
	FCO_2+Tacrolimus	Tacrolimus		FCO_2+Tacrolimus	Tacrolimus	
Head and neck (*n*=23)	8 (34.9)	7 (30.43)	0.236	12.3	6.6	0.533
Acral (*n*=26)	4 (19.04)	3 (15)	0.73	12.1	2.9	0.034*
Upper limb (*n*=18)	4 (26.66)	2 (13.33)	0.361	12.2%	10.14	0.481
Trunk (*n*=4)	1 (25)	1 (25)	1	3.3	2.5	0.823
Lower limb (*n*=5)	0 (0)	0 (0)		0	0	
P value	0.7	0.52		0.464	0.24	

VASI, Vitiligo Area Scoring Index. *$P<0.05$ is considered significant.

between all regions regarding the number of improved lesions, or the reduction in VASI scores (*P*=0.71 and 0.35, respectively) (Table 2).

Among the lesions treated with tacrolimus only, the highest improvement in the number of lesions treated was noted in the head and neck region followed by the trunk, acral, and then upper limb lesions. No improvement was noted in the lower limb lesions. Again, no statistically significant differences were noted between different regions regarding the number of improved lesions or the reduction in VASI scores (*P*=0.52 and 0.46, respectively) (Table 2).

Acral lesions treated with combined treatment, however, showed significant more reduction in VASI scores when compared with acral lesions treated with tacrolimus only (*P*=0.03).

No significant relationship was found between the age or the sex of patients and the response to treatment in both groups (*P*=0.53, 0.84, 0.26 and 0.22, respectively). However, vitiligo lesions with shorter duration showed significant more reduction in VASI scores in lesions treated with combined FCO_2 and tacrolimus (*P*=0.02), but this finding was not noted in lesions treated with topical tacrolimus only (*P*=0.27). The smoking habits of the patients did not influence the response to treatment in either groups. None of our patients experienced any complications during the study in either group apart from mild erythema that disappeared within a few days.

Follow-up for 3 months after completion of the treatment showed no change in the clinical picture. The results were maintained, with no worsening or improvement.

Discussion

In the current work, vitiligo lesions responded slightly better to combined FCO_2 and topical tacrolimus than to topical tacrolimus only, in the form of a higher number of responding lesions and more reduction in VASI scores, yet with no statistically significant difference except in acral sites, where combined treatment was superior in terms of reduction in VASI scores. The pattern of repigmentation in responding lesions was mainly a diffuse pattern in the form of tanning or repigmentation from the periphery of lesions or both, with very few lesions showing perifollicular pigmentation. Both treatment protocols showed the best results in the head and neck areas. Early vitiligo lesions showed significantly better response than older lesions with the combined treatment, compared with tacrolimus only.

A number of studies evaluated tacrolimus in the treatment of vitiligo with variable efficacy. In more than one study, around 50% repigmentation of vitiligo patches was achieved after 3 months of treatment with topical tacrolimus twice daily [24,25]. These results were better than the results obtained by tacrolimus only in our study. This may be explained by the fact that in the current study, we only included patients with stable vitiligo, which is known to be resistant to most forms of treatment.

FCO_2 was shown to improve vitiligo in several studies. Hélou *et al.* [11] demonstrated that FCO_2 laser followed by sun exposure improved vitiligo lesions, especially in sunny regions. Shin *et al.* [12] showed that the mean improvement scores were significantly higher for those treated with FCO_2 laser therapy followed by NB-UVB phototherapy, compared with those treated with NB-UVB alone. Moreover, combining FCO_2 monthly sessions with 5FU showed marked improvement in vitiligo lesions when compared with 5FU alone or FCO_2 alone, particularly on the dorsum of fingers [13], and combining ablative FCO_2 with topical betamethasone and NB-UVB led to excellent improvement compared with very minimal effect

with combining them with non-ablative fractional 1565 nm laser [26].

The results of the previous studies endorse the idea of combining ablation with topical drugs to improve and enhance their efficacy, as in this work.

In our study, although the side treated by FCO_2 showed better clinical results, these differences were not statistically significant. This might be attributed to the fact that the lesions included in our study were stable for a relatively long time. In our study, we chose to work on stable lesions to avoid koebnerization, which may occur with the use of FCO_2 in active vitiligo and was reported by Kanokrungsee *et al.* [27].

In the current work, both treatment protocols showed better results in the head and neck areas than other areas of the body. In general, the cephalic region seems to be the best and the most rapid areas to repigment compared with other regions when using different therapeutic modalities [28–30], which may be owing to more exposure of these areas to the sun [11].

Vitiligo lesions on acral areas and joints are considered difficult to treat with a poor response to conventional lines of treatment as well as surgical interventions [31–33]. In the current work, acral lesions showed significantly more improvement in the VASI score when combined treatment was used in comparison with tacrolimus only treatment.

The reason behind the favorable response of the acral lesions to the combination significantly more than to tacrolimus only compared with other body parts may be attributed to the synergistic effect of the FCO_2 facilitating penetration of the tacrolimus in addition to the exposure to the sun. The skin in acral areas is somewhat thicker than the skin in other body parts [34], and it was reported that the thicker the skin, the less the penetration of tacrolimus [35]. As FCO_2 facilitates penetration of tacrolimus ointment in acral skin, hence potentiates its effect on the combination side in contrast to the other side, where the penetration is relatively limited by the thick skin. Sill larger scale studies are needed to verify this finding.In the current work, vitiligo lesions with shorter durations demonstrated better improvement in term of reduction in VASI scores and the number of lesions responding. This comes in concordance with Hallaji *et al.* [36], who reported that early treatment of vitiligo has a better chance of success. Moreover, Yuan *et al.* [26], who performed a study very similar to ours, reported that the combination treatment is effective for progressive vitiligo and has limited effect with stable vitiligo. Our results regarding stable vitiligo improvement though statistically not significant, still better improvement was noted compared with their results, probably owing to the bigger number of patients in the stable group in our study, compared with theirs.

In contrast to our findings, Rokni *et al.* [30] reported that older patients show slower response to tacrolimus. The age of the patients in our study did not influence the results of treatment in both groups.

In conclusion, treatment of stable vitiligo with tacrolimus 0.03% ointment combined with FCO_2 results in only mild improvement of lesions, which is not significantly different from the use of tacrolimus only. The combination, however, gives a significantly better response compared with tacrolimus only in lesions of shorter duration, suggesting that the earlier the start of treatment the better. Moreover, improvement in the acral regions with the combination treatment was significantly better than tacrolimus only, which suggests that using FCO_2 on acral lesions in stable vitiligo gives hope for those patients, yet this observation still needs to be thoroughly investigated on a large-scale study.

Limitations in this study are the short duration of treatment (3 months) as well as the short period of follow-up, in addition to the low concentration of tacrolimus ointment (0.03%) used.

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Conflicts of interest
There are no conflicts of interest.

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