

Fractional Er:YAG laser assisting topical betamethasone solution in combination with NB-UVB for resistant non-segmental vitiligo

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Abstract Resistant non-segmental vitiligo is difficult to be treated. Ablative erbium-YAG (Er:YAG) laser has been used in the treatment of vitiligo, but the ablation of entire epidermis frustrated the compliance of patients. The purpose of this study is to investigate the effects of fractional Er:YAG laser followed by topical betamethasone and narrow band ultraviolet B (NB-UVB) therapy in the treatment of resistant non-segmental vitiligo. The vitiligo lesions of each enrolled patient were divided into four treatment parts, which were all irradiated with NB-UVB. Three parts were, respectively, treated with low, medium, or high energy of Er:YAG laser, followed by topical betamethasone solution application. A control part was spared with laser treatment and topical betamethasone. The treatment period lasted 6 months. The efficacy was assessed by two blinded dermatologists. Treatment protocol with high energy of 1800 mJ/P of fractional Er:YAG laser followed by topical betamethasone solution and in combination with NB-UVB made 60% patients achieve marked to excellent improvement in white patches. The protocol with medium energy of 1200 mJ/P of laser assisted approximate 36% patients achieve such improvement. The two protocols, respectively, showed better efficacies than NB-UVB only

protocol. However, fractional Er:YAG laser at low energy of 600 mJ/P did not provide such contributions to the treatment of vitiligo. The fractional Er:YAG laser in combination with topical betamethasone solution and NB-UVB was suitable for resistant non-segmental vitiligo. The energy of laser was preferred to be set at relatively high level.

Keywords Vitiligo · Er:YAG laser · Fractional

Vitiligo is a common acquired pigmentary disorder of the skin and mucous membranes. It has significant effects on quality of life and remains a persistent burden for the patients [1, 2]. Traditional treatments for vitiligo included topical or systematic medication, photo(chemo)therapy, surgery, camouflage, et al. [3]. However, seldom of them gives satisfactory results in refractory vitiligo. Thus, combination treatments incorporating conventional modalities and new therapeutic approaches are required.

There have been several reports introducing ablative laser-assisted trans-epidermal delivery of topical medicine in the treatment of vitiligo [4–6]. The ablative lasers including carbon dioxide (CO₂) laser and erbium-YAG (Er:YAG) laser were considered superior to mechanical dermabrasion, owing to the controlled ablation [7, 8]. The effects of CO₂ laser or Er:YAG laser ablation followed by topical application of 5-fluorouracil (5-FU) or hydrocortisone 17-butyrate cream combined with narrow band ultraviolet B (NB-UVB) phototherapy or not were, respectively, reported for treating non-segmental vitiligo [9–11]. However, ablation of the entire epidermis frustrated the skin healing process.

In 2004, fractional photothermolysis was introduced by Manstein and colleagues as a new concept for laser skin rejuvenation. Fractional laser do not ablate the entire epidermis and thereafter leave intact skin between coagulated necrotic

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columns [8]. This characteristic facilitates the skin healing process. Recently, fractional CO₂ laser was reported to be used in the treatment of refractory vitiligo followed by NB-UVB or sun exposure [12, 13]. In 2015, we reported a triple combination treatment with fractional CO₂ laser assisting delivery of topical betamethasone solution plus NB-UVB phototherapy for refractory vitiligo and achieved some preliminary and encouraging results [14].

Fractional laser with the Er:YAG laser was introduced as a gentler alternative to the CO₂ laser. Under proper parameter setting, the Er:YAG laser could create less residual thermal damage and faster healing [8, 12]. In the present study, we investigated the effects of fractional Er:YAG laser followed by topical application of betamethasone solution and NB-UVB on resistant non-segmental vitiligo. We provided an alternative choice for the treatment of vitiligo.

Materials and methods

Patients

All procedures performed in the study were in accordance with the ethical standards of the Institutional Medical Ethics and Human Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Patients with more than 1 year of stable, non-segmental and resistant vitiligo were recruited. The resistant vitiligo was defined as poor response to conventional treatments, including topical or systematic medication, phototherapy, or surgery. The age ranged from 18 to 60. Patients who received any treatments within 3 months prior to enrollment or had other dermatological or systemic disorders were excluded. Pregnancy or lactation females were excluded.

Treatment protocols

Vitiligo patches of each patient were equally divided to four parts to receive different treatment protocols. Fractional ablative photothermolysis treatment was carried out using a 2940-nm Er:YAG laser (Pixel, Alma Lasers Ltd., Caesarea, Israel) that incorporates a microlens aligned in a matrix of 9 × 9 (81) dots (pixels), which emits 17 mJ/P per pixel. The single-pass ablation microzone of each pixel measures approximately 150 μm in diameter and 120–140 μm in depth. The pulse widths of the device were preset at short, medium, and long. Three parts of white patches were monthly treated with the fractional 2940 nm Er:YAG laser at energy of 600, 1200, and 1800 mJ/P, respectively. The 600, 1200, and 1800 mJ/P in the pulse referred to the energy over the “entire larger spot” and not the “micro spot” (17 mJ/P). Two stacked laser passes were performed and the pulse

width was set at medium. Immediately after the laser treatment, compound betamethasone solution (Schering Pharmaceutical Company Limited, Shanghai, China) was topically applied under occlusion with a plastic film for 30 min. One part, as a positive control, was spared with laser treatment and topical betamethasone. A NB-UVB instrument (SS03, Shanghai Sigma High-tech Co., Ltd., Shanghai, China) was used for phototherapy to all the four parts according to previous description [14]. Briefly, the phototherapies were performed twice to thrice a week, beginning at a dose of 100–150 mJ/cm² and elevating by approximately 10–15% each time (up to a maximum of 1500 mJ/cm²). A summary of the treatment was given in Table 1. The total period of treatment was 6 months.

Measurement

Standard photos were taken with a digital camera (D40S, Nikon Corporation, Tokyo, Japan) at baseline (M0) and 1 month after each laser treatment. Three months post-treatment (M3) and 6 months post-treatment (M6), the response to the treatment was assessed by two blinded dermatologists. Depending on the extent of re-pigmentation, the response to the treatment was graded as: no or minimal (<25%), moderate (25–49%), marked (50–74%), excellent (≥75%) [12, 13]. Any side effect was recorded during the procedure.

Statistic analysis

Data were analyzed using SPSS for software (version 20.0, SPSS Inc., Chicago, IL, USA). Comparisons were performed by Kruskal-Wallis Test or Mann-Whitney analysis. A *P* value <0.05 was considered as statistically significant.

Results

Patients

A total of 22 patients completed the study, including 10 men (45%) and 12 women (55%). The mean age was 48 years (range 21–60). Thirty-five paired vitiligo lesions were involved in different body sites: 6 on the neck, 10 on the trunk, 11 on the anterior region of leg, and 8 on the hands or feet. Figures 1, 2, and 3 showed the representative photographs of the patients pre- and post-treatments. Table 2 presents the patients' responses to different treatment protocols.

Table 1 Treatment description of each protocol

	Protocol 1	Protocol 2	Protocol 3	Protocol 4
Fractional Er:YAG laser	–	low energy (600 mJ)	medium energy (1200 mJ)	high energy (1800 mJ)
Betamethasone solution	–	+	+	+
NB-UVB	+	+	+	+

The overall comparison of four protocols

The four protocols produced significantly different responses to the treatments, at whatever M3 ($\chi^2 = 19.501$, $P < 0.001$) or M6 ($\chi^2 = 19.716$, $P < 0.001$). No significant difference was seen between protocol 2 and protocol 1 at either M3 ($P = 0.300$) or M6 ($P = 0.196$). The overall response to therapy of protocol 3 was significantly better than that of protocol 1 (M3, $\chi^2 = 6.689$, $P = 0.010$; M6, $\chi^2 = 6.142$, $P = 0.013$). The protocol 4 was also better than protocol 1 (M3, $\chi^2 = 14.710$, $P < 0.001$; M6, $\chi^2 = 16.106$, $P < 0.001$). No difference was seen between protocol 3 and protocol 4 at M3 ($P = 0.113$) or M6 ($P = 0.078$).

The comparison of M6 with M3

The response of M6 was significantly better than that of M3 treated by protocol 2 ($Z = 2.084$, $P = 0.037$), protocol 3

($Z = 1.976$, $P = 0.048$), or protocol 4 ($Z = 3.014$, $P = 0.003$). The results of protocol 1 at M6 and at M3 showed no statistical difference ($P = 0.079$).

The various responses to four protocols at different sites

Different sites, including the neck, trunk, and the anterior region of leg and hands/feet, showed various responses to the four protocols. Table 3 showed the detailed statistic results.

On the trunk and the anterior region of leg, four protocols produced significant different responses at whatever M3 or M6 ($P < 0.05$). Protocol 3 and protocol 4 showed better efficacy than protocol 1, respectively ($P < 0.05$). However, no significant difference was seen between protocol 2 and protocol 1 ($P > 0.05$).

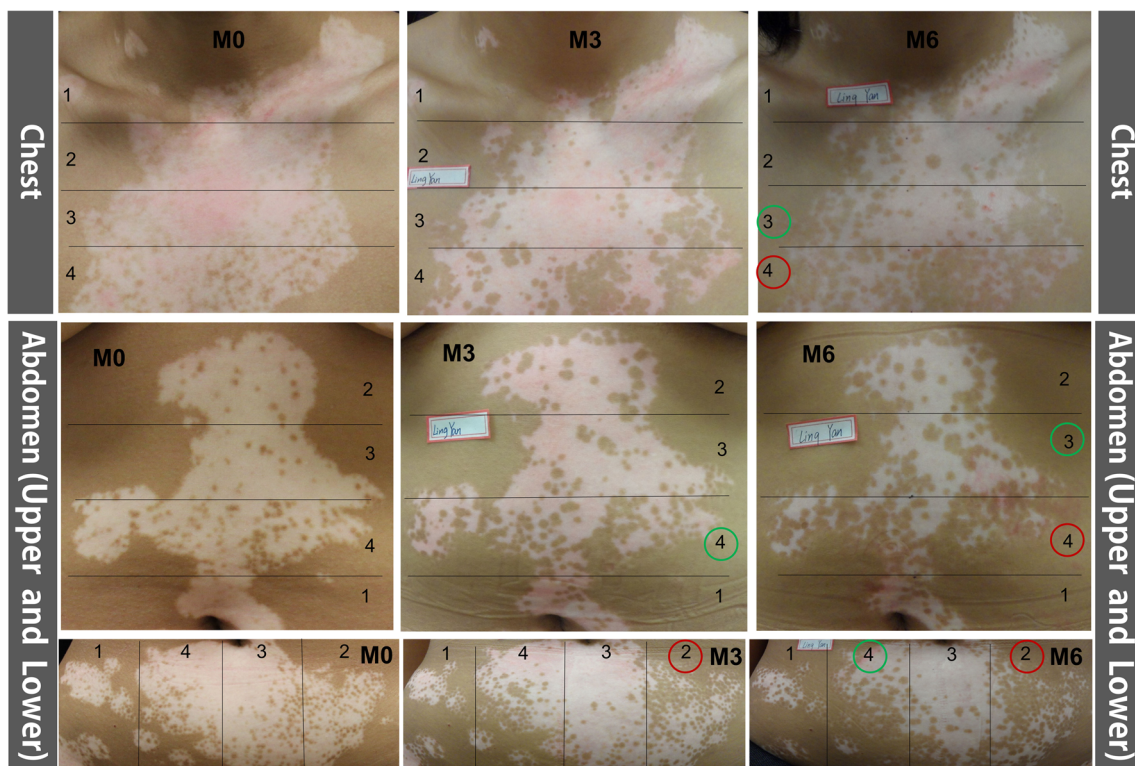


Fig. 1 The pre- and post-treatment photographs on the chest and the abdomen of a 36-year-old female vitiligo patient. Note: M0, pre-treatment; M3, 3 months post-treatment; M6, 6 months post-treatment; the Arabic numerals 1, 2, 3, and 4 were referred to as protocols 1, 2, 3, and

4, respectively. Red circle of the Arabic numeral meant excellent ($\geq 75\%$) response to treatment; green circle of the Arabic numeral meant marked (50–74%) response to treatment

Fig. 2 The pre- and post-treatment photographs on the hands and the anterior regions of two legs of a 42-year-old female vitiligo patient. Note: *M0*, pre-treatment; *M3*, 3 months post-treatment; *M6*, 6 months post-treatment; the Arabic numerals 1, 2, 3, and 4 were referred to as protocols 1, 2, 3, and 4, respectively. *Red circle* of the Arabic numeral meant excellent ($\geq 75\%$) response to treatment; *green circle* of the Arabic numeral meant marked (50–74%) response to treatment

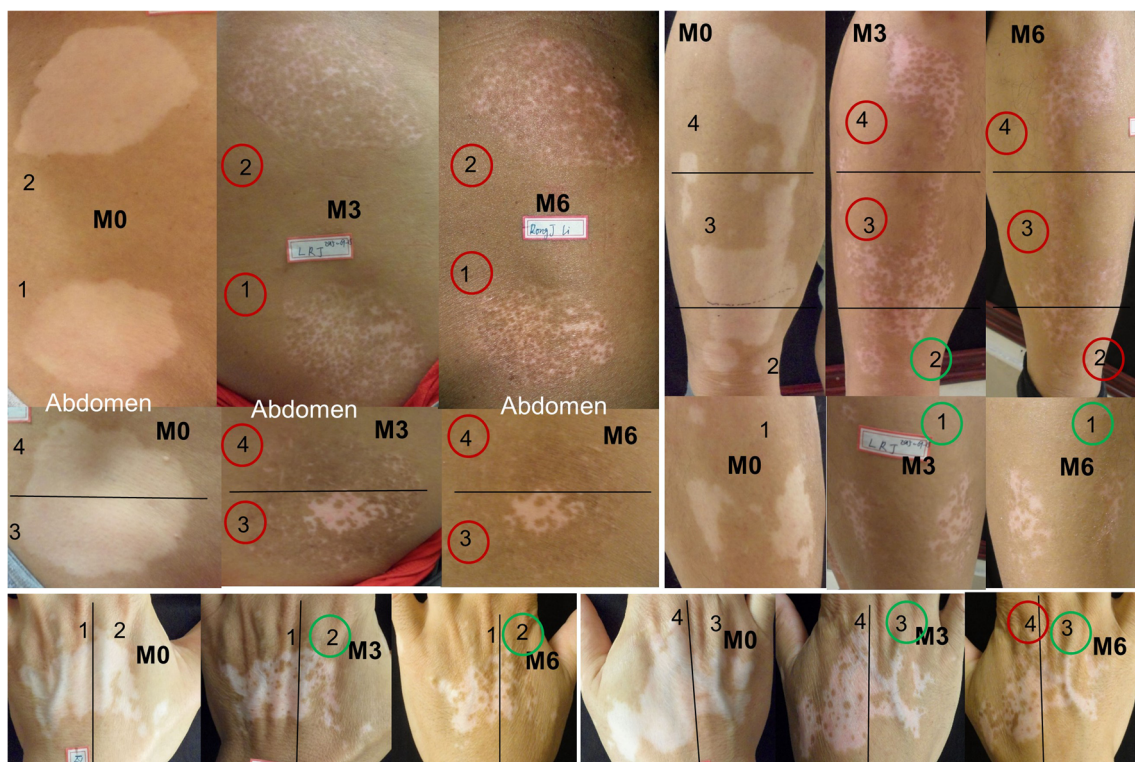
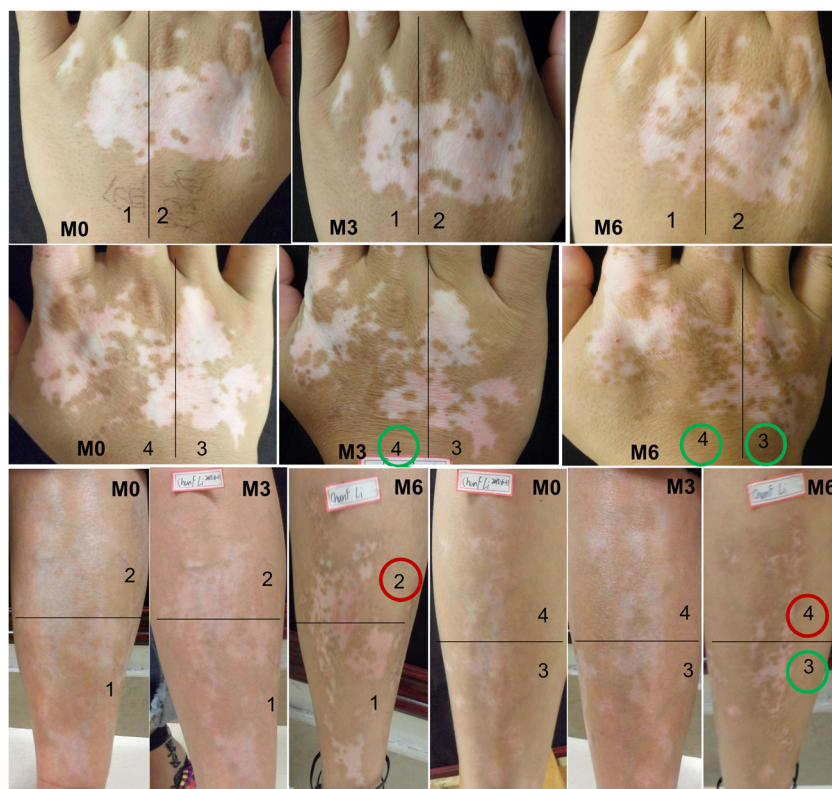


Fig. 3 The pre- and post-treatment photographs on the abdomen, the anterior regions of two legs and the hands of a 60-year-old male vitiligo patient. Note: *M0*, pre-treatment; *M3*, 3 months post-treatment; *M6*, 6 months post-treatment; the Arabic numerals 1, 2, 3, and 4 were referred

to as protocols 1, 2, 3, and 4, respectively. *Red circle* of the Arabic numeral meant excellent ($\geq 75\%$) response to treatment; *green circle* of the Arabic numeral meant marked (50–74%) response to treatment

Table 2 The patients' responses to different treatment protocols (*n* (%))

	Protocol 1	Protocol 2	Protocol 3	Protocol 4
Three months post-treatment				
No or minimal (<25%)	21 (95.45%)	19 (86.36%)	14 (63.64%)	9 (40.91%)
Moderate (25–49%)	1 (4.55%)	3 (13.64%)	8 (36.36%)	12 (54.54%)
Marked (50–74%)	0 (0%)	0 (0%)	0 (0%)	1 (4.55%)
Excellent (≥75%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Six months post-treatment				
No or minimal (<25%)	17 (77.27%)	13 (59.09%)	10 (45.45%)	5 (22.73%)
Moderate (25–49%)	4 (18.18%)	7 (31.81%)	4 (18.18%)	4 (18.18%)
Marked (50–74%)	1 (4.55%)	1 (4.55%)	6 (27.27%)	8 (36.36%)
Excellent (≥75%)	0 (0%)	1 (4.55%)	2 (9.09%)	5 (22.73%)

On both the neck or hands/feet portion, however, no difference was seen among the four protocols at whatever M3 or M6 ($P > 0.05$).

Safety evaluation

No patients developed noticeable side effects, including local infection, scarring, Koebner phenomenon, and aggravation of vitiligo. All patients experienced slight pain and burning sensation during the laser treatment, as well as erythema and slight edema after laser treatment. The symptoms were tolerable and relieved within a day. About 50% patients developed micro-crust on the area with high energy (1800 mJ/P) of laser treatment, and disappeared within 3–5 days.

Discussion

In recent years, various combination treatments have been attempted in an effort to enhance efficacy in patients with resistant vitiligo [5]. New treatment

approaches such as using ablative lasers followed by topical application of 5-FU or steroids combined with NB-UVB phototherapy were studied and increased the repigmentation rate of vitiligo [9, 10]. Despite their promising effects of the ablative lasers, ablation of the entire epidermis raises difficulties in the regulation of resurfacing depth and wound care, and involves a high risk of potential scars. In 2004, fractional laser therapy was developed to reduce the side effects of total ablative lasers. Recently, the fractional CO₂ laser followed by topical steroids combined with NB-UVB or sun exposure has been proved to be effective in the treatment of refractory vitiligo [12, 13]. In 2015, we also reported a triple combination treatment with fractional CO₂ laser assisting delivery of topical betamethasone solution plus NB-UVB phototherapy for refractory vitiligo and achieved some encouraging results [14]. Till now, another type of fractional ablative laser, i.e., fractional Er:YAG laser has not been investigated in the treatment of vitiligo.

In this study, the protocols treating with high energy (1800 mJ/P) or medium energy (1200 mJ/P) of fractional

Table 3 The comparison of the four protocols at different sites

	Neck		Trunk		Anterior region of leg		Hands/feet	
	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>	χ^2	<i>P</i>
Three months post-treatment								
Overall comparison	2.332	0.506	15.239	0.002*	14.587	0.002*	0.969	0.809
Protocol 2 vs. protocol 1	0.314	0.575	3.736	0.053	1.167	0.280	0.292	0.589
Protocol 3 vs. protocol 1	0.815	0.367	7.246	0.007*	5.091	0.024*	0.292	0.589
Protocol 4 vs. protocol 1	1.956	0.162	12.095	0.001*	11.094	0.001*	0.937	0.333
Six months post-treatment								
Overall comparison	6.112	0.106	10.543	0.014*	11.580	0.009*	2.705	0.439
Protocol 2 vs. protocol 1	0.399	0.527	2.609	0.106	0.524	0.469	0.380	0.538
Protocol 3 vs. protocol 1	2.842	0.092	5.417	0.020*	6.056	0.014*	1.221	0.269
protocol 4 vs. protocol 1	4.694	0.030*	8.205	0.004*	7.429	0.006*	2.368	0.124

* $P < 0.05$

Er:YAG laser produced significantly better responses comparing to the protocols treating with low energy (600 mJ/P) of laser or NB-UVB only. At M3, at least 25% re-pigmentation was, respectively, seen on approximate 36 and 60% patients under the former protocols. At M6, the two protocols enabled about 55 and 77% patients achieve such re-pigmentation, and about 36 and 60% patients achieve at least 50% re-pigmentation. The results indicated that fractional Er:YAG laser did provide additional contribution to the treatment of vitiligo, but the energy should be set at the level of at least 1200 mJ. Although no significant difference was seen between medium energy protocol and high energy protocol, the *P* value of 0.078 at M6 almost approached the statistic difference. It implied that fractional Er:YAG laser at the high energy of 1800 mJ may produce better efficacy than the medium one. Increasing the patients' sample size may arise such results. The results of M6 were better than those of M3, suggesting a sufficient treatment period should be no less than 6 months.

Re-pigmentation in vitiligo is known to be initiated by activation, proliferation, and migration of melanoblasts from the outer root sheath of hair follicles or melanocytes from the border area of vitiligo lesions into the depigmented epidermis [15]. The mechanisms of re-pigmentation induced by our triple combination treatment protocol may be explained as follows: (1) the fractional Er:YAG laser treatment, which is performed over the vitiligo lesion, allows for resettlement of migrating melanocytes [4]; (2) the topical corticosteroid used here contains betamethasone disodiumphosphate (fast-acting) and betamethasone dipropionate (slow-acting) [14]. Aiding by the fractional Er:YAG laser, the compound formulation is expected to induce both rapid and lasting re-pigmentation responses [14]. (3) Matrix metalloproteinase-2 (MMP-2) is known to enhance migration of melanocytes from adjacent normal skin. Previous study showed that NB-UVB irradiation could significantly increase the activity of MMP-2 in melanocytes [16]. Dermabrasion using fractional Er:YAG laser followed by NB-UVB irradiation would enhance the stimulation of MMP-2 activity in vitiligo lesion.

In 2012, Bayoumi et al. used Er:YAG laser dermabrasion followed by NB-UVB and hydrocortisone 17-butyrate cream therapy to treat vitiligo for 3 months, and at least 50% re-pigmentation improvement was seen in 46% of lesions [10]. In our stud, using high energy of fractional Er:YAG laser, although such degree of re-pigmentation was only seen in 4.55% patients at M3, it was seen in almost 60% patients at M6. Despite the quick response and high rate of re-pigmentation of total ablative Er:YAG laser, the pain during the procedure and the slow healing process strongly impaired the tolerance and limited its use in current practice. In our study, no patients developed

noticeable side effects, and all the patients were willing to continue the laser treatment with no need of anesthetic. Theoretically, fractional Er:YAG laser has the same mechanisms of re-pigmentation as ablative lasers, but can minimize the side effects.

In 2015, we reported a triple combination treatment with fractional CO₂ laser assisting delivery of topical betamethasone solution plus NB-UVB phototherapy for refractory vitiligo [14]. In that study, over 50% re-pigmentation was seen on 40% patients at M3 and 44% at M6. The results of the two time points were similar, indicating that three-month treatment was sufficient to induce the re-pigmentation response and continuous treatment to 6 months just maintained the efficacy. In our study using high energy laser, the patients who achieved at least 50% re-pigmentation significantly increased from 4.55% at M3 to about 60% at M6. Comparing to fractional CO₂ laser, fractional Er:YAG laser was a gentler and "cooler" one. So the laser induced-response was relatively slower than the fractional CO₂ laser, but continuous treatment to 6 months induced a comparative response. Although there were limitations in the present study, including the lack of a betamethasone-only control and a laser-only control, we did find that fractional Er:YAG laser may contribute to the treatment of vitiligo. Combination treatment with fractional Er:YAG laser plus topical betamethasone solution and NB-UVB therapy is suitable for resistant non-segmental vitiligo. The energy of laser is preferred to be set at relatively high level. More studies with large sample size are required to verify the results.

Compliance with ethical standards

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

Informed consent Informed consent was obtained from all individual participants included in the study.

Ethical approval All procedures performed in the study were in accordance with the ethical standards of the Institutional Medical Ethics and Human Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Ethical responsibilities of authors The manuscript has not been submitted to more than one journal for simultaneous consideration. The manuscript has not been published previously (partly or in full). A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time.

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