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ORIGINAL CONTRIBUTION

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Combined treatment with fractional carbon dioxide laser, autologous platelet-rich plasma, and narrow band ultraviolet B for vitiligo in different body sites: A prospective, randomized comparative trial

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Summary

Background: Multiple treatment options are introduced in treatment of vitiligo but the response is unsatisfactory.

Objective: In this prospective, randomized, comparative trial, we studied the effect of combined treatment with fractional carbon dioxide (CO_2) laser, platelet-rich plasma (PRP) injection, and narrowband ultraviolet B (NB-UVB) for stable nonsegmental vitiligo regarding repigmentation grade, patient's satisfaction, and side effects.

Methods: Eighty adult patients with localized nonsegmental vitiligo were enrolled in this study. The patients were randomly categorized to receive 4 lines of treatment; fractional CO_2 laser, PRP, combined fractional CO_2 laser and PRP, and combined fractional CO_2 laser and NB-UVB. The treatment period was 2 months. Patients were clinically evaluated 3 months after the last treatment. Outcome was evaluated by 5-point scale for repigmentation, 10-point visual analog scale for patient's satisfaction, and side effects.

Results: Laser and PRP group achieved the best results regarding repigmentation and patient's satisfaction. Sixty percent of the patients developed repigmentation >50% and 40% of patients developed repigmentation >75%. In laser and NB-UVB group, 5% developed repigmentation >75% and 25% developed repigmentation >50%. Only 10% of patients developed repigmentation >75% in laser group and only 20% of patients developed repigmentation >75% in PRP group.

Conclusions: Combination of fractional CO_2 laser with PRP injection is a promising treatment for vitiligo, followed by combination of fractional CO_2 laser with NB-UVB phototherapy. Both fractional CO_2 laser and PRP injection gave poor results if they received alone.

KEYWORDS
laser, platelet-rich plasma, vitiligo

1 | INTRODUCTION

Vitiligo is an acquired disorder of pigmentation that occurs due to loss of epidermal melanocytes and presented clinically by depigmented macules and patches.[1] It affects about 0.5%-1% of the population but its prevalence has geographic variation.[2] Vitiligo seriously impacts the quality of life specially if the lesion affects sites of cosmetic concern, for example, face and extremities.[3,4] Multiple treatment modalities are established but the response is variable, unsatisfactory, and requiring a prolonged course.[1] This problem is

exaggerated by the multifactorial and polygenic nature of the pathomechanism of the disease.[5] These facts pave the way to combination therapy that showed better repigmentation response than monotherapy.[6] There are multiple clinical trials that prove the benefits of adding fractional CO_2 laser (10 600 nm) in treatment of vitiligo. It is hypothesized that fractional CO_2 laser can stimulate migration of melanocytes and differentiation of melanocyte stem cells.[7-9] Narrowband ultraviolet B (NB-UVB) phototherapy (with a peak emission range of 311-313 nm) has been introduced since 1997, and become the first-line treatment of generalized vitiligo, with multiple studies establishing demonstrating its efficacy.[10-12] Prolonged course of UVB phototherapy leads to poor patient compliance.[13,14] Also, presence of extremely resistant lesions as acral lesions and those located over the bony prominence, for example, knees and elbows,[15] adversely impact the success of UVB phototherapy. Platelet-rich plasma (PRP) is an autologous preparation of platelets in concentrated plasma that is characterized by the presence of several growth factors.[16] These growth factors are known to regulate many processes including cell migration, attachment, proliferation, and differentiation.[17] Accordingly, we investigated the safety and efficacy of combined treatment with fractional CO_2 laser, autologous PRP, and NB-UVB for stable nonsegmental vitiligo as a prospective randomized comparative study.

2 | MATERIALS AND METHODS

This prospective, randomized, parallel group, comparative study was approved by the research ethics committee of Al-Azhar University Hospitals. Eighty adult patients with localized nonsegmental vitiligo and Fitzpatrick skin type III-IV were enrolled in this study. They were recruited from the Outpatient Clinic of Dermatology and Venereology Department, Al Zahraa University Hospital, Faculty of medicine for girls, Al-Azhar University, Cairo, Egypt, during the period from January 2016 to January 2017. Patients with history of photosensitive conditions, keloid or hypertrophic scar, and Koebner's phenomenon on laser-treated areas were excluded from the study. Pregnant and lactating females and those with history of skin cancer and bleeding tendency were also excluded. Patients were excluded if they receive any topical medications, phototherapy, or laser for vitiligo within 6 months prior to enrollment. The treated patients had stable lesions; the absence of new lesions or enlargement of the already present lesions for 12 months.

The patients were randomly categorized into 4 groups; each group was composed of 20 patients, and the treated sites were also categorized into 4 categories; face, neck, trunk, acral, and extremities.

2.1 | Group 1: Laser group

Patients received 4 sessions of fractional CO_2 laser (DEKA, SmartXide DOT, Italy), with 2-week interval. Topical anesthetic cream (Pridocaine®; a mixture of Lidocaine 25% and Prilocaine 25%) was applied under occlusion 30 minutes before the session. Fractional CO_2 laser was performed over the vitiligo lesion and over the perilesional apparently healthy skin of about 5 cm around the lesion. The treatment settings were power 6 Watt, dot mode with spacing 550 μm, dwell time 400 μs, scanning mode, smart track, single stack, square shape, ratio 10/10, and size 100%. Theses parameters are equivalent to fluence 0.3 J/cm, density 11.9%, and energy/dot 2.4 mJ. Patients were advised to apply only emollients twice per day after sessions.

2.2 | Group 2: PRP group

Patients received 4 sessions of autologous intradermal PRP injection with 3-week interval. Injection was lesional and 5 cm perilesional, 0.1 mL/injection, 0.5 cm spacing between injection sites, with maximum of 1 mL/session. Topical anesthetic cream (Pridocaine®) was applied under occlusion 30 minutes before the session. Injection was carried out using 30 gauge needle.

2.3 | PRP preparation method

Ten to 20 mL of venous blood was withdrawn from the anticubital vein under complete aseptic conditions. The whole blood sample was collected in tubes containing sodium citrate as an anticoagulant (sodium citrate 9NC, VACO MED, containing sodium citrate 3.2% as anticoagulant). Then, the citrated whole blood was subjected to the double spin method. The first centrifugation was slow to avoid spinning down of the platelets and to isolate plasma. The centrifugation was at 252 *g* for 10 minutes. Platelets are mostly concentrated on top of the buffy coat layer. The supernatant plasma was withdrawn and re-centrifuged. The subsequent centrifugation was faster at 448 *g* for 10 minutes, so that platelets were spun down and separated as a pellet at the bottom of the tube from platelet-poor plasma (PPP) above. The final platelet concentration depends on the volume reduction of PPP. Approximately 3/4 of the supernatant is discarded and the platelet-rich pellet is re-suspended in the remaining amount of plasma.[18] The resultant plasma was subsequently aspirated and prepared for activation by calcium chloride ($CaCl_2$) in the proportion of 0.1 mL of $CaCl_2$ per 0.9 mL of PRP, thus obtaining a concentration of activated PRP. This method of preparation was chosen on the basis that double-centrifugation protocol using the correct g-forces and spin times results in higher platelet concentrations than the single centrifugation protocol.[19]

2.4 | Group 3: Laser and PRP group

Patients received 4 sessions of fractional CO_2 laser with 2-week interval using the same laser settings as group 1. One week after each laser session, the patients received intradermal injection of autologous PRP using the same injection protocol as group 2. Total PRP sessions was 4.

2.5 | Group 4: Laser and NB-UVB group

Patients received 4 sessions of fractional CO_2 laser with 2-week interval using the same laser settings as group 1 and group 3. One week after each laser session, the patients received NB-UVB phototherapy sessions twice per week for a maximum of 2 months. Total NB-UVB sessions was 8. The NB-UVB source was 8 NB fluorescent tubes (Philips TL 100, Hamburg, Germany) with a spectrum of 310-315 nm and a maximum wave length of 311 nm installed in a Waldmann UV-100 unit. The initial dose of UVB was 130 mJ/cm^2 with subsequent 15% increment of the previous dose in the following sessions. If the patient developed erythema persisted for more than 48 hours, we decreased the dose to the highest dose that did not produce persistent erythema.

2.6 | Evaluation of the treatment

Photographs were obtained at baseline, before each treatment session, and 3 months after the final treatment. Objective clinical assessments of repigmentation were performed by 2 blinded dermatologists using a 5-point scale; grade 0 (no repigmentation), grade 1 (1%-5%), grade 2 (6%-25%), grade 3 (26%-50%), grade 4 (51%-75%), grade 5 (76%-100%). Patients satisfaction was performed 3 months after the last treatment using a 10-point visual analog scale (VAS, 0-10; the 0 level was defined as "Not satisfied at all," while a level of 10 was defined as "completely satisfied"). Patients were also asked about any side effects as erythema, itching, burning sensation, and ecchymosis.

2.7 | Statistical analysis

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 performed using Mann-Whitney *U* test for independent samples for comparing 2 groups and Kruskal-Wallis test in comparing more than 2 groups. For comparing categorical data, chi-square (χ^2) test was performed. Exact test was used instead when the expected frequency is <5. *P* values <.05 was considered statistically significant. All statistical calculations were performed using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

3 | RESULTS

This prospective, randomized, parallel group, comparative study included a total of 80 patients with localized, stable, and nonsegmental vitiligo on different body sites. Basic data of the patients are summarized in Table 1.

Distribution of the treated sites were as follows; 21 patients (26.3%) had treatment for facial lesions, 21 patients (26.3%) had

TABLE 1 Basic data of the patients

Group	Age		Skin phototype		Disease duration		Size of the lesion	
	Mean ± SD	Female	Male	III	IV	<2 y	>2 y	<5 cm	>5 cm
Laser group	29.60 ± 10.802	*n* = 14, 70.0%	*n* = 6, 30.0%	*n* = 8, 40.0%	*n* = 12, 60.0%	*n* = 8, 40.0%	*n* = 12, 60.0%	*n* = 8, 40.0%	*n* = 12, 60.0%
PRP group	34.90 ± 15.386	*n* = 11, 55.0%	*n* = 9, 45.0%	*n* = 10, 50.0%	*n* = 10, 50.0%	*n* = 10, 50.0%	*n* = 10, 50.0%	*n* = 9, 45.0%	*n* = 11, 55.0%
Laser and PRP group	33.90 ± 11.889	*n* = 12, 60.0%	*n* = 8, 40.0%	*n* = 2, 10.0%	*n* = 18, 90.0%	*n* = 8, 40.0%	*n* = 12, 60.0%	*n* = 8, 40.0%	*n* = 12, 60.0%
Laser and NB-UVB group	36.95 ± 13.044	*n* = 13, 65.0%	*n* = 7, 35.0%	*n* = 9, 45.0%	*n* = 11, 55.0%	*n* = 8, 40.0%	*n* = 12, 60.0%	*n* = 16, 80.0%	*n* = 4, 20.0%

treatment for lesions over extremities, 15 patients (18.8%) had treatment for lesions over the trunk, 12 patients (15%) had treatment for acral lesions, and 11 patients (13.8%) had treatment for lesions over the neck. Twenty-one patients (26.3%) developed perifollicular repigmentation, 19 patients (23.8%) developed diffuse repigmentation, 18 patients (22.5%) developed marginal repigmentation, 5 patients (6.3%) developed perifollicular and diffuse repigmentation, 2 patients (2.5%) developed marginal and diffuse repigmentation, while the nonresponders were 15 patients (18.8%).

Evaluation of repigmentation and VAS in each group was as follows: In laser and PRP group, the mean of repigmentation was 4.40 ± 0.503 SD and the mean of VAS was 8.20 ± 0.616 SD (range, 8-10). Of total 20 patients in this group, 8 patients (40%) developed repigmentation of more than 75% (repigmentation grade 5) and 12 patients (60%) developed repigmentation of more than 50% (repigmentation grade 4). The best response developed in lesions over the trunk followed by facial lesions then lesions over extremities. Acral and neck lesions came in the last. The mean of repigmentation and the mean of VAS in each treated site are summarized in Table 2.

In laser and NB-UVB group, the mean of repigmentation was 2.6 ± 1.635 SD and the mean of VAS was 5.65 ± 3.422 SD (rang, 0-10). Of total 20 patients in this group, 1 patient (5%) developed repigmentation of more than 75% (repigmentation grade 5), 5 patients (25%) developed repigmentation of more than 50% (repigmentation grade 4), 9 patients (45%) developed repigmentation of more than 25% (repigmentation grade 3), and 5 patients (25%) did not respond to the treatment. The best response developed in lesions over the neck followed by lesions over extremities, then truncal lesions and facial lesions. Acral lesions came in the last with no response. The mean of repigmentation and the mean of VAS in each treated site are summarized in Table 3.

Response in laser group was as follows: the mean of repigmentation was 1.9 ± 1.334 SD and the mean of VAS was 4.5 ± 2.763 SD (range, 0-10). Of total 20 patients in this group, only 2 patients (10%) developed repigmentation of more than 75% (repigmentation grade 5), 14 patients (70%) developed repigmentation of more than 5% (repigmentation grade 2), while the nonresponders were 4 patients (20%). The best response developed in lesions over the extremities followed by facial, truncal, and neck lesions. Acral lesions came in the last with no response. The mean of repigmentation and the mean of VAS in each treated site are summarized in Table 4.

Regarding PRP group, the mean of repigmentation was 1.5 ± 1.850 SD and the mean of VAS was 3.85 ± 3.675 SD (range, 0-10). Of total 20 patients in this group, only 4 patients (20%) developed repigmentation of more than 75% (repigmentation grade 5), 10 patients (50%) developed repigmentation of <5% (repigmentation grade 1), while the nonresponders were 6 patients (30%). The best response developed in lesions over the trunk followed by lesions over extremities, then neck lesions. Acral and facial lesions came in the last with no response. The mean of repigmentation and the mean of VAS in each treated site are summarized in Table 5.

The mean ranking of repigmentation between the 4 groups was 63.40 in laser and PRP group, 39.70 in laser and NB-UVB group, 31.65 in laser group, and 27.25 in PRP group. Also, the mean ranking of VAS between the 4 groups was 61.60 in laser and PRP group, 40.73 in laser and NB-UVB group, 31.70 in laser group, and 27.98 in PRP group. There was a statistically difference in repigmentation grade and VAS among the 4 groups (*P* value = .000; Kruskal-Wallis test).

Photographic examples of repigmentation response in patients within each group are demonstrated in Figures 1-4.

TABLE 2 The mean of repigmentation and the mean of VAS in each treated site in laser and PRP group

	Treated site	VAS	Repigmentation grade
Acral (*n* = 3)	Mean ± SD	8.00 ± 0.000	4.00 ± 0.000
	Mean rank*	9.50	6.5
Extremities (*n* = 7)	Mean ± SD	8.00 ± 4.183	4.29 ± 0.488
	Mean rank*	9.50	9.36
Face (*n* = 6)	Mean ± SD	8.00 ± 0.000	4.67 ± 0.516
	Mean rank*	9.50	13.17
Neck (*n* = 2)	Mean ± SD	8.00 ± 0.000	4.00 ± 0.000
	Mean rank*	9.50	6.50
Trunk (*n* = 2)	Mean ± SD	10.00 ± 0.000	5.00 ± 0.000
	Mean rank*	19.50	16.50
Total (*n* = 20)	Mean ± SD	8.20 ± 0.616	4.40 ± 0.503
Chi-square		19.000	8.067
df		4	4
P value		.001†	.089

†Kruskal-Wallis test.
*P value < .05.

TABLE 3 The mean of repigmentation and the mean of VAS in each treated site in laser and NB-UVB group

Treated site		VAS	Repigmentation grade
Acral (*n* = 3)	Mean ± SD	0.00 ± 0.000	0.00 ± 0.000
	Mean rank*	3.00	3.00
Extremities (*n* = 3)	Mean ± SD	5.33 ± 4.619	2.67 ± 2.309
	Mean rank*	12.33	12.33
Face (*n* = 7)	Mean ± SD	7.00 ± 0.000	3.00 ± 0.000
	Mean rank*	10.00	10.00
Neck (*n* = 2)	Mean ± SD	8.00 ± 0.000	4.00 ± 0.000
	Mean rank*	17.00	17.00
Trunk (*n* = 5)	Mean ± SD	6.40 ± 3.782	3.00 ±1.871
	Mean rank*	12.00	12.00
Total (*n* = 20)	Mean SD	5.56 ± 3.422	2.60 ± 1.635
Chi-square		8.975	8.975
df		4	4
P value		.062	.062

*Kruskal-Wallis test.

TABLE 4 The mean of repigmentation and the mean of VAS in each treated site in laser group

Treated site		VAS	Repigmentation grade
Acral (n = 3)	Mean ± SD	0.00 ± 0.000	0.00 ± 0.000
	Mean rank†	2.50	2.5
Extremities (n = 5)	Mean ± SD	6.00 ± 4.183	2.80 ± 2.168
	Mean rank†	12.90	12.90
Face (n = 6)	Mean ± SD	5.00 ± 0.000	2.00 ± 0.000
	Mean rank†	11.50	11.50
Neck (n = 2)	Mean ± SD	5.00 ± 0.000	2.00 ± 0.000
	Mean rank†	11.50	11.50
Trunk (n = 4)	Mean ± SD	5.00 ± 0.000	2.00 ± 0.000
	Mean rank†	11.50	11.50
Total (n = 20)	Mean ± SD	4.50 ± 2.763	1.90 ± 1.334
Chi-square		10.239	10.239
df		4	4
P value		.037*	.037*

†Kruskal-Wallis test.
*P value < .05.

TABLE 5 The mean of both repigmentation and VAS in each treated site in PRP group

Treated site		VAS	Repigmentation grade
Acral (n = 3)	Mean ± SD	0.00 ± 0.000	0.00 ± 0.000
	Mean rank†	4.00	3.5
Extremities (n = 6)	Mean ± SD	5.17 ± 2.401	1.67 ± 1.633
	Mean rank†	13.42	12.67
Face (n = 2)	Mean ± SD	0.00 ± 0.000	0.00 ± 0.000
	Mean rank†	4.00	3.50
Neck (n = 5)	Mean ± SD	3.60 ± 4.099	1.60 ± 1.949
	Mean rank†	9.90	11.50
Trunk (n = 4)	Mean ± SD	7.00 ± 3.484	3.00 ± 2.309
	Mean rank†	15.00	15.00
Total (n = 20)	Mean ± SD	3.85 ± 3.675	1.50 ± 1.850
Chi-squared		11.113	12.125
df		4	4
P value		.025*	.016*

†Kruskal-Wallis test.
*P value < .05.

No side effects were reported except for erythema that occurred after laser and NB-UVB phototherapy. Erythema resolved spontaneously within 24 hours post-treatment.

4 | DISCUSSION

Lasers are novel therapeutic modality in treatment of vitiligo. Multiple studies investigated the beneficial effect of ablative Erbium:YAG (2940 nm) laser in treatment of vitiligo.[20,21] Inspite of its successful results, Er:YAG resurfacing has many obstacles as difficulty in regulation of the resurfacing depth and wound care, and possibility of scars due to excessive skin injury.

Therefore, fractional CO_2 laser was evaluated in combination with other treatment, for example, NB-UVB phototherapy, sun exposure, topical corticosteroids, or salicylic acid in order to enhance its efficacy and avoid the side effects of Er:YAG laser resurfacing.[7-9,22,23] In this prospective comparative study, we aimed to investigate the safety and efficacy of combined treatment with fractional carbon dioxide laser, autologous PRP injection, and narrow band ultraviolet B for stable nonsegmental vitiligo.

According to this study, combination of fractional CO_2 laser and intradermal injection of autologous PRP has superior and promising results, as 60% of the patients achieved repigmentation of more than 50% and 40% of the patients achieved repigmentation of more than 75% with VAS ranged from 8 to 10 and no reported side effects. We thought that the effect of fractional CO_2 laser was due to release of cytokines during inflammation and wound healing process, and these cytokines stimulate proliferation and migration of melanocytes from the perilesional skin. Also, matrix metalloproteinase-2 is released and postulated to stimulate melanocyte stem cells migration from perilesional skin, hair bulb, and outer root sheath.[7-9,22,23]

In addition, the beneficial effect of autologous PRP in repigmentation is hypothesized to be due the presence of multiple growth factors, for example, platelets-derived growth factor, epidermal growth factors, basic fibroblast growth factor, and matrix metalloproteinase-2 that bind to the transmembrane receptors of the target cells leading to activation of intracellular signal proteins and expression of gene sequence that results in cellular proliferation and new matrix formation, new collagen formation, or epidermal cell proliferation.[24,25] We suggested that this mechanism could also occur with keratinocytes and fibroblasts in vitilagenous lesions and perilesional skin leading to improvement of their interaction with melanocytes and ensuring melanocytes stabilization. It was found that PRP has anti-inflammatory effect that suppress release of cytokines as interleukin-1, interferon-γ, and tumor necrosis factor-α which have a great role in pathogenesis of vitilig.[26-30] We found that trunk followed by facial lesions then lesions over extremities showed better response than acral and neck lesions.

We decided to treat lesional and 5-cm perilesional areas because Brazelli et al,[31] confirmed that apparently normal perilesional skin in vitiligo is lighter than normal skin as far as 5 cm from the vitiligo spot. To the best of our Knowledge, this study is the first one investigating the efficacy and safety of combining fractional CO_2 laser and intrademal injection of autologous PRP in stable, nonsegmental vitiligo, Also, There were no reported trials that applied treatment to the 5-cm perilesional skin.

This study found that combination of fractional CO_2 laser and NB-UVB came in the second rank, as 5% of patients developed repigmentation of more than 75%, 25% developed repigmentation of more than 50%, 45% developed repigmentation of more than 25%, and 25% did not respond to the treatment, with VAS ranged from 0

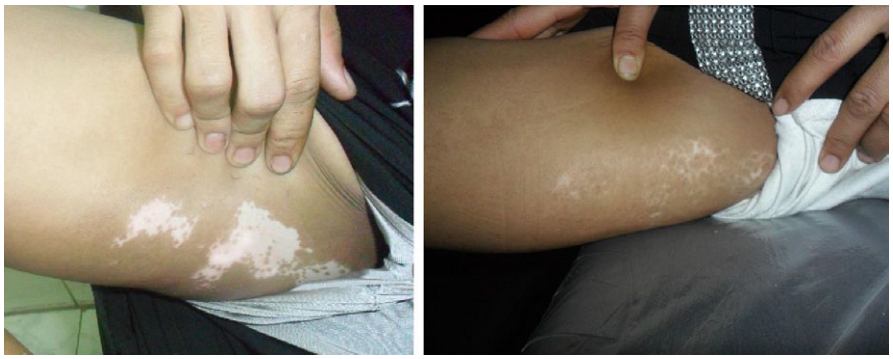

FIGURE 1 Combined treatment with fractional CO_2 laser and PRP injection for lesions in the medial aspect of the thigh; Right: before treatment, Left: 3 months after the treatment with repigmentation >75%

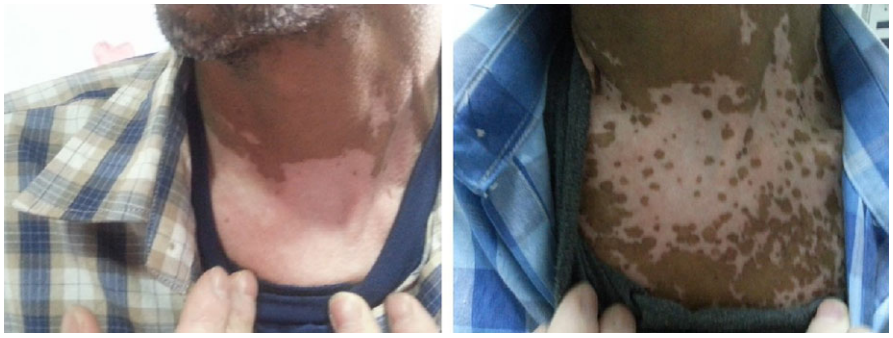

FIGURE 2 Combined treatment with fractional CO_2 laser and NB-UVB for neck lesions; Right: before treatment, Left: 3 months after the treatment with repigmentation >50%

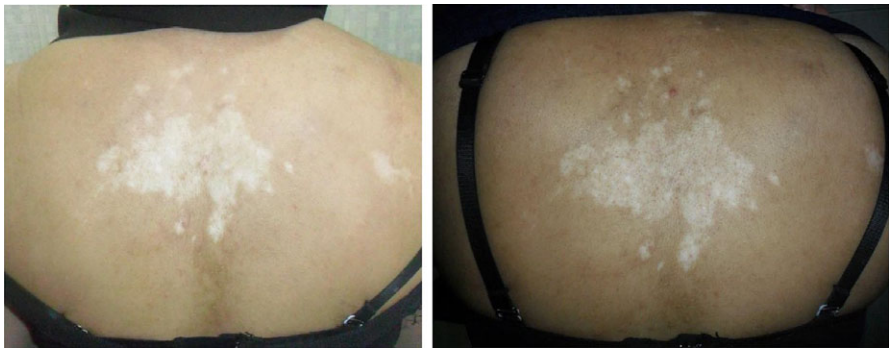

FIGURE 3 Treatment with fractional CO_2 laser for lesions on the back; Right: before treatment, Left: 3 months after the treatment with repigmentation >5%

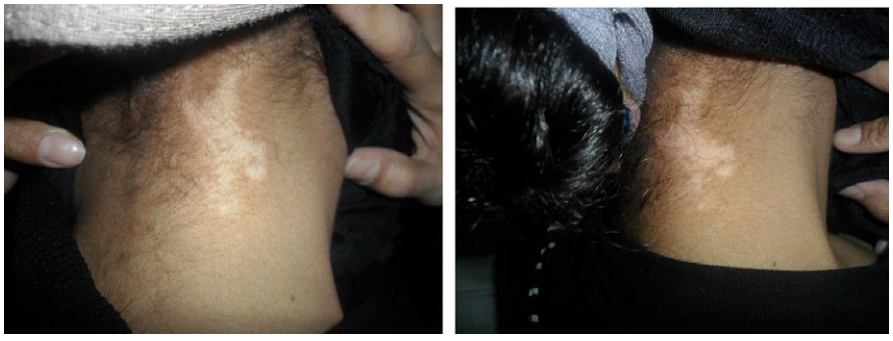

FIGURE 4 Treatment with PRP injection for lesions on the neck; Right: before treatment, Left: 3 months after the treatment with repigmentation <5%

to 10 and no reported side effects. The best response developed in lesions over the neck followed by lesions over extremities, then truncal lesions and facial lesions. Acral lesions showed no response. According to the study conducted by Shin et al,[7] half-body fractional CO_2 laser therapy was performed at a 2-month interval. NB-UVB phototherapy was then administered to the whole body 5 days after each fractional laser treatment twice a week, resulting in significantly higher repigmentation in comparison with contralateral side which did not receive fractional CO_2 laser treatment. Another study carried out by Li et al,[8] also confirmed the superior result of triple combination of fractional CO_2 laser, NB-UVB, and topical betamethasone solution. Haelou et al,[9] found that combination of fractional CO_2

laser treatment and sunlight exposure on daily basis resulted in repigmentation of vitiligo. On the same basis, Vachiramon et al,[22] confirmed that applying fractional CO_2 laser as an additive treatment to NB-UVB phototherapy and topical 0.05% clobetasol propionate cream could increase the repigmentation rate of vitiliginous lesions on difficult to treat areas.

These findings together with our results proved the beneficial effect of adding fractional CO_2 laser to the treatment of vitiligo. Addition of fractional CO_2 laser to the conventional therapy of vitiligo gave better results than addition of nonablative fractional lasers.[32] As regards the group who received fractional CO_2 laser alone, only 10% of the patients developed repigmentation of more than 75%, 70% developed repigmentation of more than 5%, while the nonresponders were 20% with VAS ranged from 0 to 10 and no reported side effects. The best response developed in lesions over the extremities followed by facial, truncal, and neck lesions. Acral lesions came in the last with no response. Similar results were achieved by El Mofty et al,[33] who found that 10% of the patients treated with fractional CO_2 laser alone developed repigmentation after 4 weekly sessions of fractional CO_2 laser using low powers. According to these data, fractional CO_2 laser alone has poor results in treatment of vitiligo.

The patients received autologous intradermal PRP injection alone came in the last rank. Only 20% of the patients developed repigmentation of more than 75%, 50% developed repigmentation of <5%, while the nonresponders were 30%. The best response developed in lesions over the trunk followed by lesions over extremities and then neck lesions. Acral and facial lesions came in the last with no response. PRP was tried in treatment of vitiligo in 2011 by Lim HK, Sh MK, and Lee MH (Clinical application of PRP in vitiligo: a pilot study. Official 1st international pigment cell conference). They treated 20 patients with vitiligo by 10 sessions of intradermal injection of PRP with 1-week interval, and they suggested that PRP was not effective in the treatment of vitiligo. Ibrahim et al[34], investigated the effect of PRP injecting on the outcome of short-term NB-UVB phototherapy in the treatment of vitiligo. They found a statistically highly significant improvement in the repigmentation in the combination group (PRP plus NB-UVB) compared with NB-UVB group. According to these data, autologous intradermal PRP injection alone has poor results in treatment of vitiligo. Limitations of this study were the small sample size, lack of control lesions, and short follow-up period. Further intra-individual studies with larger sample size, longer follow-up periods, and histopathologic or immunohistochemical examination are needed to reach the optimal results.

5 | CONCLUSION

This study demonstrated that combination of fractional CO_2 laser with autologous intradermal PRP injection is a promising treatment that produced significant improvement of stable nonsegmental vitiligo in different body sites without side effects, followed by combination of fractional CO_2 laser with NB-UVB phototherapy. Both of fractional CO_2 laser and autologous intradermal PRP injection gave poor results if used alone.

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