Section: DVL

Original Research Article

COMPARATIVE STUDY OF THE EFFICACY OF COMBINED FRACTIONAL CO_2 LASER AND TOPICAL 0.1% TACROLIMUS OINTMENT VERSUS TOPICAL 0.1% TACROLIMUS OINTMENT ALONE IN NON-SEGMENTAL VITILIGO

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ABSTRACT

Background: The clinical manifestation of Vitiligo, an acquired pigmentary condition, is depigmented macules brought on by melanocyte loss. Although there are many other treatment options, fractional carbon dioxide (CO2) lasers, which operate on the basis of photothermolysis, have lately been tested with positive results. **Objective:** To evaluate the effectiveness of topical 0.1% tacrolimus ointment plus fractional CO2 laser versus topical 0.1% tacrolimus ointment alone in non-segmental vitiligo.

Materials and Methods: Eighty patients with stable non-segmental vitiligo were enrolled in this interventional prospective study conducted over a period of two years. They were divided into two groups of forty patients each. For three months, Group A received topical 0.1% tacrolimus twice daily and fractional CO2 laser treatments every month while Group B received only 0.1% topical tacrolimus twice daily. Re-pigmentation was evaluated clinically and dermoscopically at monthly intervals for the first three months and again at the conclusion of six months.

Results: Of the eighty patients in this study, thirty-two were males, and forty-eight were females. Group A's mean age was 33.25 ± 11.8 years, while group B's was 31.98 ± 11.13 years. In both groups, the average duration of disease was 4.8 ± 4.83 years. According to the Quartile Grading Scale (QGS), at six months, there was a statistically significant difference ($p < 0.05$) in QGS with fifteen patients (60%) in Group A and nine patients (27.2%) in Group B showing more than 50% re-pigmentation. On dermoscopic evaluation at the end of six months, eighteen patients (72%) in Group A and fourteen patients (42.4%) in Group B showed moderate to good response in DAG which was statistically significant ($p < 0.05$).

Conclusion: Both clinical and dermoscopic assessments demonstrated that re-pigmentation was significantly enhanced with the combination of fractional CO_2 laser and topical tacrolimus 0.1%. These findings suggest that fractional CO_2 laser may serve as an effective therapeutic option for the management of stable vitiligo.

Keywords: Fractional CO2 laser, 0.1% Tacrolimus, Vitiligo.

INTRODUCTION

Vitiligo is a chronic, polygenic, acquired, autoimmune disorder of depigmentation causing selective loss of melanocytes, leading to depigmentation over affected skin.[1,2] Its prevalence is 0.4 to 2% of the population worldwide and 0.25% to 4% in India.[3] These lesions affect both genders and are totally amelanotic white macules, having a significant psychological impact on day-to-day life.[4] Clinically, it manifests as depigmented, chalky white macules with distinct borders that can develop at any age or site on the body but are most common on the face, forearms, hands, fingers, and scalp. The lesions vary from a few millimetres to several centimetres in size, with well-demarcated convex borders. Numerous pathogenic processes have been proposed to cause vitiligo, including oxidant-antioxidant hypotheses, autoimmunity, neural theory, and melanocytorrhagy.[5] Many treatment modalities have been used based on disease severity, disease stability as well as lesional stability, personal preference, financial status, and convenience of follow-up.[6] Topical therapy is preferred for localized lesions, whereas systemic treatment is indicated for patients with more than 10% body surface area (BSA) involvement or those with unstable and progressive disease. Surgical modalities are reserved for stable vitiligo that is recalcitrant to medical treatment.

Despite the treatments available, many patients with vitiligo remain in a refractory state. Therefore, various modalities combining conventional and newer therapeutic regimens are essential for enhancing the treatment outcome. Recently, a combination of fractional carbon dioxide (CO2) laser with topical tacrolimus, 5-fluorouracil (5-FU) and narrow band ultraviolet-B (NB-UVB) therapy has been used to enhance early response. Topical tacrolimus has been preferred for ages for the treatment of vitiligo due to its immunosuppressive action by calcineurin inhibition and fewer side effects.[7] However, the single use of topical tacrolimus alone may not be practical due to inadequate penetration due to its high molecular weight. Ablative therapies, like fractional CO2 laser, have been tried to enhance the penetration of topical drugs by creating microscopic thermal zones (MTZ). Laser produces fractional photo-thermolysis, which activates the cytokine inflammatory cascade and is responsible for the proliferation and differentiation of melanocytes [8]. In this study, the effectiveness of fractional CO2 laser and topical 0.1% tacrolimus versus topical 0.1% tacrolimus ointment alone was compared for non-segmental vitiligo.

MATERIALS AND METHODS

This interventional prospective study on eighty patients of non-segmental vitiligo attending the Dermatology, Venereology, and Leprosy outpatient department of a tertiary care center, was conducted from August 2022 to May 2024. The patients were divided into two groups of forty patients each. Group A was treated with a combination of fractional CO2 laser and topical 0.1% tacrolimus. Group B was treated with topical 0.1% tacrolimus only. Patients with non-segmental vitiligo aged 10–50yrs who had not received topical or systemic therapy in the previous three months were included. Exclusion criteria were, presence of leucotrichia, pregnancy and lactation, keloids and hypertrophic scars, and any current infections, eczema or psoriasis. The institutional ethics committee approved the study with the reference number – IEC-79/2022. Prior to the study, all patients provided written informed consent, demographic data was recorded, complete history was obtained, general and systemic examination was performed. A thorough dermatological assessment was done and details recorded. Baseline investigations like complete blood count, fasting and post prandial blood sugar, thyroid function tests were done. Photographs were taken at baseline and monthly intervals using a smartphone (One Plus 11 R 5G, Model CPH2487). An area of <10 cm2 was marked with respect to anatomical landmarks for assessing re-pigmentation at subsequent visits. Dermoscopy was performed using handheld Dermlite DL4 Dermoscope 4th generation Inc, USA.

In group A patients, before laser irradiation, the area for intervention was marked, and topical anaesthetic cream (comprising of lidocaine and prilocaine 2.5% each) was applied for 30 to 40 minutes. The site was cleaned, and eyes were protected using a gauze piece and eye shield. Laser irradiation was done by Futura RF-RF excited CO2 laser 50W (fractional CO2 laser) single pass at marked site with following laser parameters – energy – 15-20mJ, duration – 1ms, number of passes – spot distance 0.6mm. Post laser session, an ice pack was kept for ten minutes, and the site was examined for any pain, erythema, oedema & blistering. Three sessions of laser were done at monthly intervals, and topical 0.1% tacrolimus ointment was applied twice daily for three months.

In group B, the patients were advised to use 0.1% tacrolimus twice daily for three months. Patients in both groups were given treatment for the first three months and followed up for the next three months. Assessment for re-pigmentation and monitoring of side effects was done at monthly intervals for three months and at the end of sixth month. The treatment response was assessed clinically by Quartile Grading Scale (QGS) & by calculating the number of perifollicular spots on dermoscopy using Dermoscopic Assessment Grading (DAG).

Statistical Analysis

The statistical analysis was performed using the Statistical Package for Social Sciences (SPSS Version 26). Descriptive statistical analysis was used to look at the distribution of specific quantitative and categorical variables. n (%) summarised categorical variables, and means and standard deviations summed quantitative factors. Each outcome was

tallied and, if required, graphically depicted using bar or pie diagrams. Categorical variables were assessed for statistical significance in the difference between the two groups using the chi-square test. A p-value <0.05 was considered statistically significant, assuming that all statistical test rules were followed.

RESULTS

The study enrolled 80 patients with non-segmental vitiligo, comprising 32 males (40%) and 48 females (60%). Patients were randomized into two equal groups (n = 40 each). The mean age was 33.25 ± 11.80 years in Group A (fractional CO_2 laser with topical 0.1% tacrolimus) and 31.98 ± 11.13 years in Group B (topical 0.1% tacrolimus alone). The mean disease duration was similar in both groups (4.84 ± 5.29 years for Group A; 4.90 ± 4.38 years for Group B). Nine patients (11.3%) had comorbidities (3 with diabetes mellitus, 3 with hypertension, 3 with hypothyroidism). The most commonly affected anatomical sites were the legs, face/neck, forearms, hands, and trunk. A family history of vitiligo was reported in one patient from each group. The baseline Vitiligo Area Scoring Index (VASI) scores were 171.1 ± 127.5 for Group A and 136.5 ± 71.4 for Group B. Of the 80 enrolled patients, 22 were lost to follow-up (15 from Group A, 7 from Group B).

Table 1: Socio-Demographic and Baseline Characteristics

Characteristic	Group A (n = 40)	Group B (n = 40)	p-value
Mean Age (years)	33.25 ± 11.80	31.98 ± 11.13	0.94
10-20 years, n (%)	8 (20.0)	5 (12.5)	
21-30 years, n (%)	8 (20.0)	17 (42.5)	
31-40 years, n (%)	13 (32.5)	7 (17.5)	
41-50 years, n (%)	11 (27.5)	11 (27.5)	
Gender			
Male, n (%)	15 (37.5)	17 (42.5)	0.50
Female, n (%)	25 (62.5)	23 (57.5)	0.50
Mean Duration (years)	4.84 ± 5.29	4.90 ± 4.38	0.99
Comorbidities, n (%)			
Diabetes mellitus	3 (7.5)	0 (0)	0.12
Hypertension	1 (2.5)	2 (5.0)	0.50
Hypothyroidism	2 (5.0)	1 (2.5)	0.50
Lesion Site, n (%)			
Face and neck	6 (15.0)	9 (22.5)	0.83
Trunk	6 (15.0)	4 (10.0)	
Hands	5 (12.5)	4 (10.0)	
Forearms	5 (12.5)	7 (17.5)	
Foot	1 (2.5)	2 (5.0)	
Legs	17 (42.5)	14 (35.0)	
Family history, n (%)	1 (2.5)	1 (2.5)	0.73
VASI (mean ± SD)	171.1 ± 127.5	136.5 ± 71.4	0.14

Table 2: Quartile Grading Scale for Clinical Re-pigmentation

Grade	Percentage of Re-pigmentation	Clinical Response
0	0%	Nil
1	1–25%	Minimal
2	26–50%	Moderate
3	51–75%	Good
4	>75%	Excellent

Table 3: Comparative Quartile Grading Scale (QGS): Percentage of Patients Achieving ≥50% Re-pigmentation at Each Follow-up

Time Point		Group A (n = 25)	Group B (n = 33)	p-value
First month	<50% re-pigmentation	24 (96.0%)	31 (93.9%)	0.70
	≥50% re-pigmentation	1 (4.0%)	2 (6.1%)	
Third month	<50% re-pigmentation	18 (72.0%)	28 (84.8%)	0.20
	≥50% re-pigmentation	7 (28.0%)	5 (15.2%)	
Sixth month	<50% re-pigmentation	10 (40.0%)	24 (72.7%)	**0.01***
	≥50% re-pigmentation	15 (60.0%)	9 (27.3%)	

Statistically significant at p < 0.05

Table 4: Dermoscopic Assessment Grading (DAG) Criteria

Grade of Re-pigmentation	Number of Perifollicular Spots
No response	0
Minimal	1–2
Moderate	3–6
Good	>6

Table 5: Comparative Dermoscopic Assessment Grading (DAG): Patients Showing Moderate to Good Response at Follow-up

Time Point	Response Category	Group A (n = 25)	Group B (n = 33)	p-value
First month	No to minimal response	23 (92.0%)	27 (81.8%)	0.20
	Moderate to good	2 (8.0%)	6 (18.2%)	
Third month	No to minimal response	12 (48.0%)	20 (60.6%)	0.30
	Moderate to good	13 (52.0%)	13 (39.4%)	
Sixth month	No to minimal response	7 (28.0%)	19 (57.6%)	**0.02***
	Moderate to good	18 (72.0%)	14 (42.4%)	

Statistically significant at p < 0.05. Percentages are based on patients who completed follow-up at each interval.

The baseline demographic and clinical variables were comparable between the two treatment groups (Table 1). The mean ages were 33.25 ± 11.80 years for Group A and 31.98 ± 11.13 years for Group B (p = 0.94). Gender distribution, disease duration, and lesion sites showed no significant differences, indicating balanced groups for comparison. Comorbidities such as diabetes mellitus, hypertension, and hypothyroidism were infrequent and similarly distributed (p > 0.05). The mean Vitiligo Area Scoring Index (VASI) scores were also comparable (171.1 ± 127.5 in Group A versus 136.5 ± 71.4 in Group B, p = 0.14), confirming equivalent disease severity at baseline.

The Quartile Grading Scale (QGS) categorizes re-pigmentation into five grades ranging from nil (0% repigmentation) to excellent (>75%) (Table 2). This scale was used to quantify clinical response, facilitating a standardized, semi-quantitative assessment of treatment efficacy based on percentage of re-pigmentation.

Clinical evaluation demonstrated progressively increasing proportions of patients achieving ≥50% re-pigmentation in both groups over time (Table 3). At 1 and 3 months, differences between Group A and Group B were not statistically significant (p = 0.70 and 0.20, respectively). However, by 6 months, Group A showed a significantly higher rate of patients with ≥50% re-pigmentation (60.0%) compared to Group B (27.3%) (p = 0.01), indicating superior clinical response with the combination of fractional CO_2 laser and topical tacrolimus.

The DAG system quantifies perifollicular pigmentation via the number of perifollicular pigmented spots, categorizing re-pigmentation response as no response (0 spots), minimal (1–2 spots), moderate (3–6 spots), or good (>6 spots) (Table 4). This dermoscopic grading offers an objective and sensitive method to evaluate follicular re-pigmentation during treatment monitoring.

Dermoscopic analysis mirrored clinical findings, with an increasing percentage of patients showing moderate to good perifollicular re-pigmentation over time in both groups (Table 5). At 1 and 3 months, no statistically significant differences were observed between groups (p = 0.20 and 0.30, respectively). By 6 months, however, Group A demonstrated a significantly higher proportion of moderate to good responses (72.0%) compared to Group B (42.4%) (p = 0.02), supporting enhanced treatment efficacy with combined fractional CO_2 laser and topical tacrolimus therapy.

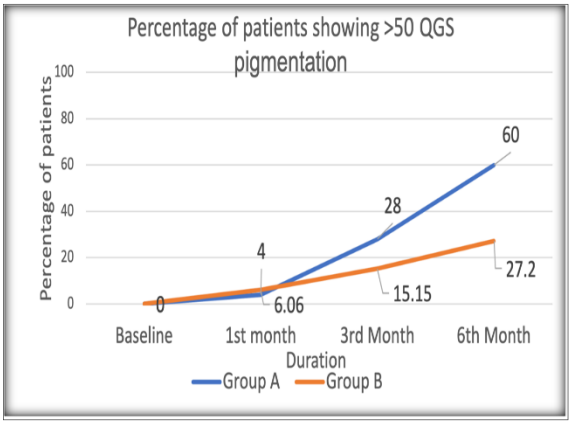

Figure 1: Line graph showing re-pigmentation in QGS between Group A and Group B in the first, third and sixth month

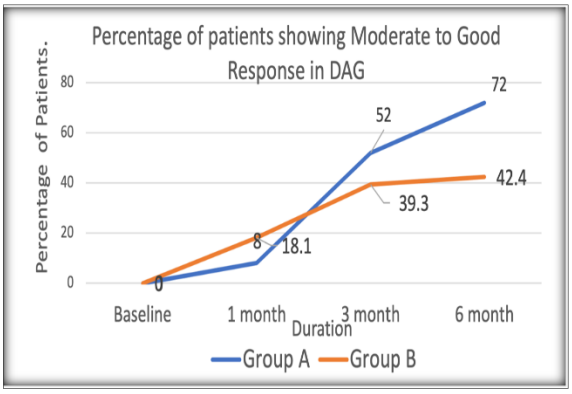

Figure 2: Line graph showing re-pigmentation in DAG between Group A and Group B in the first, third and sixth month

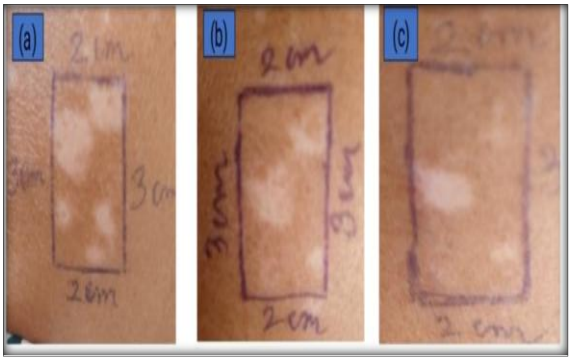

Figure 3: Clinical pictures showing QGS response in Group A at baseline (3a), third month (3b), and sixth month (3c) on the upper back

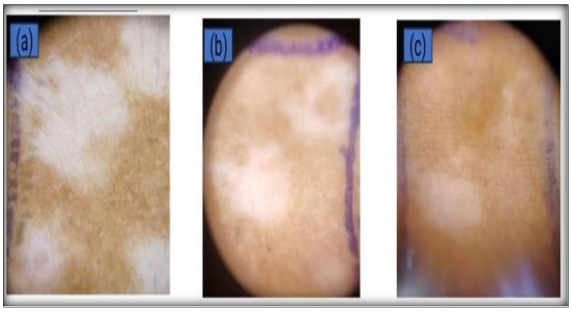

Figure 4: Dermoscopy pictures showing DAG response in Group A at baseline (5a), third month (5b), sixth month (5c) over the upper back

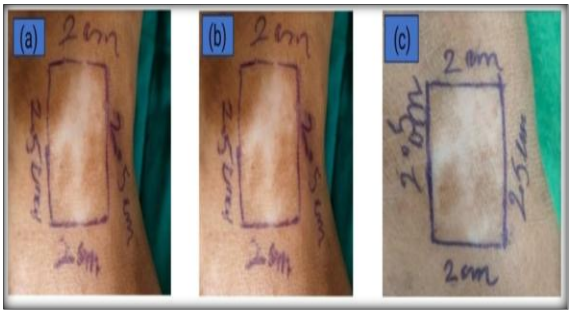

Figure 5: Clinical pictures showing QGS response in Group B at baseline (4a), third month (4b), sixth month (4c) on the left leg

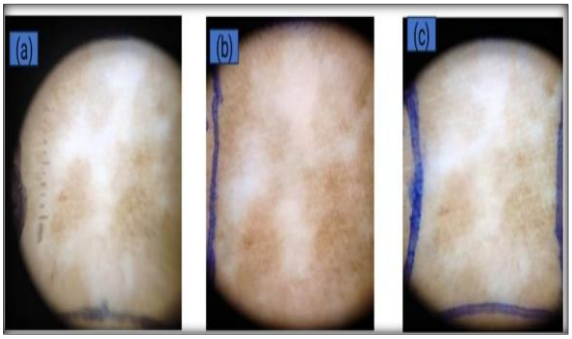

Figure 6: Dermoscopy pictures showing DAG response in Group B at baseline (6a), third month (6b), and sixth month (6c) on the left leg

DISCUSSION

Vitiligo is a chronic, autoimmune disorder of pigmentation presenting with multiple, polysized depigmented macules. It is associated with deep psychological stress and social stigma pertaining to its unpleasant appearance.[9]

The mean age was 32.61 ± 11.46 years, out of which the majority of patients were females, comparable to research published by Zeid et al. and Silpa-Archa et al.[10,11] This could be due to the cosmetic and social reasons faced by the women in society, which makes them come early for the treatment. However, the study by Bae et al. showed male predominance.[12] In contrast to the study by Silpa-Archa et al., the mean disease duration was 4.87 ± 4.83 years.[11]

In this study, out of eighty patients, nine patients had an association with comorbidities. Among these nine patients, three (3.75%) were diabetic, three (3.75%) hypertensive, and three (3.75%) hypothyroid. In a systematic review and meta-analyses by Ji ha Lee et al., vitiligo patients had an increased chance of developing diabetes mellitus compared to hypertension. Also, vitiligo patients had increased chances of having autoimmune thyroiditis followed by hypothyroidism & hyperthyroidism.[13] Only three of the eighty patients in this study had hypothyroidism, although a study by Gopal et al. found a 12% correlation between vitiligo and thyroid disorders.[14] In contrast to the study by Shajil EM et al., which found familial occurrence to be between 6.25% to 30%, only 5% of the study population had a family history of vitiligo, with just one patient per group having this condition.[15]

According to the Quartile Grading Scale (QGS), seven patients (28%) in Group A and five patients (15.15%) in Group B achieved more than 50% re-pigmentation at the end of third month. At the end of the sixth month, more than 50% re-pigmentation in QGS was demonstrated in 60% of Group A patients and 27.2% of Group B patients, thus showing significant ($p<0.05$) re-pigmentation in group A than group B. A study by Manzoor et al. on one-hundred and twenty patients of segmental vitiligo showed significant re-pigmentation with an efficacy of 56.7% in the fractional CO2 laser group than tacrolimus alone.[16] Similarly, a study by Putta et al. showed significant re-pigmentation when a combination therapy of fractional CO2 laser, topical 0.1% tacrolimus and NB-UVB was used.[17]

In this study, lesions over the lower extremities and trunk showed early improvement in QGS and DAG in group A as compared to the acral areas, contrary to Zeid et al. study, where both the groups showed better re-pigmentation at head and neck areas.[10] In a study by V Brazelli et al. grade 4 (75%) response was seen in both young and older age groups over the face, but over neck grade 4 (75%) re-pigmentation occurred earlier in younger patients as compared to older patients.[19] Similar to the study by Patil et al., males showed poor therapeutic prognosis compared to females.[20]

On dermoscopy, various patterns of re-pigmentation were seen, such as perilesional, perifollicular, and reticular pigmentation. Among these, a perifollicular pattern of re-pigmentation was seen in both groups, which was assessed using DAG. In a study by Lui Mei et al., the efficacy of treatment was assessed using dermoscopic perifollicular re-pigmentation, showing that dermoscopic assessment was superior to conventional visual observation.[18] On dermoscopic evaluation at the end of third month using Dermoscopic Assessment Grading (DAG), thirteen patients each in Group A (52%) and Group B (39.3%) demonstrated a moderate to good response. At the end of the sixth month, moderate to good response in DAG was shown by 72% of group A patients and 42.4% of group B patients, projecting a significant difference ($p<0.05$). Early perifollicular re-pigmentation was appreciated in group A rather than group B.

Moderate to good improvement (more than 50% response) in QGS was more in the fractional CO2 laser and tacrolimus group (group A) compared to tacrolimus alone group (group B). The reasons for re-pigmentation could be, first, CO2 laser ablation, causing tissue retraction and shrinkage due to collagen bundle denaturation.[17] Second, initiating re-pigmentation by proliferation, activation & movement of melanoblasts from the outer root sheath of hair follicles on melanoreceptors from the border area towards the depigmented area. Third, it enhances matrix metalloproteinases 2 and 9 production, causing melanocyte migration from adjacent skin.[10] The lesser response in group B may be attributed to the "500 Dalton rule", impairing effective absorption of tacrolimus due to its high molecular weight (822Da). Response in the fractional CO2 group was better than tacrolimus alone, which could be due to the better penetration of tacrolimus through MTZ's created by fractional CO2 laser.[8] More than 50% re-pigmentation was seen in patients aged more than fifteen years.

In this study, pain and erythema were the common side effects seen in group A and pruritus in group B, which subsided spontaneously during subsequent visits. Crusting was present in most of our patients post-laser, and it disappeared in 7-10 days. Similarly, in the Zeid et al. study, all the patients receiving fractional CO2 laser experienced pain and erythema as a common side effect.[10] Few patients were lost to follow-up due to various reasons like financial constraints, slower response, frequent hospital visits, and long-term follow-up.

The limitations of this study include a small sample size, which restricts the generalizability of the findings; challenges in patient follow-up due to the slow therapeutic response, cost constraints, and the need for frequent hospital visits; and the non-randomized study design.

CONCLUSION

A combination of fractional CO2 laser and topical tacrolimus 0.1% showed significant improvement in re-pigmentation both clinically and dermoscopically. Hence, the fractional CO2 laser is a ray of hope for the treatment of vitiliginous patches for focal cosmetically substantial sites. Still, more studies with larger samples and long-term follow-up are required to implement it as a new mode of intervention.

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