

ORIGINAL ARTICLE

Evaluation of Combination of Microneedling with Tacrolimus in the Treatment of Stable Vitiligo

Avaliação da Combinação de Microagulhamento com Tacrolimus no Tratamento de Vitiligo Estável

Received/Recebido
2021/01/20Accepted/Aceite
2021/03/14Published/Publicado
2021/09/30Saurabh Sharma¹, Parwaaz Matharoo¹, Roopam Bassi²¹Department of Dermatology, Venereology & Leprosy, Sri Guru Ram Das Institute of Medical Sciences & Research, Amritsar, Punjab, India²Department of Physiology, Sri Guru Ram Das Institute of Medical Sciences & Research, Amritsar, Punjab, India

ABSTRACT – Introduction: Vitiligo is an amelanotic disorder that can manifest as localized patches to near total body depigmentation. It is considered a cosmetic disease but it is accompanied by extensive psychological effects. Multiple treatments have been tried until now but no single method has achieved the desired response. Tacrolimus is frequently used for vitiligo treatment with few adverse effects. Microneedling is a newer modality of therapeutic wounding that augments drug absorption. Combination of microneedling and tacrolimus have been tried to improve the repigmentation response in vitiligo. Our objective was to assess the safety and efficacy of this combined modality.

Methods: Forty patients were subjected to combination of tacrolimus 0.1% ointment and microneedling at 2 week intervals for maximum 10 sessions with a follow-up period of 6 months. Assessment was based on grading of repigmentation and clinical improvement according to vitiligo noticeability scale.

Results: Excellent repigmentation was observed in 30% cases. Improvement was significantly higher in lesions present on the head and neck. Mucosal vitiligo also showed good to excellent response. No severe side effects were reported. No complications or recurrences were observed during the follow-up period.

Conclusion: Combination of microneedling and tacrolimus was found to be safe and efficacious in the treatment of stable vitiligo.

KEYWORDS – Dry Needling; Tacrolimus; Vitiligo.

RESUMO – Introdução: O vitiligo é uma doença amelanótica que pode se manifestar como manchas localizadas até quase à despigmentação total. É considerada uma doença cosmética, mas é acompanhada por extensos efeitos psicológicos. Vários tratamentos foram tentados até agora, mas nenhum método atingiu a resposta desejada. O tacrolimus é um agente frequentemente usado para o tratamento do vitiligo com um perfil mínimo de efeitos colaterais. O microagulhamento é uma modalidade mais recente de ferimento terapêutico que aumenta a absorção de fármacos. A combinação de microagulhamento e tacrolimus tem sido tentada para melhorar a resposta de repigmentação no vitiligo. Nosso objetivo foi avaliar a segurança e eficácia desta modalidade combinada.

Métodos: Quarenta pacientes foram submetidos à combinação de tacrolimus 0,1% pomada e microagulhamento em intervalos de 2 semanas por um máximo de 10 sessões com um período de acompanhamento de 6 meses. A avaliação foi baseada na graduação de repigmentação e melhora clínica de acordo com a escala de perceptibilidade do vitiligo.

Resultados: Excelente repigmentação foi observada em 30% dos casos. A melhora foi significativamente maior nas lesões presentes na cabeça e pescoço. O vitiligo mucoso também mostrou boa a excelente resposta. Não foram relatados efeitos colaterais graves. Nenhuma complicação ou recorrência foi observada durante o período de acompanhamento.

Conclusão: A combinação de microagulhamento e tacrolimus é considerada segura e eficaz no tratamento do vitiligo estável.

Compreender e estudar o risco de infecção na utilização de terapêutica imunossupressora permite a sua aplicação de forma mais informada e segura.

PALAVRAS-CHAVE – Agulhamento Seco; Tacrolimus; Vitiligo.

INTRODUCTION

Vitiligo is an acquired disorder characterized by loss of pigmentation due to autoimmune destruction of melanocytes. It has a varied morphology manifesting as depigmented patches involving skin, hair and mucous membranes.¹ Although it is an ancient disease, the pathogenesis still remains an enigma. Multiple hypothesis have been suggested to explain the etiopathogenesis including autoimmune theory, self-destructive theory, neural theory, biochemical theory,

growth factor reduction hypothesis, melanocytorrhagy and stress.² It has been accepted that all these factors synergistically play a role in melanocyte loss. Vitiligo is classified as segmental and nonsegmental. Other phenotypes include trichrome, pentachrome, red vitiligo, blue vitiligo or follicular vitiligo.³ Despite the fact that it is a benign disease, it carries the burden of social stigmatization leading to negative impact on quality of life of the patient. Several pseudo beliefs prevail in the society causing depression, low self-esteem and isolation amongst sufferers.⁴ With a plethora of therapeutic modalities

available, none seems to give the ideal response. Management includes both non-surgical and surgical options. Medical treatment includes oral and topical corticosteroids, topical calcineurin inhibitors, phototherapy, topical vitamin D analogues, antioxidants and immunosuppressants.⁵ Tacrolimus is a frequently used immunosuppressive agent. It is a calcineurin inhibitor that has a direct effect on melanocyte growth and migration. It inhibits T cell activation and restores the altered cytokine network. It is commonly used in children and in sun exposed areas. Better outcomes have been observed with combination treatments.⁶ Microneedling is a method of transdermal drug delivery by creating micropores through the stratum corneum. It augments drug absorption as well as causes mechanical migration of melanocytes from margins to amelanotic areas. It leads to therapeutic wounding and stimulates the release of multiple growth factors.⁷ Dermapen is a fractional microneedling device. Its automated needling motion increases effectiveness, minimizes pain and discomfort and promotes an even absorption of topicals.⁸ Microneedling has been tried with several drugs and reported to be more beneficial than monotherapy.⁷

MATERIAL AND METHODS

Forty patients with localized and stable vitiligo for more than one year were enrolled in this study. A written informed consent was obtained from each patient. This was a hospital based interventional study conducted in the outpatient Department of Dermatology, Venereology & Leprosy during the period from March 2019 to July 2020.

Exclusion criteria were as follows: pregnancy or lactation, history of keloid / hypertrophic scarring, active infection, bleeding or coagulation disorders, disease duration less than 1 year and patients receiving any treatment for vitiligo within the last 6 months. This study included patients aged 12-60 years with vitiligo diagnosed clinically for >1 year who were willing to come for regular follow-up. Single vitiligo patch measuring less than 10 cm² was selected in each patient. Skin biopsy was performed in difficult to diagnose cases.

Under aseptic precautions local anaesthesia was infiltrated in the selected vitiligo lesion following which, a layer of tacrolimus ointment 0.1% was applied and microneedling was done with dermapen using the lowest speed with needle penetration depth ranging from 0.25 to 0.5 mm over face and 1 to 2 mm over body in horizontal and vertical directions until pin point bleeding was observed. After cleaning with a povidone iodine solution, tacrolimus ointment 0.1% was applied under occlusive dressing for the remaining of the day. This procedure was repeated every 2 weeks for a maximum of 10 sessions. Patients were advised to apply tacrolimus ointment twice daily in between the sessions and were followed up for 6 months after the last session. Any adverse effects or complications were recorded.

Repigmentation was assessed by two dermatologists (other than those involved in this study) with the help of photographs which were taken at baseline, before each session and at follow-ups. Repigmentation response was graded as: G0 <25% - poor; G1 25%-50% - fair; G2 50%-75% - good; G3 >75% - excellent.

Vitiligo noticeability scale (VNS) was also used to measure the outcome from the patient's perspective. Scoring was done as followed: 1 more noticeable; 2 as noticeable; 3 slightly less noticeable; 4 a lot less noticeable; 5 no longer noticeable.

Statistical methods: Collected data was organized and statistically analyzed using SPSS software version 19. Frequency, mean and

standard deviation were calculated. Pearson Chi Square test was used in this study. P value <0.05 was considered statistically significant.

Ethical considerations: Informed written consent was taken on a prestructured proforma from every adult patient and from guardians in case of minors. The study conducted was in accordance with the amended Declaration of Helsinki and approval for the study was obtained from the institutional ethics committee in February 2019. (Reference no. Patho 182/19)

RESULTS

This study comprised 40 patients with clinically diagnosed stable vitiligo. There were 23 females and 17 males with ages ranging from 12 to 60 years. Demographic and clinical data are presented in table 1. All patients completed the required treatment and follow-up period.

With regard to repigmentation evaluation, excellent response (>75%) was observed in 30% of the cases and good response (50%-75%) in 45% (Fig. 1-3). In terms of patient's vitiligo noticeability scale scoring (VNS), 20% cases had a score of 5 (no longer noticeable) and 10% had a score of 4 (a lot less noticeable). The start of repigmentation was seen after a single session and excellent response was achieved within 2 months in 2 cases. Minimum number of sessions required to achieve excellent response was 4. Predominant pattern of

Table 1 - Exposure time to onset of dermatitis.

		Number of cases	Percentage
Age (years)	<30	23	57.5
	>30	17	42.5
7 - 12 > 12	Male	17	42.5
	Female	23	57.5
Family history of vitiligo	Present	6	15
	Absent	34	85
Type of vitiligo	Generalized	30	75
	Segmental	7	17.5
	Mucosal	3	7.5
Disease duration (years)	<4y	13	32.5
	>4y	27	67.5
Localization of lesions treated	Head and neck	10	25
	Limbs*	14	35
	Trunk	7	17.5
	Acral areas	9	22.5
Previous treatment	Present	18	45
	Absent	22	55

* Arm/forearm or thighs/legs.

Table 2 - Clinical response to microneedling and tacrolimus and types of response.

	Number of cases	Percentage
Age (years)		
Excellent >75%	12	30
Good 50%-75%	18	45
Fair 25%-50%	8	20
Poor <25%	2	5
Age (years)		
Marginal	15	37.5
Follicular	20	50
Diffuse	5	12.5
VNS**		
5 No longer noticeable	8	20
4 A lot less noticeable	4	10
3 Slightly less noticeable	18	45
2 Noticeable	10	25
Number of sessions		
4	2	5
5	4	10
6	4	10
7	2	5
10	28	70

** VNS – Vitiligo Noticeability Scale Scoring.



Figure 1 - Excellent repigmentation on the neck after microneedling.

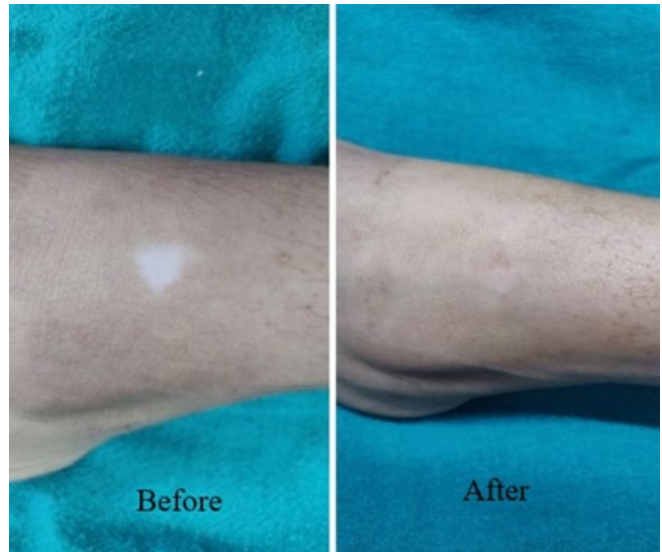


Figure 2 - Repigmentation of an acral lesion.



Figure 3 - Partial repigmentation on the lips after microneedling.

repigmentation was perifollicular in 50% cases, followed by marginal in 37.5% cases and diffuse in 12.5% cases.

Comparison between the type of response and site is shown in Table 3. Maximum number of lesions were present over limbs (arms/forearms or thighs/legs) followed by head and neck, acral areas and trunk. Good to excellent (>50%) response was achieved in all head and neck cases and in 92.8% of limbs. Of the head and neck cases, 3 (7.5%) had mucosal vitiligo involving the lips, out of which 2 had excellent response and 1 had good response. Trunk showed excellent response in 71.4% cases and fair response in 28.6% cases. None of the cases showed excellent response over acral areas. The statistical comparison was highly significant ($p = 0.0001$).

Pain, reported in 1 case, was present for a few hours after the procedure and gradually subsided. No side effects were observed in the remaining 39 cases. Repigmentation was stable and neither recurrences nor long-term complications were reported during the follow-up period of 6 months.

DISCUSSION

Vitiligo is a depigmenting disorder due to autoimmune destruction of melanocytes. It affects 1%-2% of the world's population.¹

Table 3 - Relation between the site of the lesion and type of response.

Site	Number	Type of response								P value
		Excellent		Good		Fair		Poor		
Cobalt	10	5	50%	5	50%	0	0	0	0	0.0001
Nickel	14	2	14.3%	11	78.6%	1	7.1%	0	0	
Thiuram mix	7	5	71.4%	0	0	2	28.6%	0	0	
Total studied	9	0	0	2	22.2%	5	55.6%	2	22.2%	

* Arm/forearm or thighs/legs.

In a country of darker skin type, overwhelming psychological burden is seen due to the social outcast and stigma faced by affected patients. It has been observed that repigmentation decreases the suffering and improves quality of life. The natural course of the disease is unpredictable and difficult to control. Many treatment options have been tried in the past but none has produced satisfactory results. Medical treatment remains the mainstay in most of the cases, namely- tacrolimus. Recently described methods of therapeutic wounding have gained attention and microneedling is one such modality.⁹ It is an effective and simple technique that aims at stimulating and repopulating the melanocytes into depigmented lesion. It is a safe, simple to learn and easily accessible procedure. Great potential has been shown by this technique in recalcitrant lesions especially when used in combination with other drugs.⁷ Our aim was to use microneedling as a complementary approach to the traditional therapy in vitiligo.

The beginning of repigmentation was seen after the first session in the form of erythema or mild pigmentation, and excellent repigmentation was attained in 5% of cases within 2 months (4 sessions). This was faster than the results observed by Ebrahim HM *et al* who compared tacrolimus and microneedling with tacrolimus monotherapy and observed initiation of repigmentation after 3 to 4 sessions.¹⁰ This can be attributed to the difference in procedure as, with our technique, tacrolimus was applied prior to microneedling. This ensures uniform and deeper penetration of the drug. Mina M *et al.* studied the use of tacrolimus 0.03% with microneedling and observed initiation of repigmentation within 6 weeks.¹¹ Good to excellent response was seen in 75% cases. In terms of patient's vitiligo noticeability scale scoring (VNS), 20% cases had a score of 5 (no longer noticeable) and 10% cases had a score of 4 (a lot less noticeable). This was comparable to the grading of repigmentation of excellent response (>75%), which was seen in 30% of cases. A total of 45% cases had VNS score of 3 (slightly less noticeable) which was comparable to good response (50%-75%) seen in 45% of cases. Our study showed better response than Ebrahim HM *et al* where good to excellent response was seen in 70.8% cases.¹⁰ In a different study by Ebrahim HM *et al*, microneedling combined with tacrolimus showed a better outcome (excellent response in 66.6%) than isolated microneedling or tacrolimus.¹² Good to excellent response (>50%) in 10 (40%) lesions with tacrolimus was reported by Mina M *et al.*¹¹ Passerson T *et al* combined tacrolimus and 308 nm excimer laser and observed excellent repigmentation in 70% lesions.¹³ Nistico S *et al* combined tacrolimus with excimer laser and showed excellent response in 50% patients.¹⁴ Fai D *et al* concluded that combination of tacrolimus and NB UVB had better outcome

than NB UVB alone. Their study showed excellent repigmentation in 53.3% cases.¹⁵

In our study, a better response was observed on the head, neck and limbs and a poorer response on the trunk and acral areas. We also evaluated the response of this combination in mucosal (lip) vitiligo: Out of 3 cases, 2 had excellent response and 1 showed good response. It was very well tolerated overall, with only one reported case of pain. As tacrolimus is a safe drug to treat mucosa, this combination may provide a scope of investigation for treatment of mucosal vitiligo, considered a hard to treat area and a possible indication for surgical management. In comparison to various surgical options, microneedling seems to be the safer, easier to perform and less time consuming with minimum complications.

Previous studies have shown that tacrolimus is an effective therapy for head and neck areas due to the greater density of hair follicles.¹⁶ Our findings were almost similar to Ebrahim HM *et al*, who reported that tacrolimus and microneedling showed maximum repigmentation on the face, followed by limbs, trunk and acral areas.¹² In a different study by the same authors, best results were seen on face followed by legs, trunk, elbows and knees. Acral areas also showed satisfactory improvement.¹⁰ In our study, none of the lesions present over acral areas showed excellent response and good response was only observed in 22.2% cases. The pattern of repigmentation was perifollicular in 50% of the cases followed by marginal in 37.5% and diffuse in 12.5%, which was consistent with previous studies.^{11,12}

No major adverse effects were observed in our study. Pain was reported by one patient but it gradually decreased within a few hours. Some authors reported mild pain, itching and erythema in few patients but the procedure was also generally considered as well tolerated.¹¹

Multiple options have been suggested for therapeutic wounding like TCA, phenol, needling, excimer laser, cryotherapy or dermabrasion. From these, microneedling stands out as a simple, quick and cost-effective method that can be used as a surgical intervention in stable vitiligo.⁹ It is a minimally invasive procedure that does not require hospital stay. There is no major blood loss and the patient is comfortable during the entire procedure. Microneedling is usually free of complications like cobblestoning, pseudomembrane formation, atrophy, scar formation or variegated appearance which are regularly observed with other surgical modalities. Response in mucosal vitiligo was positive in our study but these findings warrant further studies with larger samples. This technique should be considered as a viable option for dermatologists to treat resistant vitiligo lesions.

CONCLUSION

Microneedling with tacrolimus is an efficacious option for the treatment of stable vitiligo. The simplicity of the procedure and the results obtained can help patients and reduce the psychological burden due to the stigmatization associated with the disorder. Further research - should be undertaken to evaluate the response in mucosal vitiligo, the long-term stability of repigmentation and complications.

Conflicts of Interest: The authors have no conflicts of interest to declare. **Financing Support:** This work has not received any contribution, grant or scholarship. **Confidentiality of Data:** The authors declare that they have followed the protocols of their work center on the publication of data from patients. **Protection of Human and Animal Subjects:** The authors declare that the procedures were followed according to the regulations established by the Clinical Research and Ethics Committee and to the 2013 Helsinki Declaration of the World Medical Association. **Provenance and Peer Review:** Not commissioned; externally peer reviewed.

Conflitos de Interesse: Os autores declaram a inexistência de conflitos de interesse na realização do presente trabalho. **Fontes de Financiamento:** Não existiram fontes externas de financiamento para a realização deste artigo. **Confidencialidade dos Dados:** Os autores declaram ter seguido os protocolos da sua instituição acerca da publicação dos dados de doentes. **Proteção de Pessoas e Animais:** Os autores declaram que os procedimentos seguidos estavam de acordo com os regulamentos estabelecidos pelos responsáveis da Comissão de Investigação Clínica e Ética e de acordo com a Declaração de Helsinquia de 2013 da Associação Médica Mundial. **Proveniência e Revisão por Pares:** Não comissionado; revisão externa por pares.



ORCID

Saurabh Sharma: <https://orcid.org/0000-0003-2808-136X>
Parwaaz Matharoo: <https://orcid.org/0000-0003-0722-4659>
Roopam Bassi: <https://orcid.org/0000-0001-8600-0818>

Corresponding Author: Parwaaz Matharoo

Address: Sri Guru Ram Das Institute of Medical Sciences & Research
Mehta road, Vallah, Amritsar, Punjab, India 143501
E-mail: pmatharoo14@gmail.com

© Author(s) (or their employer(s)) 2021 SPDV Journal. Re-use permitted under CC BY-NC. No commercial re-use.
© Autor (es) (ou seu (s) empregador (es)) 2021 Revista SPDV. Reutilização permitida de acordo com CC BY-NC. Nenhuma reutilização comercial.

REFERENCES

1. Mahajan VK, Vashist S, Chauhan PS, Mehta KIS, Sharma V, Sharma A. Clinico-Epidemiological Profile of Patients with Vitiligo: A Retrospective Study from a Tertiary Care Center of North India. *Indian Dermatol Online J.* 2019;10:38-44. doi: 10.4103/idoj.IDOJ_124_18.
2. Arora AK, Kumaran MS. Pathogenesis of vitiligo: An update. *Pigment Int.* 2017;4:65-77. doi: 10.4103/2349-5847.219673.
3. Sehgal VN, Srivastava G. Vitiligo: compendium of clinico-epidemiological features. *Indian J Dermatol Venereol Leprol.* 2007;73:149-56. doi: 10.4103/0378-6323.32708.
4. Xu AE, Zhang DM, Wei XD, Huang B, Lu LJ. Efficacy and safety of tacrolimus cream 0.1% in the treatment of vitiligo. *Int J Dermatol.* 2009;48:86-90. doi: 10.1111/j.1365-4632.2009.03852.x.
5. Majid I. Vitiligo Management: An Update. *British Journal of Medical Practitioners.* 2010;3:a332.
6. Sisti A, Sisti G, Oranges CM. Effectiveness and safety of topical tacrolimus monotherapy for repigmentation in vitiligo: a comprehensive literature review. *An Bras Dermatol.* 2016;91:187-95. doi: 10.1590/abd1806-4841.20164012.
7. Salloum A, Bazzi N, Maalouf D, Habre M. Microneedling in vitiligo: A systematic review. *Dermatol Ther.* 2020;17:e14297. doi: 10.1111/dth.14297.
8. Singh A, Yadav S. Microneedling: Advances and widening horizons. *Indian Dermatol Online J.* 2016;7:244-54. doi: 10.4103/2229-5178.185468.
9. Savant SS. Surgical therapy of vitiligo: Current status. *Indian J Dermatol Venereol Leprol.* 2005;71:307-10. doi: 10.4103/0378-6323.16778.
10. Ebrahim HM, Elkot R, Albalate W. Combined microneedling with tacrolimus vs tacrolimus monotherapy for vitiligo treatment. *J Dermatolog Treat.* 2020;10:1-6. doi: 10.1080/09546634.2020.1716930.
11. Mina M, Elgarhy L, Al-Saeid H, Ibrahim Z. Comparison between the efficacy of microneedling combined with 5-fluorouracil vs microneedling with tacrolimus in the treatment of vitiligo. *J Cosmet Dermatol.* 2018;17:744-51. doi: 10.1111/jocd.12440.
12. Ebrahim HM, Albalate W. Efficacy of microneedling combined with tacrolimus versus either one alone for vitiligo treatment. *J Cosmet Dermatol.* 2020;19:855-62. doi: 10.1111/jocd.13304.
13. Passeron T, Ostovari N, Zakaria W, Fontas E, Larrouy JC, Lacour JP, et al. Topical tacrolimus and the 308-nm excimer laser: a synergistic combination for the treatment of vitiligo. *Arch Dermatol.* 2004;140:1065-9. doi: 10.1001/archderm.140.9.1065.
14. Nisticò S, Chiricozzi A, Saraceno R, Schipani C, Chimenti S. Vitiligo treatment with monochromatic excimer light and tacrolimus: results of an open randomized controlled study. *Photomed Laser Surg.* 2012;30:26-30. doi: 10.1089/pho.2011.3029.
15. Fai D, Cassano N, Vena GA. Narrow-band UVB phototherapy combined with tacrolimus ointment in vitiligo: a review of 110 patients. *J Eur Acad Dermatol Venereol.* 2007;21:916-920. doi: 10.1111/j.1468-3083.2006.02101.x.
16. Bhuvana K, Sarala N, Singh G, Kumar TN. Effect of 0.1% tacrolimus ointment in localized vitiligo: an open uncontrolled trial. *Indian J Dermatol.* 2011;56:445-6. doi: 10.4103/0019-5154.84735.