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Original article

Comparative Study between the Efficacy of Microneedling Combined with Trichloroacetic Acid versus Microneedling with Tacrolimus in the Treatment of Stable Vitiligo

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ABSTRACT

Background: Many treatment options were used for Vitiligo. But there is no consensus on the precise treatment modality.

Aim of the work: The study aimed to assess and compare the efficacy of microneedling combined with trichloroacetic acid [TCA] versus micro-needling with tacrolimus in the treatment of Vitiligo,

Patients and Methods: 60 Vitiligo were included and classified into two equal groups. Group [A] included 30 patients who received micro-needling with dermapen then tacrolimus 0.03 ointments every two weeks for six sessions. Group B included thirty patients who received micro-needling with dermapen then TCA 25% every two weeks for six sessions. The outcome was documented.

Results: The repigmentation was slightly higher in TCA-treated patches than with tacrolimus. Excellent response in repigmentation occurred in 43.3% of TCA patches. In contrast, in 16.7% of tacrolimus-treated patches, Good improvement occurred in 13.3% of TCA-treated patches while 23.3% of tacrolimus-treated patches. Moderate improvement occurred in 10.0% TCA -treated patches while 30.0% of tacrolimus-treated patches, mild improvement occurred in 13.3% of TCA -treated patches. In comparison, only in 16.7% of tacrolimus-treated patches, However, there was a great difference between the two drugs regarding erythema, inflammation, and exfoliations, which occurred mainly with TCA.

Conclusion: The mix of microneedling with either TCA 25% or tacrolimus is effective and safe in treating Vitiligo. However, TCA achieved a slightly increased percentage of repigmentation than tacrolimus.

Keywords: Vitiligo; Response; Microneedling; Tacrolimus; Trichloroacetic acid.

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* Main subject and any subcategories have been classified according to the research topic.

INTRODUCTION

Vitiligo is a common chronic dermatologic depigmentation disorder that affects about 1% of the overall population, and it is mainly a result of functional loss of epidermal melanocytes. Its pathogenesis explains the loss of melanocyte, including genetic factors, environmental triggers, metabolic alteration, and alter in the immune and inflammatory response^[1].

Treatment of Vitiligo has two fundamental goals: the primary is to prevent disease progression, and the second is to stimulate repigmentation in lesions for achieving an appropriate cosmetic view. Within the last years, many therapeutic modalities, included drug therapy, and operative interventions, are accepted for vitiligo^[2].

In stable cases, surgery had been proven to be effective by using melanocyte transfer, which can be done by punch mini-grafting, suction blisters, epidermal curettage techniques, split-thickness skin grafting, follicle transplantation, and cultured and non-cultured autologous melanocytes^[3].

Microneedling could be a method used for transdermal medication delivery to help the absorption of topical drugs. The application of a micro-needling device by derma pen can create micro-channels through the corneum. This accustomed increase the absorption of medicines, enhance the efficacy and reduce the amount of treatment^[4].

Vitiligo was known to be an autoimmune disease in nature. Topical immunomodulatory drugs, calcineurin inhibitors [like topical tacrolimus], have a very important role during this immunomodulatory mechanism because they inhibit calcineurin action, prevent T-cell activation, and inhibit the production of many inflammatory cytokines^[4].

Chemical trauma by trichloroacetic acid [TCA] induced repigmentation by simply destroying the basement membrane, introducing pigmentary incontinence, and reversing koebner phenomenon^[5].

Koebner phenomenon is defined as "the development of lesions at sites of traumatized uninvolved skin of patients with cutaneous diseases." Clinicians must introduce the Koebner phenomenon in surgical therapies when Vitiligo is unstable^[6].

AIM OF THIS WORK

The current work aimed to evaluate and compare the efficacy of microneedling combined with trichloroacetic acid [TCA] versus microneedling with tacrolimus within stable vitiligo treatment.

PATIENTS AND METHODS

The study included sixty patients with stable Vitiligo attending the Dermatology Department's outpatient clinic, Al-Azhar University Hospital [Damietta], from February to October 2020.

Inclusion criteria: Patients of both sexes with stable Vitiligo [VIDA +1,0 or 1].

Exclusion criteria: Patients with the following conditions were excluded; unstable Vitiligo, a history of keloidal tendency or anticoagulant use, coagulation and bleeding disorders, active infections, and liver diseases.

Ethical considerations: Informed written consent was obtained. The study protocol was also approved by the Local Institutional Review Board [IRP] [Damietta Faculty of Medicine, Al-Azhar University].

Included subjects were classified into two equal groups: Group [A] received micro-needling with dermapen then tacrolimus 0.03 ointments every two weeks for six sessions. Group B received micro-needling with dermapen, then TCA 25% every two weeks for six sessions.

In group A, the position chosen was cleaned by 70% alcohol then anesthetized with topical lidocaine cream applied under occlusion for about 30 minutes. The vitiligo area's micro-needling was then performed with dermapen with needle length [0.5-1mm] consistent with the treated site from one edge to a different by multiple, parallel side to side and top to downstrokes until multiple tiny, punctate, bleeding points appear. Control of bleeding was achieved by compressing a normal saline soaked gauge on the treated area then topical tacrolimus ointment [0.03%] was applied. A plastic sheet covered the patch for occlusion for a minimum of 6 hours, and patients were instructed to use the cream on their patches nightly. Every patient has received a session every 2 weeks for a maximum of 6 sessions^[4].

In group B, anesthetic cream application on the position chosen was achieved using topical lidocaine

cream, then micro-needling as type A, and TCA [25%] was applied topically. All patients were assessed by full history taking [age, gender, onset, duration, intensity, aggravating factors, and previous treatment] and clinical examination [to evaluate and score severity and stability of disease]. Furthermore, digital photography was taken at baseline, before and after each treatment session.

Types of used equipment: Dermapen [Figure 1] was Dr. Pen [model ultima A1-W], Wireless adjustable from 0.25 to 3 mm. It has 5 speeds and acts at 16000 rpm. Titanium needle was disposable Dermapen Tip [for preventing the possibility of cross-contamination]. Each tip ensures 36 perfectly placed microneedles in it. Titanium needle [Figure 2] is a disposable Dermapen Tip [for preventing the possibility of cross-contamination]. Each tip ensures 36 perfectly placed microneedles in it.



Figure [1]: Derma Pen



Figure [2]: Titanium needle

Drugs: tacrolimus [0.03%] ointment ,TCA [25%]. We used this concentration to ensure fewer side effects in previous studies.

Treatment evaluation: It was achieved by photographs and dermoscopy taken at baseline and

before and after each session. The repigmentation responses were graded as follows: No changes [0%], Mild repigmentation [1% - 25%], Moderate repigmentation [26%-50%], Good repigmentation [51%-75%] and Excellent repigmentation. [76%-100%].

Statistical analysis: Data were analyzed using IBM®SPSS® software package version 20.0. The Kolmogorov-Smirnov test was used to test the normality of distribution. Quantitative data were described using mean, standard deviation, and range [the difference between the minimum and the maximum], and comparison between two groups had been achieved by independent samples [t] test. Categorical data were presented in frequency and percent distributions, and groups were compared by Chi-square test or Mann-Whitney [U] test. The significance of the obtained results was judged at < 5% level.

RESULTS

Table [1] demonstrated that the tacrolimus group's age ranged between 8 and 59 years; the mean value was 36 ± 11 years. On the other side, the TCA group's age was ranged between 9 and 55 years with a mean age of 33.2 ± 5.78 years. Also, 26.7% were under or equal to 20 years; and 73.3% were older than 20 years. There were no differences between groups [P=0.604].

The repigmentation pattern was as follows: 16.7% had no pigmentation, 50.0% had diffuse repigmentation, and 33.3% had follicular repigmentation. There was a significant increase of follicular and a significant decrease of diffuse pigmentation in tacrolimus than TCA group [50.0%, 36.7% vs. 16.7% and 63.3%, respectively, $p = 0.023$] [Table 2].

Regarding side effects, pain during the session was equally distributed among two groups [reported in 46.7% of each group]. Additionally, erythema was reported in 53.3% and 73.3% of tacrolimus and TCA groups, respectively, with no significant difference between groups. Finally, exfoliations were reported in 56.7% of the TCA group compared to none in the tacrolimus group, with a significant difference between groups [Table 3].

The total degree of improvement was increased in the tacrolimus group compared to the TCA group [86.7% vs. 80.0%]. However, the difference was statistically non-significant. Also, mild, moderate, and

good results were higher and excellent results were lower in tacrolimus compared to the TCA group [16.7%, 30.0%, 2.3%, and 16.7% vs. 13.3%, 10.0%, 13.3%, and 43.3% successively]. The overall score was lower in tacrolimus than in the TCA group, and the difference was statistically insignificant [Table 4]. The response

after each session showed a non-significant difference between tacrolimus and TCA groups. After the fourth session in the TCA group and after the fifth session in the tacrolimus group, the highest response was achieved [Table 5]. Figures 3 to 6 represented some of the studied patients before and after treatment.

Table [1]: Age distribution among studied groups.

	Tacrolimus	TCA	Total	Test	P-Value
≤20 years	7[23.3%]	9[30.0%]	16[26.7%]	U=415.5	0.604
>20 years	23[76.7%]	21[70.0%]	44[73.3%]		
Min.-Max.	18-35	18-36	18-36		
Mean± S.D	25.9±6.04	25.2±5.78	25.6±5.87		

TCA: Trichloroacetic Acid

Table [2]: Pattern of repigmentation among studied groups

Pattern of repigmentation	Tacrolimus [n=30]	TCA [n=30]	Total N=60]	Test	P-Value
No	4[13.3%]	6[20.0%]	10[16.7%]	7.533	0.023*
Diffuse	11[36.7%]	19[63.3%]	30[50.0%]		
Follicular	15[50.0%]	5[16.7%]	20[33.3%]		

TCA: Trichloroacetic Acid; *: Significant

Table [3]: Side effects among studied groups

Side effect	Tacrolimus	TCA	Total	Test	P-Value
Pain during Session	14[46.7%]	14[46.7%]	28[46.7%]	0.001	1.000
Erythema	16[53.3%]	22[73.3%]	38[63.3%]	2.58	0.180
Exfoliates	0	17[56.7%]	17[28.3%]	23.72	<0.001*

TCA: Trichloroacetic Acid; *: Significant

Table [4]: Degree of improvement among studied groups

Degree of improvement	Tacrolimus	TCA	Total	Test	P-Value
No	4[13.3%]	6[20.0%]	10[16.7%]	7.885	0.096
Mild	5[16.7%]	4[13.3%]	9[15.0%]		
Moderate	9[30.0%]	3[10.0%]	12[20.0%]		
Good	7[23.3%]	4[13.3%]	11[18.3%]		
Excellent	5[16.7%]	13[43.3%]	18[43.3%]		
Min.-Max.	0-99	0-100	0-100	t=0.883	0.381
Mean± S.D	44.1±28.256	51.7±37.745	47.9±33.277		

TCA: Trichloroacetic Acid

Table [5]: Response after sessions

Response	Tacrolimus	TCA	Total	Test	p-Value
After 2 session	2[6.7%]	3[10.0%]	5[8.3%]	0.21	0.64
After 3 session	4[13.3%]	7[23.3%]	11[18.3%]	1.0	0.31
After 4 session	14[46.7%]	12[40.0%]	26[43.3%]	0.27	0.60
After 5 session	6[20.0%]	2[6.7%]	8[13.3%]	2.30	0.12

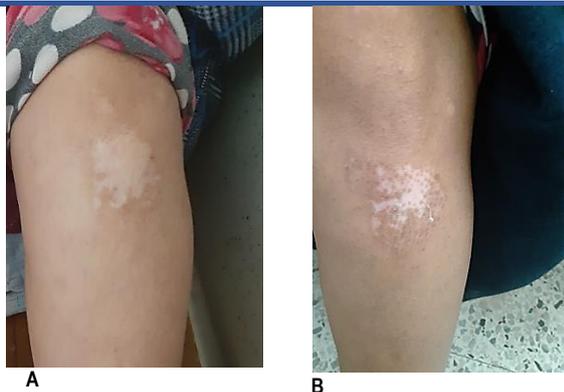


Figure [3]: A female patient, 35 years old, had Vitiligo for two years and was treated by 6 sessions of micro-needling followed by tacrolimus [A: before and B: after treatment].



Figure [4]: male patient, 63 years old, has Vitiligo for 3 years, treated by 6 sessions of micro-needling followed by TCA [A; before treatment, B: after treatment].



Figure [5]: Male patient 67 years old has Vitiligo of 1 year ago treated by 3 sessions of micro-needling followed by TCA 25%



Figure [6]: Female patient 15 years old has Vitiligo 8 years ago treated by 6 sessions of micro-needling followed by tacrolimus [A: before treatment, B: Before treatment]

DISCUSSION

Vitiligo is an autoimmune, asymptomatic depigmented disease that occurs due to loss of melanocytes function and may be related to other autoimmune disorders. It can affect the standard of life by its psychological impact [7]. Therapeutic options in Vitiligo have different modes of action, but it must be focused on the disease course and clinical presentation. Thus every patient needs a customized protocol of therapy. Till now there's no single treatment method has been found that's the foremost effective in the treatment of Vitiligo [8]. However, combined treatments have shown improvement of the repigmentation and reduction of the side effects and time needed for the treatment. This strategy has been more practical in subjects with lesions refractory or resistant to monotherapies[4].

In our study, microneedling was combined with either tacrolimus or TCA. This increased the deeper delivery of drugs through micropores created by microneedling and by pushing the functional

melanocytes, located within the normal skin around the lesions and pigmented hairs, toward the hypopigmented area. These combination therapies had worked synergistically for improving the efficacy and shorten the duration of therapy with earlier and greater regimentation. All the side effects observed in the current study were mild and acceptable. No patient had to discontinue the sessions due to the side effect, and every one of the side effects disappeared spontaneously without treatment. Erythema and Pain disappeared one day after the session, and exfoliations had disappeared within one week.

In our study, the first response was faster within the patches treated with tacrolimus than TCA. The repigmentation was appeared after 4 sessions [6 weeks] in 46.7% of the patches treated by tacrolimus combined with microneedling, and this was faster than using pimecrolimus 1% cream combined with microdermabrasion as studied by Farajzadeh *et al.*[9]. They found that 25.7% of the patches showed repigmentation after 10 sessions. This is explained by the fact that the microneedling device produced thin

vertical skin pores reaching into the mid dermis by creating small micro-channels that help deliver the topical drug to the basal's melanocytes cell layer. While microdermabrasion reaches only the stratum barrier, this needs to increase the number of sessions to deliver the melanocytes' topical drug. On the opposite hand, the primary repigmentation started in 40% of the patches treated by TCA combined with microneedling after 4 sessions, and this trusts Ibrahim *et al.*^[10]. Their study found four patients [40%] have repigmentation after the fourth session of TCA 25% peel.

In the current work, 44% of the patches treated with tacrolimus achieved a marked response, while 50% of patches treated with the tacrolimus combined with the 308-nm excimer laser showed response as studied by Kawalek *et al.*^[11]. This superiority may be due to the utilization of an excimer laser. Also, in another study, Park *et al.*^[12] found that the combination of tacrolimus plus excimer laser was significantly more effective than either therapy alone

Although this therapy is time-consuming, expensive, and its therapeutic effect on acral was poor, our finding in the tacrolimus group trusts Grimes *et al.*^[13] where they found 41.3% response. This study was done by 0.1% tacrolimus upon the group of mean age 38.4 years.

Our results differ from Bilal *et al.*^[14], who reported a good response in 53.3% by using 0.1% topical tacrolimus with narrow-band ultraviolet-B [UVB] [twice weekly for 12 weeks]. These differences are due to the various tacrolimus concentrations and the narrow band combination. This study is also different from the previous one using tacrolimus combined with excimer laser^[11] or with NB-UVB photo therapy^[17]; the acral parts dont show poor response with tacrolimus combined with microneedling. As, 58% of the acral patches [7 from 12 patches within the hands] achieved mild to moderate improvement [repigmentation 25%-50%], and 2 patches achieved good improvement [repigmentation >50%]. So, this combined therapy gave the impression to be superior to NB-UVB and excimer laser in efficacy, session duration, costs, time interval, and safety. On the opposite hand, the response to TCA during this study was 51.7%, and this was on top of the study of El-Mofty *et al.*^[15]. Two patients [20%] underwent TCA 15%, Four patients

[40%] with TCA peel 25%. The superiority in our study may be due to the employment of microneedling.

Also, it was different from Ibrahim *et al.*^[10] study which showed 70% marked response in patches treated by TCA 15%+ NB-UVB and 20% in patches treated by TCA 25% +NB-UVB, this superiority in their study may be due to the combination with NB-UVB. Our result also differ from Puri and Puri^[5] study as they reported that there is marked pigmentation [> 90%] was seen in 66.6% of patients, moderate pigmentation [61-90%] was seen in 13.3% of patients, and mild pigmentation [<60%] was seen in 20% patients. The probable causes of the difference in the degree of repigmentation are the concentration difference of TCA 25% and TCA 100% with relevance to different body sites. In the tacrolimus group, the rate of moderate to excellent repigmentation was [50%] on the knee, [75%] on an elbow, [83%] on the face, and [50%] on hands. The extremities show mild to the moderate response. as microneedling enhanced the response to tacrolimus even in resistant sites. This matches with the results of Fai D *et al.*^[16] who was reported that the response to tacrolimus plus NB-UVB was according to the location, being more responsible for face lesions [73%], then limbs [68%] and trunk [53.5%], but effects on the extremities and genital areas was quite disappointing.

Our results differ from Silverberg *et al.*^[17] findings, in which the response was 61% for head/neck regions and 47% for the trunk and/or extremities, as this study was performed upon children by 0.1% tacrolimus. Our results have also differed from Lepe *et al.*^[18]. They found tacrolimus produces over 75% repigmentation, mostly on facial areas. The probable causes of the differences are the difference of concentration in tacrolimus 0.03% versus 0.1% and the site of Vitiligo.

Regarding the side effects, Puri and Puri^[5] used TCA [100%] and reported that hyperpigmentation was seen in 20% of patients, hypopigmentation was seen in 13.3%, persistent erythema was seen in 6.6% of patients, superficial scarring and secondary bacterial infection were seen in 6.6% patients. But in our study, TCA peel used is of lower concentration [25%], it was relatively safe with neither systemic absorption nor toxicity were detected, only mild exfoliations in 17[56.7%], erythema in 22[73.3%], and none of them developed scarring, infection or koebnerization. Tacrolimus group had no complications at all the

patches treated by it during this study. Farajzadeh et al.^[9] also noted no side effects of the mix of pimecrolimus and microdermabrasion. They reported that this combination was safe and effective within vitiligo treatment.

This study's strength is that comparative test, which evaluates treatment effects in real-world settings; a positive lead to a test can inform practice because it provides evidence that the treatment/intervention is effective in usual practice. This study's weakness was the small sample size; future studies with a bigger group with different Vitiligo types are needed.

Conclusion: The microneedling plus TCA 25% and the microneedling plus tacrolimus effectively treat vitiligo with an accepted pattern of repigmentation of all age groups. Likewise, they are a safe alternative or additive method that may be used before, or together with, any approved method for stable vitiligo treatment.

Financial and Non-financial Relationships and Activities of Interest

None

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