

# Comparative study between the efficacy of micro-needling combined with 5-Fluorouracil versus micro-needling with trichloroacetic acid in treatment of stable vitiligo

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## Abstract

**Background:** Vitiligo is a common de-pigmenting disorder. Despite advancements in therapy, managing the condition remains challenging. Combining treatment approaches may improve outcomes.

**Objective:** To compare the efficacy and safety of microneedling with 5-fluorouracil (5-FU) versus trichloroacetic acid (TCA) 35% in stable vitiligo.

**Methods:** This single-blinded study was designed as interventional and comparative. Nineteen patients (43 patches) with stable vitiligo were enrolled and randomized into two groups. One group (10 patients, 22 patches) received microneedling with 5-FU, and the other (9 patients, 21 patches) received microneedling with 35% TCA. Eight biweekly sessions were performed. Follow-up was done 2 months after the final session.

**Results:** No significant difference was observed in mean re-pigmentation between the two groups. Both showed a significant reduction in lesion surface area. Excellent response was seen in 9.1% (5-FU) and 9.5% (TCA), while no or poor pigmentation occurred in 72.8% and 80.9%, respectively. Lesion site (5-FU group) and skin phenotype (TCA group) showed significant associations with pigmentation. An inverse correlation existed between re-pigmentation and lesion size in both groups.

**Conclusion:** Microneedling with either 5-FU or TCA appears safe, practical, and affordable for treating stable vitiligo. The mean repigmentation response was comparable in both groups.

**Keyword:** 5- fluorouracil; Trichloroacetic acid; Microneedling; Vitiligo.

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## Introduction

Vitiligo is a prevalent skin depigmentation disorder affecting 0.5-2% of the global population. Recent advances have identified it as an autoimmune disease linked to metabolic imbalance, oxidative stress, and genetic or environmental factors. Its psychosocial impact is often substantial, affecting patients' quality

of daily life.<sup>1</sup> Various therapies are used in vitiligo, including topical steroids, immunomodulators,

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phototherapy, oral pulses, antioxidants, surgery, lasers, and depigmentation techniques. Despite progress, outcomes remain suboptimal, with many patients experiencing partial or no re-pigmentation. Thus, combining treatments and exploring new approaches is often necessary.<sup>2,3</sup>

5-FU is a pyrimidine analog that interferes with DNA and RNA synthesis by inhibiting thymidylate synthase.<sup>4</sup> Recent studies support the use of 5-FU, topically or intralesionally, as an effective vitiligo treatment.<sup>5,6</sup> This effect may be attributed to the release of certain mediators and enzymes that enhance melanocyte activity and movement.<sup>6</sup>

Although the role of TCA in vitiligo treatment is not well studied, some reports suggest it may stimulate repigmentation by inducing inflammation of melanocytes followed by post-inflammatory hyperpigmentation.<sup>7</sup> Transdermal drug delivery techniques such as microneedling and laser-assisted methods have been shown to enhance skin permeability by creating microchannels that facilitate the absorption of topically applied agents like corticosteroids and 5-fluorouracil, while also avoiding the limitations of oral delivery such as poor bioavailability and systemic side effects.<sup>8,9</sup>

This study evaluates the safety and effectiveness of microneedling combined with either 5-FU or 35% TCA for managing stable vitiligo.

## Methods

This is an interventional, therapeutic, comparative, single-blinded study carried out at the Center of Dermatology and Venereology/ Baghdad Medical City, Baghdad, Iraq, between December 2021 and August 2023. Nineteen patients (43 patches) with stable vitiligo were included in this study. The diagnosis of vitiligo was made clinically and by the aid of Wood's light. A full history was taken from patients, including age, sex, duration of the disease, previous therapy, family history of vitiligo, previous episodes of repigmentation, other cutaneous or systemic diseases,

drug allergies, and history of hypertrophic scars, keloid, or koebnerization. In addition, a thorough dermatological examination was done for each patient to detect the skin phenotype and the type and sites of vitiligo.

The patients included in the study should have stable vitiligo for at least 6 months, a patch size of less than 10 cm and not have received topical or systemic treatment in the last 3 months.

Patients less than 10 years old, with keloidal or bleeding tendency, on anticoagulant therapy, active infections, active Koebner's phenomenon, unstable vitiligo in the last 6 months, receiving topical or systemic treatment for vitiligo for the last 3 months were excluded from the study.

Patients were fully informed about the nature, course and prognosis of the disease, the procedure, the number of sessions needed alongside the complications of micro-needling, TCA and 5-FU solution. Furthermore, informed consent was obtained from each participant or their parents at the initial visit (for being part of this study and for allowing us to share their photographs). Ethical approval was gained from the Scientific Council of Dermatology and Venereology, Iraqi Board for Medical Specializations.

The total number of participants in this study was 24 patients, 5 patients did not complete the study and 19 patients (with 43 patches) completed the study. The patients were assigned in to two groups. 5-FU Group was 10 patients (22 patches) treated with micro-needling and 5FU solution, TCA group was 9 patients (21patches) treated with micro-needling and TCA 35%. At least one patch in each patient was left as a control (a total of 25 patches). A total of eight sessions were done, consequently every two weeks (baseline, 2<sup>nd</sup> week, 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 10<sup>th</sup>, 12<sup>th</sup> and 14<sup>th</sup>). The patients were seen two months after the last session to evaluate the response and to report any side effects if present. The surface area of the treated patches was measured in centimeters using a transparent graph paper before

the first session and 2 months after the last session of treatment.

The micro-needling in both groups was done by using the derma pen Dr. Pen (model ultima A1-W supplied by Modern Technology company), with five adjustments of needle depth (from 0.25mm to 3 mm depth), five levels of speed, and works at 16000 rpm. The needle tip used was disposable and contained 36 needles with 33 gauge thickness and 0.5 mm needle size. The depth of micro-needling was chosen according to body site and as follows (0.25 mm for the eyelid, 1mm for other parts of the face and neck, 2mm for the trunk, 3mm for extremities).

***The 5-FU Group; micro-needling and 5-FU solution:***

Twenty-two patches were included in this group. Topical anesthesia Emla 5% cream (lidocaine 2.5% plus prilocaine 2.5%) (manufactured by Astra Zeneca, Sodertalje, Sweden) was put on the area intended to be treated and left there for 45 minutes. After anesthesia, the area was washed with distilled water and cleansed with 70% alcohol, micro-needling was done in the vitiliginous area and a thin surrounding rim of normal skin (about 2mm) in horizontal and vertical directions until pinpoint bleeding appeared.. Hemostasis was achieved using saline-soaked gauze over the treated area. A 50 mg/mL 5-FU solution (manufactured by Onko İlaç San, Kocaeli, Turkey) was applied using an insulin syringe and gently spread over the entire lesion. Another microneedling pass was then performed, followed by a second 5-FU layer. A semi-occlusive dressing was applied and removed after 24 hours.

***The TCA Group; micro-needling and TCA35%:***

Twenty-one patches were included in this group. The patients were treated in a similar manner as the 5-FU group apart from using TCA 35% (manufactured by THOMAS BAKER, Mumbai, India) instead of 5-FU.

Standardized photographs were captured at baseline, before each session, and 2 months after the final treatment using an iPhone XR camera, maintaining consistent distance, lighting, and location.

The evaluation was done by the same operator, the supervisor, and a third independent dermatologist. At the end of the study, depending on the percentage of re-pigmentation, the outcome was recorded according to the Investigator's Global Assessment (IGA);<sup>10</sup>

Grade I (G1) <25% repigmentation (poor);

Grade II (G2) 25%-50% repigmentation (fair);

Grade III (G3) 51%-75% repigmentation (good);

Grade IV (G4) >75% repigmentation (excellent).

Moreover, the response was measured according to the regression in the surface area (measured in square centimeters) of the treated patches.

Data were analyzed using IBM SPSS software, version 29 (IBM Corp., Chicago, IL, USA). Descriptive statistics such as frequency, percentage, mean, standard deviation, and range (minimum–maximum) were used.

To assess differences in quantitative variables, independent samples t-test was used for comparison between two unrelated groups, while paired samples t-test was applied for related data. ANOVA was employed for comparisons involving more than two groups. For categorical data, the Chi-square ( $\chi^2$ ) test was utilized, with Yates' correction or Fisher's exact test applied when appropriate. A p-value of  $\leq 0.05$  was considered statistically significant.

Pearson correlation analysis was conducted to evaluate relationships between continuous variables. Pearson correlation analysis was used to assess the relationship between two quantitative variables, and its significance was tested using a t-test. The correlation coefficient (r) could be either positive (direct) or negative (inverse). Values below 0.3 indicate no correlation, values between 0.3 and 0.5 suggest a weak correlation, between 0.5 and 0.7 indicate a moderate one, and values above 0.7 represent a strong correlation. Additionally, the coefficient of determination ( $r^2$ ) was calculated; for example, if  $r=0.58$ , then  $r^2=0.34$ , meaning that 34% of the variability in one variable can be explained by the other.

**Table 1** Baseline characteristic of the study groups.

		5FU		TCA		P value
		No.	%	No.	%	
Age (yr)	<20	6	60.0	8	88.9	0.153
	=>20	4	40.0	1	11.1	
	Mean±SD(Range)	26.0±12.8 (10-49)		18.0±5.8 (10-32)		
Sex	Male	2	20.0	3	33.3	0.510
	Female	8	80.0	6	66.7	
Skin phenotype	Type III	13	59.1	8	38.1	0.169
	Type IV	9	40.9	13	61.9	
Vitiligo type	Focal	3	13.6	5	23.8	0.689
	Segmental	8	36.4	7	33.3	
	Vulgaris	11	50.0	9	42.9	
Site	Face	4	18.2	4	19.0	0.761
	Neck	2	9.1	4	19.0	
	Trunk/ Back	6	27.3	7	33.3	
	Upper limb	1	4.5	1	4.8	
	Lower limb	9	40.9	5	23.8	

**Table 2** Re-pigmentation response in both study groups.

		5-FU		TCA		P value
		No	%	No	%	
Start of Re-pigmentation (weeks)	2	-	-	6	35.3	0.083
	4	12	75	11	64.7	
	6	4	25	-	-	
Pattern of re-pigmentation	Marginal	9	56.2	12	70.6	0.27
	Marginal & follicular	3	18.8	2	11.8	
	Marginal & diffuse	4	25	3	17.6	
Percent of re-pigmentation		22.1±26.6 (0-90)		22.6±25.7 (0-90)		0.957
Grade of re-pigmentation	No pigmentation	6	27.3	4	19.0	0.778
	Poor pigmentation (<25%)	10	45.5	13	61.9	
	Good pigmentation (25-50%)	3	13.6	1	4.8	
	Very good pigmentation (51-75%)	1	4.5	1	4.8	
	Excellent pigmentation (>75%)	2	9.1	2	9.5	

## Results

Baseline characteristics of the study groups summarized in **Table 1**.

Re-pigmentation response summarized in **Table 2**.

The surface area of patches in 5-FU group was decreased from 12 cm<sup>2</sup> before treatment to 9.9 cm<sup>2</sup> after treatment (mean decrease was 2.1±3.2) which was statistically significant (p-value=0.006). For the TCA group, the surface area of patches decreased from 5.8 cm<sup>2</sup> before treatment to 5 cm<sup>2</sup> after treatment (mean

decrease was 0.8±1.0) which was statistically significant (p-value=0.002).

In the 5-FU group, there was a statistically significant difference in the mean re-pigmentation rate in comparison with the site of the vitiligo patch (p value=0.003), the highest mean re-pigmentation was in the patches located on the face (63.8±28.7) followed by the lower limbs (17.2±19.9), upper limbs (15.0), trunk (8.7±7.3) and neck (5.0±7.1). In the TCA group, there was a statistically significant difference in mean re-pigmentation rate in comparison with skin phenotype with the highest mean re-pigmentation in



**Figure 1** Forty one years old female with segmental vitiligo on the face for 6 years duration treated with micro-needling and 5-FU with excellent pigmentation after 8 sessions. A: before and B: after of treatment.



**Figure 2** Thirty two years old female with segmental vitiligo on the face for 6 years duration treated with micro-needling and TCA with good re-pigmentation after 8 sessions, A: before and B: after treatment.



**Figure 3** 17 years old female with vitiligo on the eyelid for 2 years duration treated with microneedling and 5-FU with excellent pigmentation after only three sessions, A: before and B: after treatment.

skin phenotype III ( $40.4 \pm 34.4$ ) in comparison with ( $11.6 \pm 8.3$ ) in skin phenotype IV ( $p$  value=0.009). Regarding age, sex, type of vitiligo, duration, previous treatment, and family history, the difference was statistically not significant in both groups ( $p$ -value <0.05).

Representative clinical responses before and after treatment are shown in **Figures 1-3**. In the Pearson correlation analysis, there was a significant inverse correlation between re-pigmentation rate and lesion size in both groups ( $r = -0.66$ ,  $P = 0.001$  in the 5-FU group) and ( $r = -0.483$ ,  $p = 0.027$  in the TCA group).

Regarding the side effects, all patients in both groups experienced pain during the procedure, which was minimal, and did not need to stop treatment. In the 5-FU group, erythema was found in 14 (63.6%) patches and itching in 3 (13.6%) patches. In the TCA group, erythema was found in all patches, exfoliations in 14 (66.6%) patches, and erosions in 2 (9.5%) patches.

### Discussion

Vitiligo is a common disorder of depigmentation caused by the progressive destruction of melanocytes that affects the skin, hair, and mucous membranes, clinically presenting as depigmented macules and leukotrichia.<sup>11</sup> One of the key aspects in managing vitiligo is acknowledging that it is not merely a cosmetic concern, but a medical condition with effective and safe therapeutic options available.<sup>12</sup> A variety of treatment modalities exist, each working through distinct mechanisms, and their selection should be based on the clinical features and disease progression of each case. Therefore, treatment must be personalized to suit the needs of every patient. To date, no single therapy has been proven superior for all patients with vitiligo.<sup>13</sup> However, using combination approaches has demonstrated better outcomes in repigmentation, shortened treatment durations, and fewer side effects, particularly in patients who do not respond well to monotherapies.<sup>6</sup>

In this study, microneedling was combined with either 5-FU or TCA, enhancing drug penetration through micropores and facilitating melanocyte migration toward depigmented areas. This combination acted as a coadjuvant, improving outcomes and reducing treatment duration.

The start of response was faster in the patches treated with TCA than with 5-FU. No previous studies have compared 5-FU and TCA regarding the start of re-pigmentation. The re-pigmentation in the TCA group was faster than El-Nokaly et al. work which found that 40% of patches started pigmentation after 8 weeks (4 sessions) and 23.3% of patches after 6 weeks (3 sessions).<sup>14</sup> The faster response in this work may be explained by the higher concentration of TCA that has been used (35%) in comparison with El-Nokaly et al. study (25%). The start of re-pigmentation in the 5-FU group agrees with the Chhapra et al. study which found that 61.1% of patches started pigmentation after 1 month of treatment.<sup>15</sup> The start of the response in the present study was slightly faster than Mina et al; which found that 44% of patches started pigmentation after 6 weeks (3 sessions), 20% of patches started pigmentation after 4 weeks (2 sessions), and 28% of patches started pigmentation after 8 weeks (4 sessions).<sup>6</sup>

Regarding the mean re-pigmentation response, there was no statistically significant difference between the two groups. This finding disagrees with the Nofal et al. study, which found that combined micro-needling with TCA was associated with a higher degree of pigmentation in comparison with micro-needling and 5-FU. This higher response to the TCA group may be explained by the fact that their study used TCA at higher concentrations (30 % on the face and 50% in areas other than the face).<sup>16</sup>

Regarding the degree of re-pigmentation in the 5-FU group, the results were similar to the Pazyar et al. study, which found excellent pigmentation in only 15.8% of patches and no or poor response in 68 % of patches.<sup>13</sup> The results also agree with Nofal et al. who found excellent pigmentation in only 8% of patches and no or poor pigmentation in 60% of patches.<sup>16</sup>

The response in the present study was much lower than was observed by Chhapra et al. who found excellent pigmentation in 48.6% of patches. This may be explained by the larger sample size in that study (72

patches vs. 22 patches in the present study).<sup>15</sup> The lower response in this study compared to Mina et al. (48% excellent pigmentation) may be attributed to fewer treatment sessions (6 vs. 12) and less frequent 5-FU application (biweekly vs. daily).<sup>6</sup>

The exact mechanism of 5-FU in vitiligo remains unclear, but it is thought to promote repigmentation by enhancing melanocyte proliferation, inhibiting cytotoxic agents, and modulating immunity, thereby activating residual or follicular melanocytes.<sup>17</sup>

In the TCA group, the grade of re-pigmentation was lower than Nofal et al. who found excellent pigmentation in 28% of patches and no or poor pigmentation in only 24 % of patches, this difference may be due to the different concentrations of TCA used in their study (50% in comparison with 35 % used in the present study).<sup>16</sup>

The present work results were much lower than that of Al-Nokaly et al. study which used TCA in 25% concentration and found excellent pigmentation in 43.3% of patches and no or poor pigmentation in 33.3% of patients.<sup>14</sup>

The response was also lower than Ibrahim et al. who found good to excellent pigmentation (pigmentation >50%) in 53% of patients. The difference is that they use TCA in a concentration of 70% after micro-needling of acral patches only.<sup>18</sup>

TCA promotes repigmentation through controlled epidermal injury. The induced inflammation triggers melanogenesis, and during re-epithelialization, melanocytes migrate from hair follicles, eccrine glands, and adjacent normal skin.<sup>19</sup>

About different body sites, TCA showed excellent improvement (>75%) in lesions in different body parts (trunk and upper limbs), which means that no statistically significant difference in re-pigmentation regarding the site of the patch. This agrees with Nofal et al. and Al-nokaly et al. who did not mention the

difference in re-pigmentation rate concerning the site of the vitiligo patch.<sup>14,16</sup> On the other hand, Nofal et al. indicated that using TCA alone, at various concentrations, could serve as an affordable and well-tolerated option for vitiligo treatment, showing best results on the face and least on the hands and feet.<sup>3</sup>

While with 5-FU, there was a statistically significant relation between the site of the patch and re-pigmentation response as vitiligo of the face showed the best results (excellent improvement in 5- the FU group was only in patches located on the face). This agrees with Chhapra et al. and Pazyar et al. who also found that re-pigmentation response was different in different body parts but they did not use 5-FU on the face.<sup>15,17</sup> Facial vitiligo tends to show better therapeutic outcomes due to the increased melanocyte density, presence of follicular reservoirs, and higher sun exposure compared to other body areas.

In TCA, the mean re-pigmentation response was higher in skin phenotype III in comparison with type IV (40.4% vs 11.6% respectively) which was statistically significant ( $p$  value=0.009), this disagrees with Nofal et al. study which found that patients with darker skin phenotypes (IV, V) had better re-pigmentation than lighter skin phenotypes (I, II, III), this may be due to the larger sample size in his study (100 patches ) and different concentration used (30% for the eyelids, 50% for the face, neck, and trunk, 50% to 70% for the forearms and legs, and full strength 100% for the hands and feet).<sup>3</sup>

The present work revealed that the mean re-pigmentation rate in both groups showed no statistically significant difference regarding age, sex, duration of vitiligo, vitiligo type, and family history.

The results of this study reported that the mean surface area of the patches in the 5-FU group was significantly decreased after treatment compared to that preoperatively ( $P=0.006$ ). In addition, in the TCA group, the surface area of the patches was significantly decreased after treatment compared to preoperatively

( $P=0.002$ ). Moreover, Pearson correlation analysis revealed a significant inverse correlation between re-pigmentation rate and lesion size in both groups. In the same accordance, Santosh et al study, regarding the surface area, reported that for small patches of vitiligo, 5-FU after needling is a cost-effective, safe, and easy method of treatment with minimal adverse effects in spite of it is not reasonable for larger patches of vitiligo.<sup>20</sup> The Nofal et al. study, which used TCA in varying strengths for vitiligo therapy, found that smaller patches tended to yield better outcomes compared to larger ones.<sup>3</sup>

Side effects were mild and temporary in both groups and did not require discontinuation of therapy in any case.

## Conclusion

Micro-needling technique combined with both 5-FU and TCA is a simple, safe, and cost-effective treatment modality for stable vitiligo with negligible adverse effects. No difference was found between the 5-FU and TCA regarding the mean re-pigmentation response. Micro-needling combined with 5-FU shows excellent re-pigmentation only on the face while micro-needling combined with TCA shows excellent re-pigmentation on areas other than the face like upper extremities and trunk. Skin phenotype III showed better re-pigmentation than type IV. Both modalities of treatment showed significant re-pigmentation on small patches while little re-pigmentation on large patches.

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