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Original Article

Evaluation of Efficacy of Microneedling and Topical Methotrexate Versus Microneedling and Topical 5-Flourouracil in Treatment of Vitiligo Patients

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Abstract

Background: Vitiligo is a common skin disease, characterized by the selective loss of basal cell layer melanocytes, which in turn leads to loss of pigment in the affected areas of the skin.

Aim of work: to evaluate the efficacy of microneedling with topical methotrexate versus microneedling with topical 5-fluorouracil in management of vitiligo patients.

Subjects & methods: this randomized trial involved 30 patients aged ≥ 1۸ years with stable vitiligo. Three patches in each patient were treated by 6 sessions as follows: The 1st patch (A) was received microneedling followed by methotrexate (MTX) 25 mg\ml solution application. The 2nd patch (B) was received microneedling followed by 5-flurouracil (5-FU) 50mg\ml solution application. The 3rd patch (C) was received microneedling followed by saline application.

Result: our study revealed significantly better outcomes in patches treated by microneedling and MTX or 5-FU solutions compared to microneedling with saline. VASI scores improved more in the MTX and 5-fluorouracil groups (28.5% and 31.03%) than in the saline group (15.8%). After 3 months follow up, 60% of patients in the MTX and 5-fluorouracil groups achieved good to excellent repigmentation, while 40% in the saline group had poor results.

Conclusion: our study demonstrated that microneedling combined with either topical methotrexate or 5-fluorouracil is a safe and effective modality for vitiligo treatment. Both combination therapies showed superior efficacy compared to microneedling alone, with high patient satisfaction rates and manageable side effects.

Key words: Microneedling, Topical Methotrexate, MTX, Topical 5-Flourouracil, Treatment, Vitiligo.

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Introduction:

Vitiligo as a depigmenting skin disease is characterized by selective destruction of melanocytes, which in turn leads to loss of pigment in the diseased skin. [1] It is considered as autoimmune disorder with genetic background and environmental factors together with metabolic, oxidative stress and cell detachment abnormalities .[2] Due to its multifactorial pathomechanism, It is challenging for dermatologists to find a single therapeutic option that can correct autoim-munity, stimulate the repigmentation, and differentiation of melanocyte stem cells, and effectively prevent the recurrence of the disease. So far, modalities of vitiligo treatment ideally consists of combination therapy.[3, 4]

5-Flourouracil (5-FU) which is an antimetabolite analogue of the naturally occurring pyrimidine uracil is metabolized through the same metabolic pathways as uracil. [5] Due to its antimitotic action, topical 5-Flourouracil is a used for treat-ment of many dermatological diseases charact-erized by a high rate of mitotic proliferation. [6,7]

Clinically, it was observed that localized hyperpigmentation has occurred during systemic treatment of various cancers by 5-Flourouracil. Usually, these hyperpigmented lesions are located on the normally pigmented skin of extremities (hands and feet) and mucosa of the tongue. [8,9]

One of the most interesting observation about the biological effect of 5-Flourouracil on melanocytes has been reported by experimental studies. In the presence of low concentrations of 5-Flourouracil, epidermal keratinocytes are selectively destroyed within three weeks, while basal melanocytes continue to proliferate and to form pigment. [10]

Methotrexate as an antifolate drug with antimetabolite activity is a time-tested effective agent extensively used in treatment of various autoimmune disorders in low to moderate doses with good efficacy and tolerability on a long-term course .[11]

Methotrexate treatment leads to reduction in the number of TNF-α-producing T cells, while the number of T cells producing IL-10 after polyclonal activation increased, in another study. [12]

Further, methotrexate suppresses TNF-α-induced nuclear factor-κB activation through the release of adenosine, with resulting anti-inflammatory, antiproliferative and immunomodulatory effects [13]

Moreover, modulations of IL-6 production and reactive oxygen species synthesis also add to the therapeutic effects of methotrexate . [11]

Unlike oral methotrexate, topical preparations of the drug, that was used for the treatment of localized lesions, showed non-significant hepatotoxic or hematologic adverse effects. [14]

Microneedling technique creates abundant microwounds that directly stimulate the injured tissue to release various growth factors that play a direct role in stimulation of collagen and elastin synthesis and deposition within the dermis. [15]

This leads to the release of fibroblast growth factor , platelet derived growth factor and transforming gro-wth factor alpha and beta (TGF α and TGF-b) lead-ing to neovascularization and neocollagenesis. [16]

Trauma increase melanin synthesis through creation of inflammatory response ranging from tyrosine kinase induction in early stage to the late upregulation of growth factors, proteases, and extracellular matrix components. This leads to increase in melanin synthesis and transfer of melanosoms to the surrounding keratinocytes . [17]

Microneedling acts also by increasing transdermal drug delivery because of application of drugs using the microneedle device allows the drug molecules to cross the stratum corneum layer with more drug molecules enter the skin. The important benefits of this technology are the rapid onset of action, better patient compliance, increa-sed permeability and efficacy. [18]

This study aims to evaluate the safety and efficacy of microneedling with topical methotrexate versus microneedling with topical 5-fluorou-racil in manegement of vitiligo patients.

Patients and Methods

This study was a prospective, randomized controlled clinical study approval by research and ethical committee at faculty of medicine, Sohag university. informed written consent obtained from all participants after explanation of nature of the study. The study continued from September 2022 to January 2024. The study included 30 patients with stable vitiligo who had attended the dermatology outpatient clinics of Sohag University Hospital and Sohag General Hospital.

Inclusion Criteria were; Patients aged 18 years and older, having at least three separate patches of depigmentation and Presence of stable vitiligo, (no new lesions or extension of preexisting lesions for 6months) while

Exclusion Criteria were; Previous history of keloid formation, Presence of systemic diseases such as diabetes or bleeding disorders and Current receiving of chemotherapy or radiotherapy.

All patients in this study were subjected to: complete history taking including; Personal history of name, age, sex, residence, marital status, occupation, and relevant medical habits, Clinical History of the onset, course, duration, and treatment of vitiligo, therapeutic history of any drug intake, family history of any similar conditions and medical history of any chronic illness. General examination was done to detect any associated systemic illness and clinical examination for Assessment of the type of vitiligo. Evaluation of the extent of lesions using the Vitiligo Area Scoring Index (VASI) and photographic and dermoscopic documentation prior to start of treatment.

Intervention Protocol: The intervention focuses on comparing the efficacy of application of three different topical medications following microneedling in patients with stable vitiligo. Each patient had three separate vitiligo patches, each receiving a distinct treatment. In each enrolled patient three patches of vitiligo were randomly divided by simple randomization into three groups; Group of patch A, Group of patch B and Group of patch C

Patch A: This patch undergone microneedling, followed by the application of a methotrexate solution at a concentration of 25mg/ml. The solution had been applied immediately after microneedling. Patch B: This patch undergone microneedling, followed by the application of a 5-fluorouracil solution at a concentration of 50mg /ml, applied immediately after microneedling. Patch C: This patch undergone microneedling, followed by the application of a saline solution, serving as a control.

Microneedling Procedure: Patients undergone a total of six treatment sessions, each spaced two weeks apart. After application of topical anesthesia for half an hour before the sessions, an automatic dermapen device (vibrating frequency: 6500-10000 rpm, speed level 5, Ultima A6 model, Dr. Pen, Korea). A new needle had been used for each patient, the needle length had been adjustable from 0.25mm to 2mm, tailored to the treatment area's skin thickness, Betadine solution was used for sterilization before starting each treatment session.

Complementary Treatments: all patients will receive narrowband UVB light therapy twice a week for the duration of the treatment sessions and during follow up period. This was to enhance the treatment effects and promote repigmentation.

Patient evaluation : The effectiveness of each treatment had been assessed through various methods including VASI score, [19] photographic analysis, dermoscopic evaluation, patient satisfaction surveys, and safety assessment of treatment, These evaluation measures were done before each of the 6 sessions to monitor the progress and any adverse reactions.

Patient follow up after the end of the 6 treatment sessions had been conducted monthly, continuing for three months post-treatment including VASI score, photographic analysis, dermoscopic evaluation, and patient satisfaction surveys.

(1) VASI Score (Vitiligo Area Scoring Index) [19]:

This involves calculating the percentage of vitiligo involvement in terms of hand units. One hand unit approximately equates to 1% of the total body surface area.

The degree of pigmentation in each patch is estimated and categorized as:100% (Complete depigmentation), 90% (Minimal specks of pigment), 75%, 50%, 25% (Increasing degrees of pigmentation), 10% (Almost complete pigmentation with only specks of depigmentation).

The VASI for each body region was calculated by multiplying the area of vitiligo (in hand units) by the extent of depigmentation within each hand unit. The total body VASI is the sum of all body sites. [19]

(2) Photographic Assessment:
Standardized photographs of the treated patches were taken at each evaluation point and the visible repigmentation had been graded as follows; Poor (0-25% repigmentation), Good (26-50% repigmentation), Very Good (51-75% repigmentation), Excellent (76-100% repigmentation) **.**

(3) Dermoscopic Evaluation:
A dermoscope had been used to closely examine the skin and assess the signs of repigmentation, particularly focusing on perifollicular pigmentation. Dermoscopic evaluation was performed by Dermlite I polarized and non-polarized dermoscope at 2.1 magnification in polarized mode, and photographs were captured by Redmi Note 10S phone.

(4) Patient Satisfaction Survey:
A 5-point scale ranging from 1 (very unsatisfied) to 5 (very satisfied) was done

(5) Safety Assessment:
Comprehensive general and dermatological examination after each treatment session to detect any complications, side effects, or adverse reactions were done.

Statistical analysis: Data had been recorded in excel sheet. Data analysis had been carried out using Statistical Package for Social Science (SPSS) program. Continuous variables had been presented as mean ± standard deviation (SD) if data are normally distributed and as median (25^{th}&75^{th} percentiles) if data show wide range of variation. Categorical variables had been recorded as frequency and percentage and had been compared using Chi-square test. Statistical significance had been considered when $p < 0.05$.

Results

This randomized trial was conducted on 30 patients in whom the age ranged between 18 and 66 with a mean ±SD of 30.9 ± 12.6 years, the majority of cases were females representing about 77% (n=23) .Only 6.7% (n=2) of cases had positive family history. All cases had non-segmental vitiligo. Respecting the lesions site; 70% (n=21) had upper or lower limb lesions, 36.7% (n=11) had lesions of head/neck and 26.7% (n=8) had trunk lesions.

As regard the effect of treatment modalities on VASI score of the studied lesions; There was insignificant difference between the studied groups as regard mean VASI score before starting any of the treatment sessions ($p>0.05$). For repeated measures (from 1^{st} session till 3-months follow-up), there was significant reduction in the mean VASI score in the three studied treatment groups i.e, group patch-A (from 0.35 to 0.25, $p < 0.001$), with improvement 28.5% group patch-B (from 0.29 to 0.20, $p = 0.007$), with improvement 31.03% patch-C (from 0.19 to 0.16, $p = 0.034$), with improvement 15.8%. The VASI score reduction was more evident in patch-A and B as indicated by interaction analysis ($p = 0.021$). (photo 1,2) The variation in improvement was insignificant between groups of patch **A** and **B.** The effect of different treatment modalities on the VASI score over the study period is illustrated in (table 1) and (figure 1)

Table 1: Effect of different Treatment modalities on the VASI score among studied lesions:

	Patch-A	Patch-B	Patch-C	P-value
1st Session	0.35 ± 0.2	0.29 ± 0.1	0.19 ± 0.1	
2nd Session	0.35 ± 0.1	0.29 ± 0.2	0.19 ± 0.1	
3rd Session	0.35 ± 0.1	0.29 ± 0.2	0.17 ± 0.1	
4th Session	0.34 ± 0.1	0.29 ± 0.1	0.17 ± 0.1	
5th Session	0.31 ± 0.1	0.27 ± 0.1	0.17 ± 0.1	
6th Session	0.30 ± 0.1	0.26 ± 0.1	0.16 ± 0.1	
1-month FU	0.29 ± 0.1	0.25 ± 0.1	0.16 ± 0.1	
2-months FU	0.27 ± 0.1	0.23 ± 0.2	0.16 ± 0.1	
3-months FU	0.25 ± 0.1	0.20 ± 0.2	0.16 ± 0.1	
P-value* **	**< 0.001**	**= 0.007**	**= 0.034**	**= 0.021$**
Improvement in VASI score				
Reduction	0.10 ± 0.1	0.09 ± 0.2	0.03 ± 0.1	**= 0.040***
• **P-value****	A vs. B = 0.324	**B vs. C=0.041**	**A vs. C = 0.034**	
Percentage of improvement in VASI score %	28.6% ± 1.5	31% ± 3.5	15.8% ± 1.2	**= 0.023***
• **P-value****	I vs. II = 0.302	**II vs. III=0.026**	**I vs. III = 0.029**	

***One-way ANOVA test was used to compare the mean difference between groups**
****Post-hoc test was used for pairwise comparison with Tukey's correction**
*****Repeated measure ANOVA was used to compare the mean difference within group**
$Two-way ANOVA test was used to compare interaction between group and time

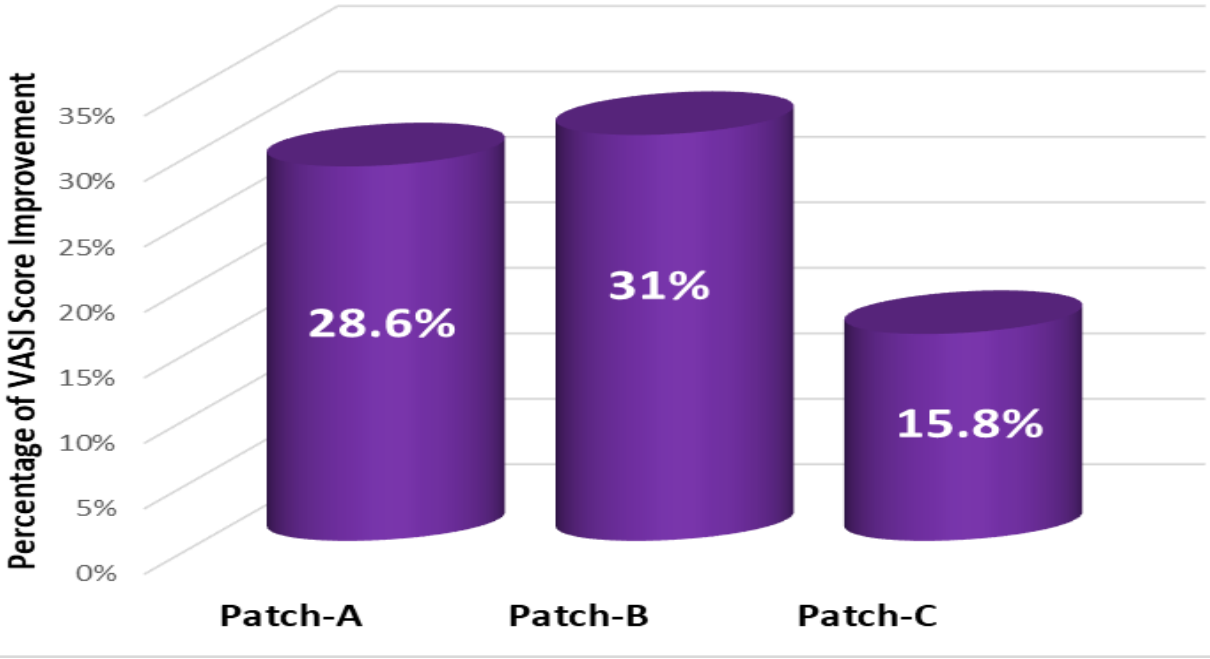


Fig. 1: Difference in the VASI score Improvement% between groups

The dermoscopic finding of the studied lesions during treatment sessions and follow up period revealed significant difference between the stud-ied groups as regard the dermoscopic findings for peri-follicular pigmentation, perilesional pigmen-tation and complete repigmentation ($p < 0.05$). (Photo 1,2) Moreover, significant difference was found as regard the change in dermoscopic findings for Paches A and B ($p < 0.05$) this illustrated in (tables2, 3).

Table 2: Effect of Treatment on the Dermoscopic Findings among studied lesions during sessions

	Pach-A	Patch-B	Patch-C	P-value
1st Session				
❖ **Milky White de-pigmen.**	30 (100%)	30 (100%)	30 (100%)	= 1.000*
❖ **Peri-Follicular Pigmen.**	2 (6.7%)	2 (6.7%)	2 (6.7%)	= 1.000*
P-value	A vs B=1.000	B vs C=1.000	A vs C=1.000	
❖ **Perilesional Pigmen.**	3 (10%)	3 (10%)	3 (10%)	= 1.000*
P-value	A vs B=1.000	B vs C=1.000	A vs C=1.000	
2nd Session				
❖ **Milky White de-pigmen.**	30 (100%)	30 (100%)	30 (100%)	= 1.000*
❖ **Hypopigmentation**	1 (3.3%)	0 (0%)	0 (0%)	= 0.954*
❖ **Peri-Follicular Pigmen.**	3 (10%)	3 (10%)	2 (6.7%)	= 0.541*
P-value	A vs B=1.000	B vs C=0.640	A vs C=0.640	
❖ **Perilesional Pigmen.**	15 (50%)	13 (43.3%)	6 (20%)	= 0.037*
P-value	A vs B=0.605	B vs C=0.052	**A vs C=0.015**	
3rd Session				
❖ **Milky White de-pigmen.**	30 (100%)	30 (100%)	30 (100%)	= 1.000*
❖ **Hypopigmentation**	1 (3.3%)	0 (0%)	0 (0%)	= 0.951*
❖ **Peri-Follicular Pigmen.**	4 (13.3%)	4 (13.3%)	2 (6.7%)	= 0.294*
P-value	A vs B=1.000	B vs C=0.389	A vs C=0.389	
❖ **Perilesional Pigmen.**	19 (63.3%)	17 (56.7%)	6 (20%)	**= 0.031 ***
P-value	A vs B=0.589	**B vs C=0.003**	**A vs C<0.001**	
4th Session				
❖ **Milky White de-pigmen.**	30 (100%)	30 (100%)	30 (100%)	= 1.000*
❖ **Hypopigmentation**	2 (6.7%)	1 (3.3%)	0 (0%)	= 0.587*
❖ **Peri-Follicular Pigmen.**	6 (20%)	4 (13.3%)	2 (6.7%)	= 0.125*
P-value	A vs B=0.488	B vs C=0.389	A vs C=0.129	
❖ **Perilesional Pigmen.**	19 (63.3%)	17 (56.7%)	6 (20%)	**= 0.038 ***
P-value	A vs B=0.589	**B vs C=0.003**	**A vs C<0.001**	
5th Session				
❖ **Milky White de-pigmen.**	28 (93.3%)	28 (93.3%)	29 (96.7%)	= 0.842*
❖ **Hypopigmentation**	2 (6.7%)	1 (3.3%)	1 (3.3%)	= 0.804*
❖ **Peri-Follicular Pigmen.**	6 (20%)	5 (16.7%)	3 (10%)	= 0.239*
P-value	A vs B=0.861	B vs C=0.345	A vs C=0.278	
❖ **Perilesional Pigmen.**	19 (63.3%)	21 (70%)	10 (33.3%)	**= 0.024***
P-value	A vs B =0.584	**B vs C=0.004**	**A vs C=0.020**	
❖ **Complete Re-pigmentation**	2 (6.7%)	2 (6.7%)	1 (3.3%)	=0.598*
P-value	A vs B=1.000	B vs C=0.389	A vs C=0.389	
6th Session				
❖ **Milky White de-pigmen.**	27 (90%)	27 (90%)	29 (96.7%)	= 0.894*
❖ **Hypopigmentation**	3 (10%)	1 (3.3%)	2 (6.7%)	= 0.666*
❖ **Peri-Follicular Pigmen.**	6 (20%)	6 (20%)	1 (3.3%)	**= 0.046***
P-value	A vs B=1.000	**B vs C=0.044**	**A vs C=0.044**	
❖ **Perilesional Pigmen.**	19 (63.3%)	21 (70%)	10 (33.3%)	**= 0.024***
P-value	A vs B =0.584	**B vs C=0.004**	**A vs C=0.020**	
❖ **Complete Re-pigmentation**	3 (10%)	3 (10%)	1 (3.3%)	= 0.408*
P-value	A vs B=1.000	B vs C=0.301	A vs C=0.301	

*Monte Carlo exact test was used to compare the difference in Frequency between.

Table 3: Effect of Treatment on the Dermoscopic Findings among studied lesions during follow up periods:

	Patch-A	Patch-B	Patch-C	P-value
1-month FU				
❖ Milky White de-pigmen.	26 (86.7%)	26 (86.7%)	28 (93.3%)	= 0.751*
❖ Hypopigmentation	3 (10%)	1 (3.3%)	2 (6.7%)	= 0.224*
❖ Peri-Follicular Pigmen.	7 (23.3%)	6 (20%)	1 (3.3%)	**= 0.047***
P-value	A vs B =0.754	**B vs C=0.044**	**A vs C=0.023**	
❖ Perilesional Pigmen.	19 (63.3%)	21 (70%)	10 (33.3%)	=0.024 *
P-value	A vs B =0.584	**B vs C=0.004**	**A vs C=0.020**	
❖ Complete Re-pigmentation	4 (13.3%)	4 (13.3%)	2 (6.7%)	=0.598*
P-value	A vs B=1.000	B vs C=0.389	A vs C=0.389	
2-months FU				
❖ Hypopigmentation	3 (10%)	1 (3.3%)	2 (6.7%)	**= 0.624***
❖ Milky White de-pigmen.	25 (83.3%)	25 (83.3%)	28 (93.3%)	= 0.524*
❖ Peri-Follicular Pigmen.	7 (23.3%)	7 (23.3%)	1 (3.3%)	**= 0.035***
P-value	A vs B=1.000	**B vs C=0.023**	**A vs C=0.023**	
❖ Perilesional Pigmen.	19 (63.3%)	21 (70%)	10 (33.3%)	**= 0.024***
P-value	A vs B =0.584	**B vs C=0.004**	**A vs C=0.020**	
❖ Complete Re-pigmentation	5 (16.7%)	5 (16.7%)	2 (6.7%)	= 0.171*
P-value	A vs B=1.000	B vs C=0.228	A vs C=0.228	
3-months FU				
❖ Milky White de-pigmen.	23 (76.7%)	24 (80%)	28 (93.3%)	= 0.123*
❖ Hypopigmentation	3 (10%)	1 (3.3%)	3 (10%)	= 0.681*
❖ Peri-Follicular Pigmen.	8 (26.6%)	7 (23.3%)	2 (6.7%)	**= 0.045***
P-value	A vs B=0.766	B vs C=0.071	**Avs C=0.038**	
❖ Perilesional Pigmen.	19 (63.3%)	21 (70%)	10 (33.3%)	= 0.024 *
P-value	Avs B=0.584	**B vs C=0.004**	**A vs C=0.020**	
❖ Complete Re-pigmentation	7 (23.3%)	6 (20%)	2 (6.7%)	= 0.186*
P-value	A vs B=0.754	B vs C=0.129	A vs C =0.071	

*Monte Carlo exact test was used to compare the difference in Frequency between groups

After 3months of follow up, clinical assessment revealed significant difference between the studied groups of patch (**A** or **B)** compared with patch **C** as regard grade of repigmentation. Group of patch **C** showed the lowest grade of repigmentation with poor repigmentation in 40% of cases. While groups of patch(**A** or **B)** had a higher degree of repigmentation with about 60% of cases achieved good to excellent repigmentation {60%, 60.1% respectively }. (table 4)

Table 4: Effect of different Treatment modalities on the Grade of Re-pigmentation.

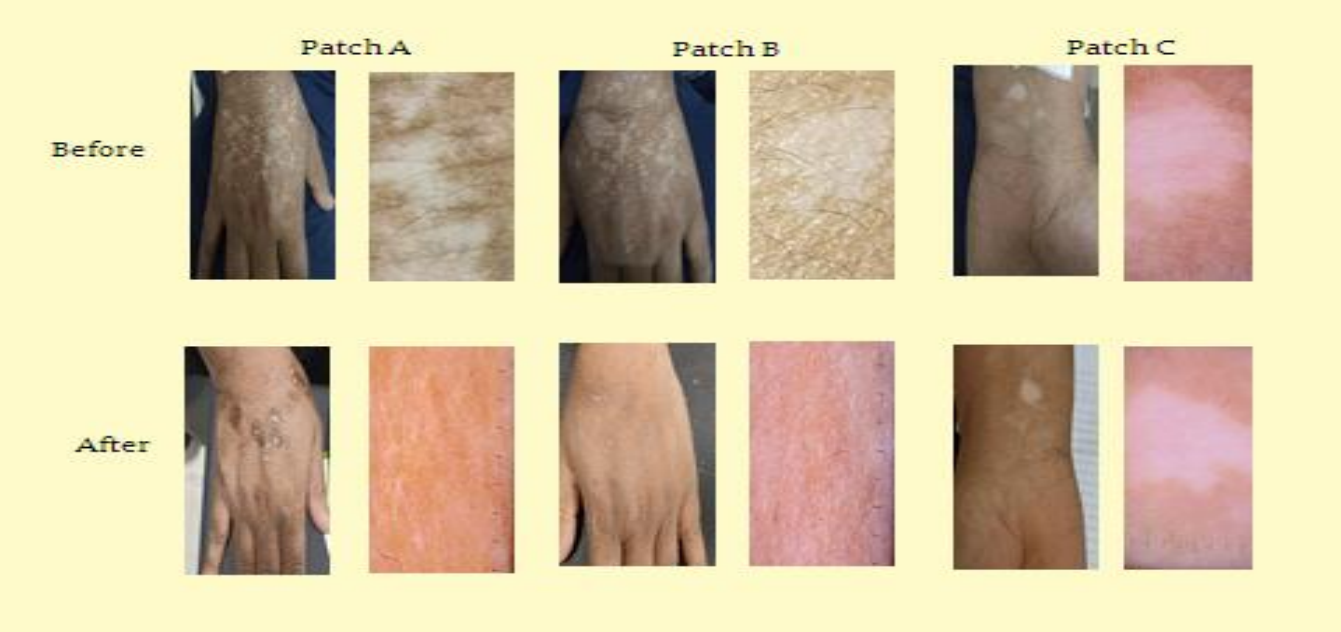
	Pach-A	Patch-B	Patch-C	P-value
Re-pigmentation Pattern				
❖ Poor (0%)	8 (26.7%)	8 (26.7%)	12 (40%)	= 0.086*
❖ Satisfactory (< 25%)	4 (13.3%)	4 (13.3%)	2 (6.7%)	
❖ Good (25% - 50%)	6 (20%)	8 (26.7%)	8 (26.7%)	
❖ Very Good (50% - 75%)	6 (20%)	2 (6.7%)	4 (13.3%)	
❖ Excellent (> 75%)	6 (20%)	8 (26.7%)	4 (13.3%)	

***Monte Carlo exact test was used to compare the difference in Frequency between groups**

Analysis of VASI score improvement in the studied lesions according to their anatomical sits showed that the improvement was insignificantly (p = 0.354) higher in UL/LL and head/Neck (32% and 30%) than trunk (26%) for Patches-A. Similarly for B (p=0.412), higher in UL/LL and head/Neck (34% and 31%) than trunk (27%). Also, it was non-significant (p = 0.214) in patch-C, in UL/LL, head/Neck and trunk (19%, 10% and 15%) (table 5)

Table 5: Relationship between Lesion Site and VASI score %. Improvement

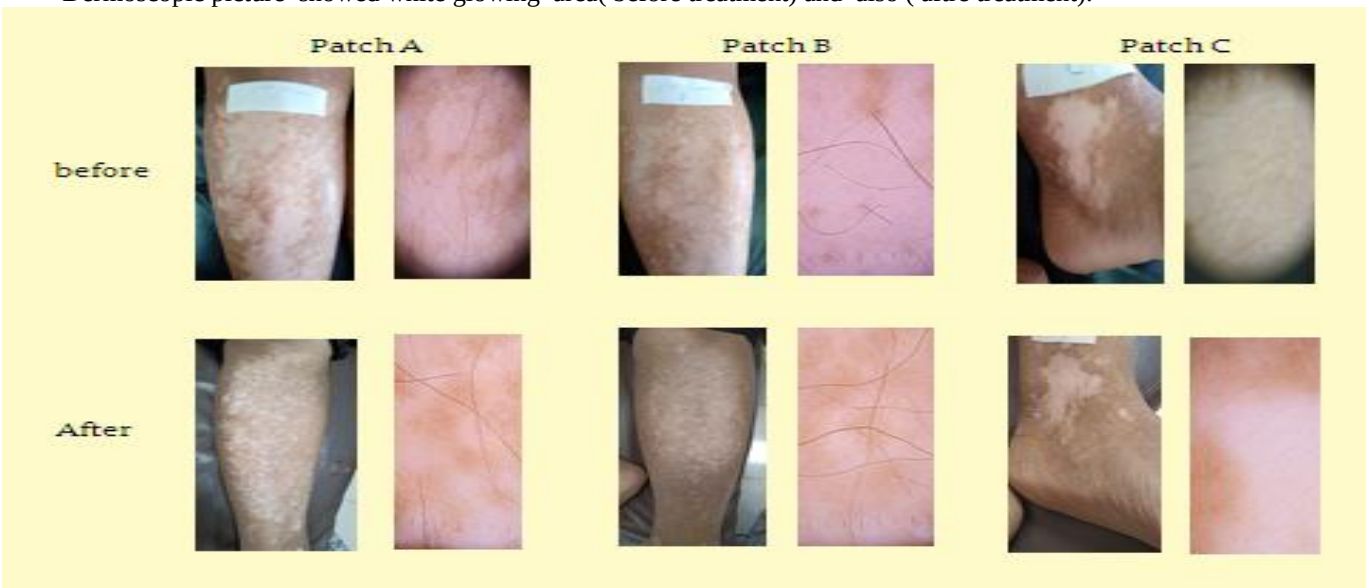
	Pach-A	Patch-B	Patch-C
VASI score% Improvement			
❖ **Head/Neck**	32%	34%	19%
❖ **Upper/Lower Limb**	30%	31%	10%
❖ **Trunk**	26%	27%	15%
P-value*	= 0.354	= 0.412	= 0.214



Patch A showed improvement in VASI score = 0.25 (before treatment) to VASI score = 0.01 (after treatment) and the Dermoscopic picture showed white glowing area(before treatment) and Complete repigmentation (aftre treatment)

Patch B showed improvement in VASI score =0.25 (before treatment) to VASI score = 0 (after treatment) and the Dermoscopic picture showed white glowing area (before treatment) and complete repegmintation (aftre treatment)

Patch C showed minimal improvement in VASI score = 0.05 (before treatment) to VASI score = 0.04 (after treatment) and the Dermoscopic picture showed white glowing area(before treatment) and also (aftre treatment).



Patch A showed improvement in VASI score = 0.25 (before treatment) to VASI score = 0.01 (after treatment) and the ermoscopic picture showed white glowing area(before treatment) and Complete repigmentation (after treatment).

Patch B showed improvement in VASI score =0.25 (before treatment) to VASI score = 0 (after treatment) and the Dermoscopic picture showed white glowing area (before treatment) and complete repegmintation (after treatment).

Patch C showed minimal improvement in VASI score = 0.05 (before treatment) to VASI score = 0.04 (after treatment) and the Dermoscopic picture showed white glowing area (before treatment) and also (after treatment).

As regard adverse reactions after sessions; the majority of patients had pain and erythema (93.3%, and100%) respectively. unlikely, no case had KP ,infection nor scars.

As regard patient's satisfaction after the end of the study duration. the majority of cases 66.6% was either satisfied or very satisfied .so there was significant difference (p = 0.042). (table 6)

Table 6: Effect of treatment on patients' satisfaction.

Patient's Satisfaction		= 0.042*
• Very Unsatisfied	0 (0.0%)	
• Dissatisfied	3 (10%)	
• Unsure	7 (23.3%)	
• Satisfied	9 (26.7%)	
• Very satisfied	11 (36.7%)	

***Binomial Z-test was used to compare Frequency**

Discussion:

Vitiligo is considered the most common disorder of skin pigmentation, It is characterized by the disappearance of skin pigment, due to the loss of basal melanocytes. Its prevalence ranged from 0.06-2.28%. worldwide. [1]

Melanocytes are located in the basal layer of the epidermis and form with the surrounding keratinocytes the melanocyte- keratinocyte epidermal unit, whose main function is to synthesize melanin by a complex process called melanogenesis and to distribute it to surrounding keratinocyts . [20]

As regard its pathogenesis, Vitiligo is considered as an autoimmune disorder with genetic background in which environmental factors together with metabolic, oxidative stress and cell detachment abnormalities play a role. [2]

Treatment strategies aim to stop the disease progression, enhance repigmentation and prevent recurrence. [21]

MTX is a folic acid antagonist that act by decreasing the number of T cells producing TNF-α, consequently having immunomodulatory , anti-inflammatory and antiproliferative actions that can play a role in management of vitiligo .[22]

Topical 5-FU helps treatment of vitiligo through stimulation of melanocytes in the hair follicles with migration during process of epithelization and by increasing the number of melanosomes reach keratinocytes through dendrites. The efficacy of 5-FU as a monotherapy has been reported by **Tsuji and Hamada.** [23]

Microneedling technique act by the creation of abundant micro holes in the skin that directly stimulates the release of multiple growth factors that play a role in stimulation of collagen synthesis and elastin production and deposition within the dermis. [15]

This leads to the secretion of fibroblast growth factor, platelet derived growth factor and transforming growth factor alpha and beta (TGF α and TGF-b) that lead to neovascularization and neocollagenesis . [16]

Microneedling affects also by transdermal drug delivery because of administration of drugs using the microneedling allows the drug particles to pass through the stratum corneum layer, thus allowing more drug particles to enter the skin. The beneficial effects of this technique are the rapid onset of action, better patient compliance, increased permeability and efficacy of therapeutic agents . [18]

The aim of this work was to evaluate the efficacy of microneedling with topical methotrexate versus microneedling with topical 5-fluorouracil in management of vitiligo patients.

Finding of our study revealed positive improving effect of combing MTX or 5-flourouracil with microneedling in treatment of vitiligo than control group who treated by microneedling alone and this is approved by assessment of VASI score, degree of improvement with repigmentation and patient satisfaction.

The positive improving action of microneedling for treatment of vitiligo is in agreement with that reported by a systematic review by **Roohaninasab et al.** [24] concluded that microneedling process is a safe and effective technique and an adjuvant therapy for vitiligo .

While the positive improving action of 5-flourouracil with microneedling was in accordance with finding of **Zahra et al.** [25] or **Pazyar et al.** [26] who reported that using micro-needling technique in conjunction with topical 5-FU could treat vitiligo patients more efficiently than topical monotherapy with tacrolimus.

Also **Hegazy et al.** [27] who compared the clinical efficacy of microneedling and topical 5-fluorouracil versus microneedling and topical latanoprost in the induction of skin repigmentation in localized stable vitiligo patients. Assessment of the VASI scores of both groups post-treatment and at the baseline, it was found that there was a significant reduction in the VASI scores due to the influence of both modalities in that study.

In agreement to our finding **Abdou et al.** [28] assessed the therapeutic effect of topical 5-FU and microneedling on VASI scores for a period of three months, observing a decrease in mean of VASI score (3.5 ± 2.8) and median (2.6) values post-treatment relative to pretreatment values (mean of 4.4 ± 2.7 and a median of 4.1) (Wilcoxon = 4.5, $p < 0.001$). Their study generated a 20% excellent response.

On other hand only one previous case report by **Abdelmaksoud et al.** [22] support with positive effect of topical MTX gel for treatment of vitiligo as a topical agent.

In a case report; application of topical MTX 1% gel twice daily for 3 months in a patient with stable vitiligo produce repigmentation. No side effects were reported. However, further studies are required to evaluate the efficacy and safety of MTX **.** [22]

The variation in results of previous study as regard the degree of improvement in comparison to our study can be attributed to variability in number of patients, duration of treatment, associated other modalities in therapy and clinical type of treated vitiligo, so that in our study we insist to compare the 3 patches in the same patient and we find that VASI score improvement is slightly higher in 5-flourouracil treatment than MTX treatment compared to control patch.

Our findings revealed a significant reduction in VASI scores across all three treatment groups, with patches A (microneedling + methotrexate) and B (microneedling + 5-fluorouracil) showing greater improvement than patch C (microneedling + saline). The mean VASI score reductions were 28.5%, 31.03%, and 15.8% for patches A, B, and C, respectively. These results suggest that the addition of topical methotrexate or 5-fluorouracil to microneedling enhances the repigmentation process in vitiligo lesions.

As regarded dermoscopic evaluation of treatment efficacy, although it was used in the few last years to evaluate efficacy of treatment as **Wang et al.** [29] who aimed to evaluate the efficacy of dermoscopy in the assessment of the therapeutic effect of a combination of 308-nm excimer laser and tacrolimus ointment in treatment of localized vitiligo. Dermoscopy revealed that repigmentation islands presents in two main forms, the marginal type of repigmentation and the central type, which refer to the appearance of pigmentation islands in the normal skin at the edge of white patch and at the center of white patch, respectively. Dermoscopy proved to be a valuable tool for accurate and scientific evaluation of vitiligo therapy. We are the first to use it in evaluation of the efficacy of microneedling and topical 5-flourouracil or MTX solution .and this revealed significant differences between treatment groups in terms of peri-follicular pigmentation, perilesional pigmentation, and complete repigmentation. The higher rates of peri-follicular and perilesional pigmentation in patches A and B compared to patch C further, this supports the enhanced efficacy of combining microneedling with active topical agents.

In our study, the predominant repigmentation pattern was marginal repigmentation followed by perifollicular repigmentation in patches A and B that showed higher grades of repigmentation compared to patch C.

This in consistent with finding of **Yang et al.** [30] who reported that , marginal and diffuse repigmentations were more commonly associated with topical therapies.

As regard the pattern of repigmentation, **Attwa et al.,** [31] agreed with the current study and found a perifollicular and marginal repigmentation patterns were noted after treatment with microneedling and 5- FU.

Also **Neinaa et al.,** [32] found that perifollicular and diffuse repigmentation are most prominent after microneedling with latanoprost.

This is somewhat different from the findings of **Ebrahim et al.** [33] who reported a mix of perifollicular and diffuse patterns with microneedling plus topical steroids. The difference might be due to the varied mechanisms of action of the topical agents used, combination with narrow band sessions or the shorter duration of our study.

In our study, after 3months of follow up we found that there was significant difference between the studied groups of patch **A** or **B** compared with patch **C** as regard grade of repigmentation. We found that group of patch **C** had the lowest grade of repigmentation with poor repigmentation in 40% of cases .while groups of patch(**A** or **B)** had a higher grade of repigmentation with about 60% of cases achieved good to excellent repigmentation {60%, 60.1% respectively).

This is in agreement with **Mina et al.** [34] who found that excellent result was 48% and 4% very good response in patients treated with microneedling and 5-FU this was significantly higher than patients treated by microneedling and tacrolimus

Also, another study conducted on fifty patients with stable yet resistant vitiligo and subjected to microneedling followed by 5- FU application every two weeks for 3 months, resulted in 60% total response of repigmentation in the lesions, complete pigmentation in very small patches and 40% did not have any response. [35]

On the other hand, in an Egyptian study by **Attwa et al.** [31] also investigated the additional effect of needling and 5-FU to needling alone in 27 localized vitiligo patients for 3 months, reported a better response in the combination of needling and topical 5-FU, yet the results of improvement were 3.7% excellent response and 3.7% very good response, higher result in our study can be explained by comping treatment with narrowband sessions and longer duration of study

Also in agreement to our study **Galal et al.**[36] who aimed to assess the efficacy and safety of topical 5-fluorouracil application in 0.5% concentration in management of resistant vitiligo either alone or in combination with microneedling. They reported excellent to very good response in 56.7% of patients treated with microneedling and 5-fluorouracil 0.5% versus 36.6% in patients treated with topical 5-fluorouracil 0.5% only. Our results revealed the same finding as the studies that used topical 5-fluorouracil in concentration 5% as **Shashikiran et al.** [37] a study in which topical 5% fluorouracil and microneedling showed good to excellent response in 75% of the patches.

Higher result than our study was reported by **Zahra et al.**[25] who aimed to evaluate the efficacy and safety of topical 5% 5-fluorouracil with microneedling and topical 5% 5-fluorouracil alone in mangement of stable vitiligo. At the end of 6 months, excellent response (>75% repigmentation) was reported in 47% patches of Group A and only in 4.3% patches of Group B patients, on the other hand <50% repigmentation was reported in 6.8% and 87% patches of Group A and Group B respectively. The higher result due to longer duration of treatment (6months) than our study (3months)

In agreement with our finding several trials that combine microneedling with another therapeutic modality have shown increased effectiveness in stimulating regimentation in vitiligo lesions. These also demonstrated reduced duration of treatment with less reported side effects. [38,39]

The speculated mechanisms of repigmentation after process of microneedling include injury-induced inflammation inciting proliferation of keratinocytes along with melanocytes during healing process, influx of cytokines leading to activation of melanocytes in the margin of the patch or outer root sheath of pigmented hair follicle,[40] and mechanical migration of melanocytes through physical transport of melanocytes with the needle from the pigmented skin to the depigmented skin so that they can serve as a source of melanogenesis. [41]

In our study, while it was not statistically significant, we observed a trend towards better improvement in extremities and head/neck areas compared to the trunk. This trend was consistent across all treatment groups.

Hegazy et al. [27] reported that, as regard to different body sites, 5-FU showed excellent to good response in different body sites specially legs, knees, arms, and one case of foot lesions showed good improvement.

Mina et al.,[34] and **Shashikiran et al.,** [37] on 5-FU, listed that truncal and extremities lesions were the best responders to 5-FU while acral lesions showed the least improvement. in our studied group there was no acral vitiligo and better response in face and extremities can be explained by presence of hair and exposure to sun in most of these locations.

In our study, the safety profile of the treatments and occurrence of side effects were generally favorable, with the majority of patients

experiencing pain (93.3%) and erythema (100%). Importantly, no cases of koebnerization, infection, or scarring were observed. The absence of more serious adverse events in our study supports the safety of combining microneedling with topical methotrexate or 5-fluorouracil in vitiligo treatment. However, the high rates of pain and erythema underscore the need for proper patient counseling and potential pain management strategies in clinical practice.

Those finding agreed with **Attwa et al.** [31] and **Mina et al.** [34] both also reported a minimal side effect with 5-FU use of transient pain and itching, and absence of systemic complication.

The adverse effects of occurrence of erythema and itching were in agreement with a study by **Shashikiran et al.** [37]. However, they reported pain with the procedure in all the patients (100%). The lesser occurrence of pain in our study can be explained by the usage of topical anesthetic cream before the procedure.

Also, this in agreement with **Mohaghegh et al.**[42] who reported that the process of microneedling was well tolerated in patients, and it was a quite simple and easy procedure for the dermatologist, and there was also no reported long-lasting adverse effect.

Our results showed high levels of patient satisfaction, with 66.6% of patients reporting being either satisfied or very satisfied with the treatment outcomes. This high satisfaction rate is encouraging and aligns with the objective improvements observed in VASI scores and dermoscopic findings. Patient satisfaction is a crucial aspect of vitiligo treatment, given the psychological impact of the disease, and our results suggest that microneedling combined with topical agents can significantly improve patient-reported outcomes.

Conclusion:

Our study demonstrated that microneedling combined with either topical methotrexate or 5-fluorouracil is an effective and safe treatment option for vitiligo. Both combination therapies showed superior efficacy compared to microneedling alone, with high patient satisfaction rates and manageable side effects. Finding from our study open a new era for usage of methotrexate solution topically in treatment of vitiligo lesions.

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