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Comparing the efficacy of Micro-needling Alone versus Micro-needling with Topical 5-Fluorouracil in Treating Stable Non-Segmental Vitiligo

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ABSTRACT

Background: Vitiligo is a commonly acquired, idiopathic, heritable white macule which increases in diameter with time due to melanocytes loss. Several treatment modalities, ranging from medical to surgical interference, are available. Many studies stated that inducing injury combined with topical 5Fluoro-uracil (5-FU) induces skin re-pigmentation in vitiligo lesions. Microneedling is a reasonably, inexpensive, easy and fast evaluating tool present in outpatient clinics as a trans-dermal drug delivery device for high molecular-weight drugs to enhance their absorption and effect.

Objectives: Evaluation of the efficacy of micro-needling plus topical 5-FU in treating stable non-segmental vitiligo and comparing its results with micro-needling alone. **Patients and Methods:** Fifty patients complaining of stable, non segmental vitiligo were recruited for our study, where we picked two patches; one patch was treated with micro-needling without adding any medicine, while we treated the other with micro-needling with 5-FU added. The sessions were every 2 weeks, and each patient received 6 sessions in 3 months.

Results: On the micro-needling alone side, none (0%) of studied participants showed repigmentation, while in micro-needling with 5FU side, 38 (76%) of patients showed repigmentation response, out of which 23 cases (46.0%) had mild repigmentation (<25%) whereas the remaining 15 cases (30.0%) had moderate repigmentation (25-50%).

Conclusion: Micro-needling with 5-FU is considered a safe, easy, and tolerable procedure for vitiligo treatment.

Significance: Micro-needling and 5 Fluorouracil are well known treatments, used frequently in aesthetic and skin malignancy fields; can be helpful also in pigmentary problems by delivering the drug and creating an inflammatory environment also helping the transportation of melanocytes to the vitiliginous area.

Keywords: Vitiligo, Micro-needling, and 5 Fluorouracil.

INTRODUCTION

Vitiligo is one of the most common acquired skin diseases that happens to 1-2 % of the people of the world [1]. Pathogenesis of vitiligo is unknown, but the auto-immune theory is supported by several factors: Many auto-immune disorders may present incoherence with it, and antibodies against melano-cytes were found in 10 % of cases with vitiligo. Also, inflammatory infiltrates were observed in the margins of the active lesion. In the bio-chemical hypothesis, the destruction of melano-cytes is contributed to changes in oxidative stress. The increase of hydrogen peroxide molecules with higher superoxide dismutase activity in vitiligo cases, re-inforces this hypothesis [2].

Another theory is the melano-cytorrhagy hypothesis, where the defect in melanocyte adhesions leads to trans-epidermal detachment and loss, leading their exposure to auto-antigens and immune system activation with subsequent melanocytic damage [3].

Re-pigmentation patterns for vitiliginous patches are: a) Peri-follicular; when the re-pigmentation is mainly follicular; b) Marginal; re-pigmentation is from the patches margins, and c) diffuse; re-pigmentation occurs diffusely across the whole patch [4]. The re-pigmentation response of patients with vitiligo to NUVB phototherapy happens by activating the dormant melano-cytes in the epidermis and hair follicles [5]. 5-fluorouracil (5-FU) is a pyrimidine analog that exerts potent anti-mitotic activity against skin tumors such as solar keratosis and some basal and squamous cell skin tumors [6]. Clinically, localized hyper-pigmentations happened during its systemic usage; in treating various malignancies. Usually, these hyper-pigmented patches appear on the customarily pigmented extremities (upper and lower limbs) and the tongue. These hyper-pigmentations may be explained as post-inflammatory hyperpigmentation in friction-related areas [7].

Aim of the work was to evaluate the efficacy of micro-needling plus topical 5-FU. in treating stable non-segmental vitiligo and comparing its results with micro-needling alone.

PATIENTS AND METHODS

Fifty cases with stable, Non segmental vitiligo participated in the research, and Wood's lamp assessed them.

Ethical consent:

The study followed the ethics approval of the Research Ethical Committee of the Faculty of Medicine, Ain Shams University (FMASU MS 20/2019). Also, an acquired approval consent from every patient interested in being included in the study after clarification of strategy. The Declaration of Helsinki for human beings, which is the international medical association's code of ethics, was followed during the conduct of this study.

Inclusion criteria:

1. Patients with non-segmental vitiligo.
2. Lesions were steady (no appearance of new lesions, change in the size of existing patches, or Koebner phenomena for one year).

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3. Diverse body locations (face, neck, upper and lower limbs, trunk, and acral areas).

Exclusion criteria:

4. Pregnant lactating patients.
5. Patient under 18 years of age.
6. Patients who used any skin or foundational treatment for 3 months earlier to our study.

Dermatological assessment:

Vitiligo Area Severity Index (VASI) is a normalized, delicate technique to measure the degree level of de / repigmentation presented by **Hamzavi *et al.*** [8].

The association level is determined in terms of hand units ; each hand unit is around identical to one % of the whole body surface region. Level of pigmentation is assessed closest to one of following rates: (100 %) = complete de-pigmentation without any pigment at all, (90%) = a shade of color is present, (75%) = de-pigmented region surpasses pigmented territory, (50%) = pigmented /de-pigmented regions are equivalent, (25%) = pigmented region surpasses de-pigmented zone, (10%) = just bits of de-pigmentation present. VASI for each body locale is determined utilizing the following recipe, which thinks about commitments of all body areas (conceivable range: 0 – 100): VASI = P (all body size) (hand units) x (de-pigmentation) [9].

Methods:

In each patient, we picked two patches; one patch was treated with micro-needling without adding any medicine, while we treated the other with micro-needling with 5-FU added. The sessions were every 2 weeks, and each patient received 6 sessions in 3 months. Micro-needling of the region was performed with dermaroller at 0.50-0.75 mm depth until numerous little punctate blood drops showed up. The second patch of the same case, we did micro-needling using 5-Fluorouracil 5 % (50 mg/mL) as ampoule [Utoral ®, E-I-M-C united Pharmaceuticals, Egypt].

A photo of the patient's lesions with each visit till the end of treatment sessions was taken.

Treatment Evaluation:

The patients were analyzed for proof of perifollicular, minimal or diffuse repigmentation, koebnerization, or appearance of new vitiliginous lesions. For target evaluation of repigmentation we applied Vitilligo Extent Score for a Target Area (VESTA) utilizing reference pictures of both peripheral perifollicular repigmentation recipe for figuring all out repigmentation rate (%) **(Figure 1)** [1].

Figure (1): VESTA scoring

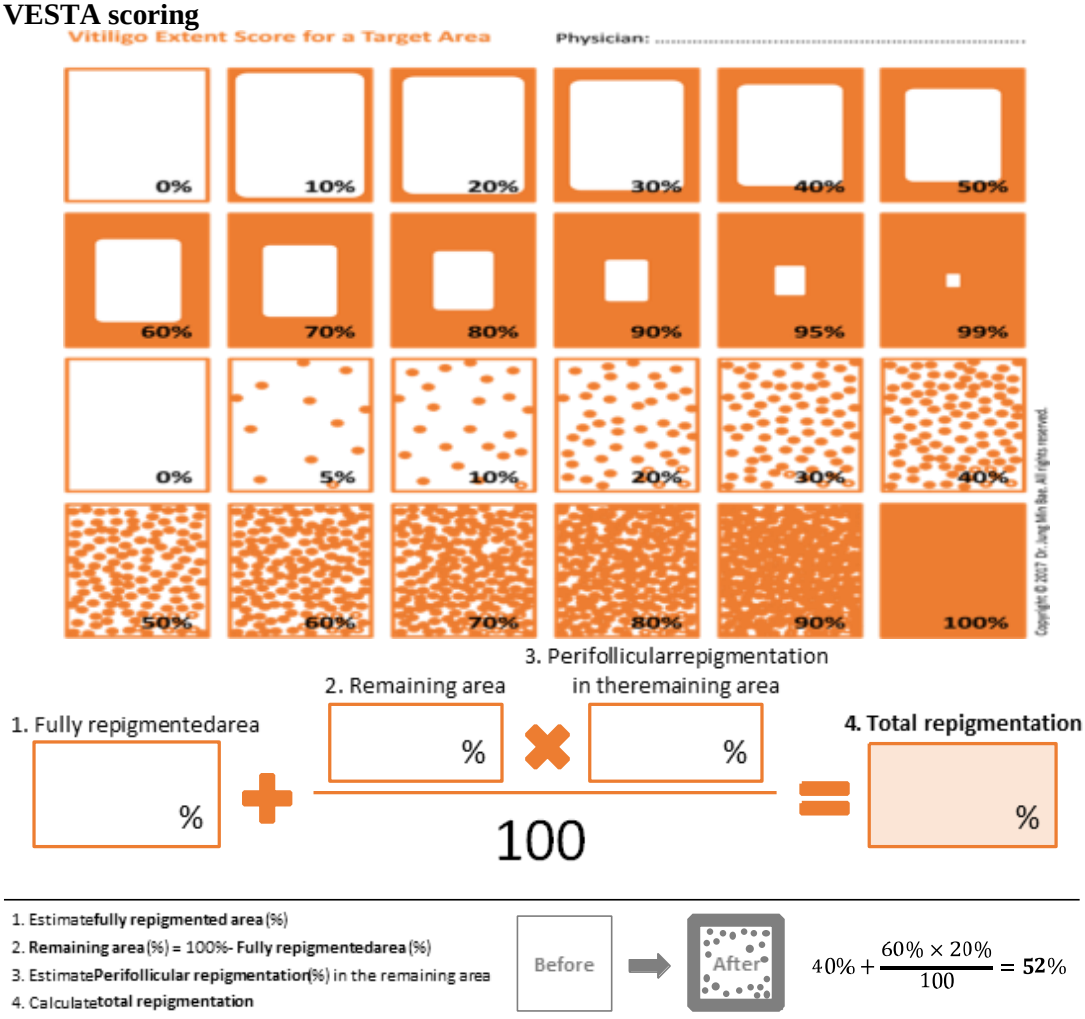

Figure (1): VESTA scoring of marginal peri-follicular re-pigmentation and calculating the rate of re- pigmentation [10].

According to Global Assessment Scale, depending level of repigmentation, the treatment reaction was reviewed as follows: No reaction (0%) repigmentation, Mellow reaction (<25%) repigmentation, Moderate reaction (25 -<50%) repigmentation, Great reaction (≥50–75%) repigmentation, Magnificent reaction (≥75–100%) repigmentation [11, 12].

Statistical analysis

The collected data were coded, processed and analyzed using the SPSS (Statistical Package for Social Sciences) version 24 for Windows® (IBM SPSS Inc, Chicago, IL, USA). Data were tested for normal distribution using the Shapiro Walk test. Qualitative data were represented as frequencies and relative percentages. Chi square test ($\chi 2$) to calculate difference between two or more groups of qualitative variables. Quantitative data were expressed as mean ± SD (Standard deviation). Independent samples t-test was used to compare between two independent groups of normally distributed variables (parametric data). P value < 0.05 was considered significant.

RESULTS

Clinical and demographic data of patients

Fifty adult patients with stable non segmental vitiligo were recruited for this study; 12 cases (24%) were males and 38 cases (76%) were females. Age ranged from 20 58 years (mean ± SD= 37.20±10.90).

11 cases (22%) with a family history, and the mean duration of the vitiligo ranged from 2-15 years (M ± SD=7.82± 2.27) years. Regarding the severity of disease cases, assessment with VASI score ranged from 10-68 (mean ± SD= 36.16±15.0).

Skin phototype of cases according to Fitzpatrick scale was (8.0%) Type II (4 cases), (54.0%) Type III (27 cases), (36.0%) Type IV (18 cases) (2.0%) Type V (only one case) **(Table 1).**

Table (1) shows; no statistical significant differences between responses microneedling plus 5-FU side according to patients' age, gender, VASI score, Fitzpatrick skin phototype, and family history disease duration.

Table (1) : Relation between repigmentation of micro-needling plus 5-FU side; regarding age, gender, VASI score, Fitzpatrick skin phototype, family history disease duration.

Parameters	No-repigmentation (n=12)	Mild (n=23)	Moderate (n=15)	t/x2#	p-value
Age (years)	35.94±7.78	36.67±11.47	35.80±8.72	0.383	0.471
Gender					
Female	9 (75%)	17 (73.9%)	12 (80.0%)	0.206#	0.561
Male	3 (25%)	6 (26.1%)	3 (20.0%)		
VASI score	31.36±7.88	36.80±13.09	32.00±11.62	1.616	0.195
Fitzpatrick skin phototype					
Type II	1 (8.3%)	2 (8.7%)	1 (6.7%)	2.823#	0.432
Type III	7 (58.3%)	12 (52.2%)	8 (53.3%)		
Type IV	4 (33.3%)	8 (34.8%)	6 (40.0%)		
Type V	0 (0.0%)	1 (4.3%)	0 (0.0%)		
Family history					
Positive	3 (25.0%)	4 (17.4%)	4 (26.7%)	1.381	0.682
Negative	9 (75.0%)	19 (82.6%)	11 (73.3%)		
Disease duration (years)	8.37±2.43	7.61±2.38	8.44±2.33	0.952	0.291

Table (2) shows; highly statistical significant differences between groups according to response; micro-needling side, no one (0%) of the studied participants showed repigmentation, while on micro-needling with 5-FU side, 12 cases (24%) showed no re-pigmentation and 38 (76%) of cases showed a re-pigmentation response.

Table (2): Comparison between micro-needling side only micro-needling plus 5-FU according to the response.

Lesion	Microneedling side only (n=50)	Microneedling & 5fu (n=50)	x2	p-value
Negative	50 (100%)	12 (24%)	58.107	<0.001**
Positive	0 (0%)	38 (76%)		

Table (3) shows that micro-needling with 5-FU side; 12 cases (24.0%) had no-repigmentation, 23 cases (46.0%) had mild repigmentation (<25%) and the remaining 15 cases (30.0%) had moderate repigmentation (25-50%).

Table (3): Distribution of cases according to their percentage of repigmentation micro-needling 5FU side (n=50)

Outcome	No.	%
No-repigmentation	12	24.0%
mild repigmentation <25%	23	46.0%
Moderate repigmentation 25-50%	15	30.0%
Total	50	100%

Twenty-seven cases (71.10%) of the positive repigmented side of patients (Micro-needling with 5-FU) showed a marginal repigmentation pattern. In comparison, the other 11 cases (28.9%) showed marginal + follicular pattern of repigmentation using the micro-needling with 5-FU side.

Lesions of upper limbs represent (36.0%), lower limbs represent (28.0%), trunk represent (14.0%), acral represent (12.0%), face neck represent (10.0%) of lesion site. A statistically significant differences between the responses of micro-needling plus 5-FU side according to the patch site; upper lower extremities truncal patches showed a better response, while acral lesions showed no repigmentation.

Figures 2 and 3 show photos of 6 patients from the microneedling with 5-FU side; 3 of them **figure (2)** showed less than 25% repigmentation, and the other **figure (3)** showed those with 25-50% repigmentation.

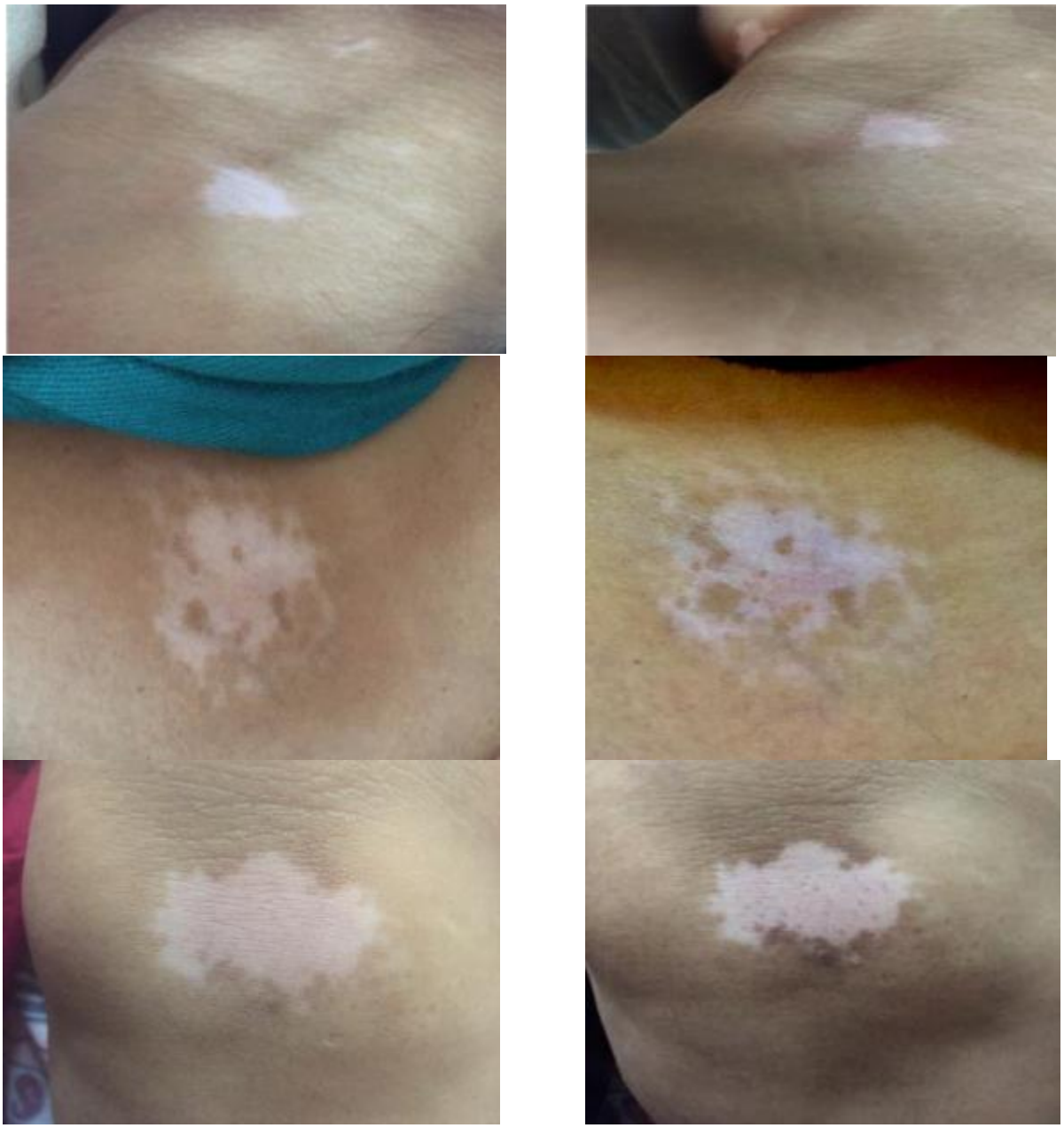

Figure (2): vitiligo lesions in three different patients; before treatment, after the lesion was treated by (microneedling & 5-FU) for six sessions showing mild response (repigmentation less than 25%).

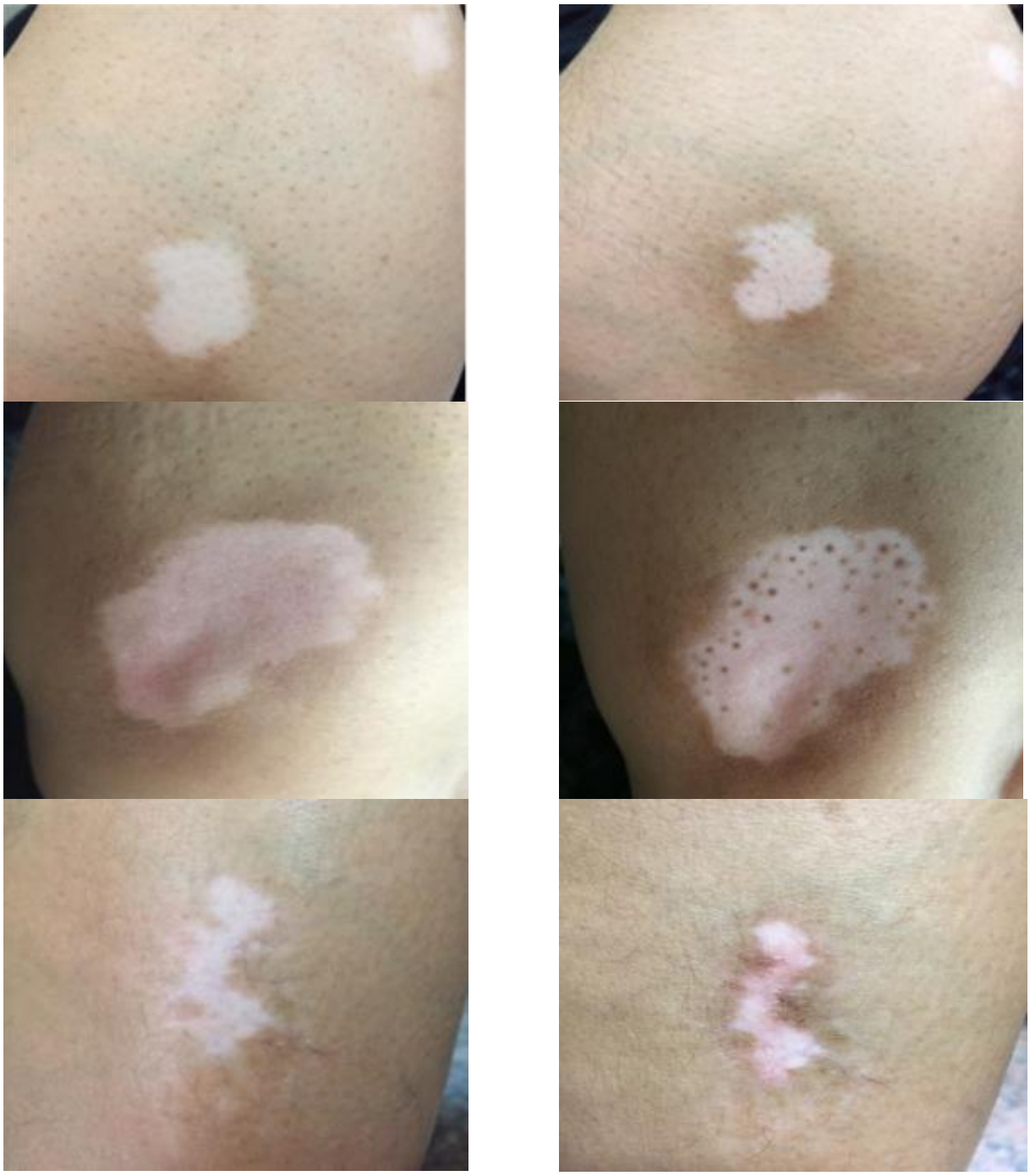

Figure (3): vitiligo lesions in three different patients; before treatment and after the lesion was treated by (microneedling & 5-FU) for six sessions showing moderate response (repigmentation 25-50 %).

A highly statistically significant difference was found between groups according to the side effects. On the microneedling side; 6 patients (12%) stated pain and burning sensations, and none of them was annoyed from the erythema, while on the micro-needling with 5-FU side; 15 patients (30%) complained of pain and burning sensation, 17 patients (34%) complained of erythema

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DISCUSSION

Vitiligo is a gained depigmenting skin disease, usually influencing human populations with no ethnic, racial, or financial inclination. It has a significant antagonistic effect on patients' mental states and personal satisfaction [13].

Many therapeutic modalities, ranging from medical to surgical interference, are available. There are two main strategies in the treatment of vitiligo. The first includes arresting the progression of the active disease to limit the area involved with de-pigmentation. The second strategy is the re-pigmentation of the de-pigmented site. Also, it is crucial to maintain the condition in a stable phase and to prevent relapse [14].

Therapeutic modalities include various types of medications, either systemic or topical, phototherapy, laser, and surgical therapies [15].

5-Fluorouracil is a significant fundamental chemotherapeutic medication for malignant tumor regimens. It has been utilized in treatment of numerous dermatological conditions such as keratoacanthomas, actinic keratosis, and skin malignant tumors [16].

Topical 5-FU has been tried as a treatment modality either alone or preceded by epidermal ablation with Erbium:YAG (2940 nm) laser [17] or carbon dioxide laser [18] or mechanically by micro-needling [19].

Intradermal injection of 5-FU also has been tried in treatment of localized non segmental vitiligo and achieved good results of repigmentation [20-21].

Micro-needling, is an essential office procedure that makes a considerable number of gaps and spaces through the epidermal layers into the papillary dermis. It is used as a developing treatment methodology in treating many dermatological diseases [22].

Fifty patients with stable non-segmental vitiligo were recruited in our study. We determined two patches in every patient; we used a dermaroller alone in treatment in one patch and the other one was treated with dermaroller but followed by topical application 5% 5-FU. One session every two weeks for three months. Re-pigmentation was evaluated by grades; ranging from zero response (0%) to excellent (>75%) response. Any adverse effect was reported every visit.

On micro-needling side, none [0%] of the studied participants showed re-pigmentation. On micro-needling and 5-FU side; twelve of our patients [24%] showed zero response, twenty three patients [46%] showed mild grade, fifteen patients [30%] showed moderate grade and no one in our study showed either good or excellent grades of re-pigmentation.

In our study, we used a dermaroller device as a reasonable, easy, inexpensive and fast outpatient clinic room procedure and can be used as an alternative procedure to epidermal ablation previously made by laser strategies, which operated as a trans-dermal drug delivery method for the 5-FU to increase their effectiveness [23].

Similar results were found by **Gauthier *et al.*** [24] who performed an animal model study with mechanical dermabrasion in addition to topical 5-FU and others were treated with topical 5-FU alone.

Another study by **Attwa *et al.*** [25] likewise demonstrated high statistical difference between effects of the microneedling alone versus microneedling with 5-FU. Microneedling response was only 18.5% of cases, while that with 5-FU added; showed response on 70.4% of cases. Similar comparable results were reported by **Santosh *et al.*** [26] **and Ghiya *et al.*** [27] reported repigmentation achieved by micro-needling with topical application of 5-FU in 67.24 % of cases.

The impact of 5-FU might be explained by its immune-modulatory effects. The inflammation created by the needling process of multi-cellular infiltration suppresses the melano-cytotoxic T-cells. Thus, this immune-modulatory process allows the re-settlement of migrating melanocytes [28]. Also, 5-FU stimulates the reservoir of the melanocytes in the hair's outer root sheath or the persistent DOPA-negative melanocytes in the de-pigmented epidermis to produce melanin [24].

A strong inflammation was reported after micro-needling with application of topical 5-FU, which leads to local oedema with increased intracellular gaps at the lower epidermal portion for a long duration, which in turn makes the migration easier for the active melanocytes with frequently vacuolated cytoplasm from the pigmented sites to the non-pigmented lesional areas through these enlarged intercellular spaces [29].

Another study revealed that 5-FU pigmentation role is contributed to the activation of change in the micro-environment to favor the migration of melanocytes from pigmented areas to the achromic sites. 5-FU stimulates fibroblasts in the dermis to produce CXCL12, which attracts CXCR4 positive melanocytes, and hence to melanocyte migration. They founded that CXCL12 up-regulation at the periphery of wounds in which 5-FU was topically applied versus the untreated control group, which leads to travelling of these melanocytes towards the fibroblasts in the dermis. In other wards CXCL12 makes the melanocyte a motile cell [2].

Another study by **Mohamed *et al.*** [30] used Carbon dioxide laser instead of micron-eedling with 5-FU solution. They reported repigmentation in 49.8% of the lesions of the cases after five successive sessions of treatment repeated monthly. However our study showed better results as it has less coast, shorter down time and less side effects.

Malik *et al.* [23] **and Mina *et al.*** [31] used another topical medications in combination with micro-needling like tacrolimus ointment with practically identical outcomes, however it is more expensive compared to 5- Fluorouracil solution.

On demonstrating the degree of improvement among our patients, we detect different grades of re-pigmentation in the microneedling with 5-FU side where 12 patients [24%] showed no re-pigmentation, 23

[46%] showed mild grade (less than 25% re-pigmentation), 15 [30%] showed moderate grade (25–50% re-pigmentation) and no one [0%] of the studied patients showed either good grade (50–75% re-pigmentation) or excellent grade (> 75% re-pigmentation).

Similar study with dermapen was done by **Attwa *et al.*** [25] showed very close results to ours, where 29.6% of patients showed no repigmentation, 51.9% showed mild grade but 11.1% showed moderate grade, 3.7% (only one case) showed good grade and 3.7% (one case) showed excellent grade.

In our study, we concluded that, the pattern of repigmentation was either marginal; the pigment extend from the edges of the lesion for few millimeters what is called perilesional hyperpigmentation in 27 cases (71.1 %) or marginal mixed with follicular pattern in form of small, brown, perifolicular macules, which then enlarged and coalesced that is reported in 11 cases (28.9%) of our participants.

Regarding different factors that could affect the re-pigmentation response, no correlation was found between the re-pigmentation response and both demographic and clinical data of the patients as (Age, Gender, Family history, duration of vitiligo, VASI score and Fitzpatrik photo-type). **Attwa *et al.*** [25] reported similar results where there were no statistical significance relations.

A statistical significant difference with better re-pigmentation on certain sites was found; were the trunk and the extremities showed better results, while the acral parts were the worst with zero response. This coincides with the study done by **Shashikiran *et al.*** [32], were the trunkal lesions had the best response while the acral lesions were the least responsive ones that hardly repigmented.

This variation in the response may be explained by the regional variation in the baseline epidermal stem cell factor (SCF), density of Langerhans cells (LC) and the density of hair follicles, which have been shown to be the reservoirs for melanocytes. The acral parts have the lowest hair follicles density [33, 34].

More studies are needed to confirm our results, using bigger number of patients and may be more number of sessions.

CONCLUSION

Micro-needling with 5-FU is a safe, easy, and tolerable procedure for treating vitiligo. It is also a low-cost modality that does not require expensive devices and can be done in a standard outpatient room.

Microneedling helps in transdermal drug delivery and makes an inflammatory environment with the inflammatory cytokines, and the 5-FU helps transport melanocytes to the vitiliginous area.

Although despite the tolerability and safety of this technique in treating vitiligo, the outcome was not very satisfactory. It is recommended to be used as an adjuvant therapy with other treatment modalities.

STRUCTURAL SUMMARY

(micro-needling with topical 5-FU showed better results than micro-needling alone), (trunk is the best area to be treated while the acral lesions are resistant to treatment), (micro-needling is safe, easy, with tolerable side effects and can be used alternatively to ablative Lasers as a safe transepidermal delivery system).

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