“AN OPEN LABEL, NON-RANDOMIZED, INTERVENTIONAL STUDY TO EVALUATE THE EFFICACY AND SAFETY OF MICRONEEDLING COMBINED WITH 5-FLUOROURACIL 5% CREAM VERSUS MICRONEEDLING COMBINED WITH TACROLIMUS 0.1% OINTMENT VERSUS MICRONEEDLING ALONE IN CLINICALLY STABLE VITILIGO PATIENTS.”

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ABBREVIATIONS LIST

S.No	Abbreviation	Full Form
1	5-FU	5-Fluorouracil
2	CVI	Continuous Venous Infusion
3	FUMP	Fluorouridine Monophosphate
4	PRPP	Phosphoribosyl Pyrophosphate
5	OPRT	Orotate Phosphoribosyltransferase
6	FUR	Fluorouridine
7	UP	Uridine Phosphorylase
8	UK	Uridine Kinase
9	FUDP	Fluorouridine Diphosphate
10	RR	Ribonucleotide Reductase
11	FdUDP	Fluorodeoxyuridine Diphosphate
12	FUTP	Fluorouridine Triphosphate
13	FdUMP	Fluorodeoxyuridine Monophosphate
14	FdUTP	Fluorodeoxyuridine Triphosphate
15	TP	Thymidine Phosphorylase
16	TK	Thymidine Kinase
17	DHFU	5,6-Dihydro-5-Fluorouracil
18	FBAL	α-Fluoro-β-Alanine

19	HFS	Hand-Foot Syndrome
20	SPSS	Statistical Package for the Social Sciences
21	TGF-α	Transforming Growth Factor-Alpha
22	TGF-β	Transforming Growth Factor-Beta
23	PDGF	Platelet-Derived Growth Factor
24	NbUVB	Narrowband Ultraviolet B
25	PUVA	Psoralen plus Ultraviolet A
26	PASI	Psoriasis Area Severity Index
27	VASI	Vitiligo Area Scoring Index
28	VETF	Vitiligo European Task Force
29	VIDA	Vitiligo Disease Activity Score
30	IL	Interleukin (e.g., IL-1β, IL-6, IL-12)
31	TNF-α	Tumor Necrosis Factor-Alpha
32	HSP70	Heat Shock Protein 70
33	NLRP1	NOD-like Receptor Protein 1
34	CXCL	Chemokine Ligand (e.g., CXCL9, CXCL10)
35	JAK	Janus Kinase
36	STAT	Signal Transducer and Activator of Transcription
37	TCI	Topical Calcineurin Inhibitors
38	TCS	Topical Corticosteroids
39	HLA	Human Leukocyte Antigen

40	GWAS	Genome-Wide Association Studies
41	XBP1	X-box Binding Protein 1
42	PTPN22	Protein Tyrosine Phosphatase Non-Receptor Type 22
43	IKZF4	IKAROS Family Zinc Finger 4
44	NFAT	Nuclear Factor of Activated T-Cells
45	FKBP	FK506 Binding Protein
46	CYP3A4	Cytochrome P450 Family 3 Subfamily A Member 4
47	IFN-γ	Interferon-Gamma
48	CD8+	Cluster of Differentiation 8 Positive T Cells
49	MART1	Melanoma Antigen Recognized by T Cells 1
50	gp100	Glycoprotein 100
51	IL-15	Interleukin-15
52	CD122	Cluster of Differentiation 122
53	Trm	Tissue-Resident Memory T Cells

ABSTRACT

Background: Vitiligo is a chronic, acquired depigmentation disorder characterized by the selective destruction of melanocytes, leading to the appearance of white macules on the skin. It affects approximately 0.5% to 2% of the global population and has a profound psychosocial impact despite its non-life-threatening nature. Various treatment modalities exist, including topical immunosuppressants, phototherapy, and surgical interventions, but none guarantee universal efficacy. Microneedling has emerged as a promising approach to enhance repigmentation, particularly when combined with pharmacological agents such as 5-Fluorouracil (5-FU) and Tacrolimus. However, limited comparative studies exist evaluating the efficacy and safety of these combinations in stable vitiligo.

Aim and Objective: The primary objective of this study is to compare the efficacy of microneedling alone versus microneedling combined with 5-FU 5% cream and microneedling combined with Tacrolimus 0.1% ointment in patients with stable vitiligo. The secondary objective is to assess the safety profile and patient satisfaction associated with these treatment modalities.

Materials and Methods: This open-label, non-randomized, interventional study was conducted over one year at KLE's Dr. Prabhakar Kore Hospital and Medical Research Centre. A total of 72 stable vitiligo patches from 24 patients aged 18–50 years were included. Each patient had three vitiligo patches assigned randomly to one of the three treatment groups: Group A (microneedling + 5-FU 5%), Group B (microneedling + Tacrolimus 0.1%), and Group C (microneedling alone). The procedure was performed every four weeks for four sessions, followed by a follow-up at the fifth month. Treatment efficacy was assessed using the Global Physician Assessment Scale, while

patient satisfaction was measured using the Visual Analog Scale. Side effects were recorded to evaluate safety.

Results: Among the 72 treated vitiligo patches, the highest repigmentation rate was observed in Group A (microneedling + 5-FU), where 66.7% of patches showed significant repigmentation (>50%). Group B (microneedling + Tacrolimus) demonstrated moderate improvement, with 50% of patches showing repigmentation between 25-50%. Group C (microneedling alone) exhibited the least improvement, with 75% of patches showing <25% repigmentation. Patient satisfaction was highest in Group A, followed by Group B, with Group C showing the least satisfaction. Side effects, including mild erythema, ulceration, and hyperpigmentation, were more common in the 5-FU group but were transient and manageable.

Limitation: The study was limited by its small sample size and short follow-up duration. Additionally, the non-randomized design may introduce selection bias. Long-term effects and relapse rates were not assessed.

Conclusion: Microneedling combined with 5-FU 5% cream is the most effective treatment modality among the three groups, demonstrating superior repigmentation rates and patient satisfaction. Microneedling with Tacrolimus 0.1% ointment also showed moderate efficacy, while microneedling alone had the least impact. The procedure was well-tolerated, with manageable side effects. Larger, randomized controlled trials with extended follow-up periods are recommended to validate these findings and establish standardized treatment protocols for vitiligo.

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INTRODUCTION

Vitiligo, a prevalent depigmenting skin disorder that affects 0.5% to 2% of people worldwide, is typified by the selective loss of melanocytes, which results in chalky-white macules. It is recognized as an immunological disorder with complex causes. Unlike albinism, vitiligo does not increase the risk of non-melanoma skin carcinoma. Though often considered a cosmetic issue, it has significant psychological impacts. A 2011 global consensus classified segmental vitiligo separately from nonsegmental forms. Various treatments exist to halt progression and promote repigmentation, but none guarantee success for all patients. [1]

Treatment should be evaluated after at least six months and may take 6-24 months for visible results. Topical immunomodulators like tacrolimus and pimecrolimus are effective for sensitive areas, while strong topical steroids are used for the body. Ruxolitinib, a JAK inhibitor, is the first FDA- and EMA-approved treatment, showing strong efficacy in clinical trials. Phototherapy, especially narrow-band UVB, enhances repigmentation and is now available for home use. Sun exposure, combined with immunomodulators, is beneficial, with no risk of skin cancer.[2] Individualized management is crucial, and ongoing research seeks to improve therapeutic option.[1]

Microneedling (Mn) is a minimally invasive procedure that creates micro-punctures in the skin, triggering platelet-derived growth factors that promote neovascularization and collagen production. Microneedling (Mn) also facilitates drug delivery through the stratum corneum and promotes vitiligo repigmentation by transferring melanocytes from pigmented to depigmented areas. The skin perforation process triggers wound healing, cytokine release, and melanocytic autoinoculation,

enhancing melanogenesis. The procedure involved applying an electronic dermapen with needle depths of 1-2 mm after a topical anesthetic, with 6-12 sessions at two-week intervals.[3]

Tsuji and Hamada introduced the use of 5- fluorouracil (5-FU) after therapeutic wounding for vitiligo treatment. 5-FU selectively affects keratinocytes while sparing melanocytes, leading to melanocyte overstimulation and migration, promoting repigmentation. In vitro studies show that low concentrations of 5-FU destroy keratinocytes in three weeks, allowing melanocytes to proliferate. Microneedling enhances this process by inducing inflammation, increasing basal layer permeability, and facilitating melanocyte migration. Inflammatory mediators like leukotrienes and matrix metalloproteinases further aid this process. Clinical trials combining microneedling with other therapies have shown improved repigmentation, faster results, and fewer side effects.[4]

Tacrolimus, an immunosuppressant targeting T cells and mast cells, inhibits inflammatory cytokines and enhances vitiligo repigmentation, especially when combined with other treatments. Studies show that combining tacrolimus with excimer laser, CO_2 fractional laser, or narrowband UVB (NB-UVB) improves repigmentation rates compared to monotherapy. Combining tacrolimus with microneedling may enhance efficacy and shorten treatment duration, particularly for localized, treatment-resistant vitiligo.[5]

Although microneedling has been investigated in conjunction with 5-FU and tacrolimus independently, comparative studies evaluating the effectiveness of Mn alone, Mn with 5-FU, and Mn with tacrolimus in a controlled environment are scarce. Standardizing protocols for needle depth, session frequency, and drug application are

essential to optimize Mn-based treatments for vitiligo. By addressing these gaps, this current study aims to provide stronger clinical evidence supporting microneedling-based approaches as effective and standardized treatment options for vitiligo.

AIMS AND OBJECTIVES

- **Primary objective**

To evaluate the efficacy. of microneedling combined with 5-fluorouracil. 5% cream versus the efficacy of microneedling combined with tacrolimus. 0.1% ointment versus microneedling alone in clinically stable vitiligo patients.

- **Secondary objective**

To evaluate the safety. of microneedling combined with 5-fluorouracil. 5% cream versus the safety of microneedling combined with tacrolimus. 0.1% ointment versus microneedling alone in clinically stable vitiligo patients.

REVIEW OF LITERATURE

The largest organ of the human body is skin, and a person's individual identity as well as the race and ethnicity are determined by the pigmentation of their skin.[6] The three elements essential in determining a person's skin pigmentation are hemoglobin content in both oxygenated and deoxygenated blood, carotenoids and melanin. Melanin is the primary pigment that determines an individual's color among these three major determinants.[7,8]

The two primary forms of melanin are eumelanin and pheomelanin. Animals, microbes, and certain fungi all contain eumelanin. It is either dark or black in color and is formed from tyrosine. [9] Birds, mammals, and higher animals are endemic for pheomelanin. It is red or yellow color and a derivative of tyrosine. Unlike eumelanin, which is composed of indole units, pheomelanin is composed of sulfur-containing monomer units, primarily benzothiazine and benzothiazole.[10] Melanin giving skin its color, is produced by cells called melanocytes.

VITILIGO

Vitiligo is a chronic acquired condition defined by depigmentation of the skin secondary to destruction/loss of melanocytes. [11] It has detrimental psychological impact even though it does not affect life expectancy.[12] Melanocyte destruction in vitiligo has been attributed to several processes, including oxidative stress, autoimmune response, heredity, and the generation of inflammatory mediators.[13] Numerous studies indicate that vitiligo is linked to dyslipidemia, insulin resistance, and other metabolic disorders.[14] But the precise etiology of vitiligo remains unclear.

HISTORY

Around 2200 B.C., in the time of the Aushooryan era, vitiligo was initially documented in writing as Kilăsa. Additionally, vitiligo information from the Egyptian Ebers Papyrus goes back to 1550 B.C.[15] Although it has been recognized for thousands of years, the first formal medical description was made in 1765. Early observations, such as Kaposi's in 1879, identified a lack of pigmented cells in affected areas. Addison's 1855 description of Addison's disease noted that some patients also had vitiligo, suggesting a potential autoimmune link. In 1965, DeMowbray proposed that autoimmune diseases, like vitiligo, might share a genetic predisposition.

The genetics of vitiligo were first explored in 1950 with the identification of affected families, leading to early theories of complex inheritance. Research intensified in the 1960's, moving from blood protein studies to genetic analysis. Research through genome-wide association studies (GWAS) has achieved notable success, pinpointing 50 genetic loci linked to vitiligo in populations from Europe and Han Chinese. These studies have provided insights into vitiligo's biological pathways, environmental triggers, and potential new treatment strategies.[16]

EPIDEMIOLOGY

It affects approximately 0.1% to 2% of people worldwide, and there are no appreciable variations in its prevalence by gender, ethnicity, or geography.[17] According to numerous Indian studies, vitiligo's prevalence in dermatological outpatients ranges from 0.25 to 4%, with rates as high as 8.8% in Gujarat and Rajasthan.[18]

PATHOGENESIS OF VITILIGO

I. GENETICS

The main changes of genetics involved in the pathogenesis of vitiligo are enlisted (table 1)

Table 1- Genes and Histocompatibility antigens (HLA)[19]

Gene	**Expression**
NLRP1	+
XBP1	+
FOXD3	+
PDGFRA	+
PTPN22	+
IKZF4	+
FOXP3	+
DDR1	-
HLA	**Risk**
HLA-A*02	↑
HLA-Aw*31	↑
HLA-A*32	↑
HLA-A*33	↑
HLA-A*09	↓
HLA-Aw*19	↓
HLA-DQB1*06	↑
HLA-DQB1*0303	↑
HLA-DR4	↑
HLA-DRB1*07	↑
HLA-DR7	↑

Vitiligo is a polygenic, multifactorial disease with 75-83% genetic risk. Key loci include NLRP1, XBP1, and HLA genes. GWAS identified over 50 associated loci, including PTPN22 and IKZF4. Mechanisms involve autoimmunity, melanocyte

adhesion, and oxidative stress. Polygenic risk scores predict vitiligo with 71% accuracy.[19–22]

II. OXIDATIVE. STRESS

Oxidative. stress plays a major role in vitiligo by damaging melanocytes, exacerbated by UV radiation and pollutants. The imbalance between pro-oxidants (e.g., superoxide dismutase, malondialdehyde) and antioxidants (e.g., glutathione peroxidase) leads to melanocyte destruction. Exogenous stressors like UV and chemicals, alongside internal processes like melanogenesis, trigger excessive ROS production. [23–25]

ROS induce mitochondrial damage and apoptosis, while melanocytes in patients of vitiligo who are more sensitive to the oxidative stress.[26] Impaired Nrf2 defense and autophagy disruption further contribute to damage.[27] Oxidative stress may also be involved in the Köbner phenomenon, where trauma triggers inflammatory mediators and ROS.[28,29]

III. AUTOIMMUNITY

1.Innate. immunity

The innate. immune system plays a pivotal role in vitiligo pathogenesis, linking oxidative stress with the adaptive immune response.[30] Activated dendritic cells, macrophages, NK cells, and increased proinflammatory cytokines (e.g., IL1α, IL1β, IL6, IL8, IL12, TNFα) are found in vitiligo patients, indicating global immune activation.[31,32]

Oxidative stress may trigger autoimmunity in vitiligo, with melanocyte-derived exosomes delivering autoantigens to dendritic cells, promoting their maturation. [33] Heat shock protein 70 (HSP70) is a critical DAMP (damage-associated molecular patterns) in vitiligo, inducing dendritic cell activation and proinflammatory cytokine release.[34] In animal models, HSP70 accelerates skin depigmentation.[35] and a mutant HSP70 has been shown to increase repigmentation by causing inactivation of dendritic cells and inhibiting T-cell responses.[36]

Furthermore, NLRP-1, a key innate immune regulator, stimulates the generation of IL1β and activates the inflammasome, both of which are involved in T-cell polarization and the development of vitiligo.[37,38] For vitiligo, IL1β suppression may be a viable treatment target.[37]

2.**Adaptive. immunity**

CD8+ T. cells play a key role in destruction of melanocyte in vitiligo, with higher numbers found in blood and lesional skin, correlating with disease activity.[38] These cells target melanocyte proteins (gp100, MART1, tyrosinase) and produce IFN-γ, which recruits more CD8+ T cells to the skin via chemokines (CXCL9, CXCL10, CXCL11).[39,40] IFN-γ activates JAK1/2 and STAT pathways, and elevated JAK1 expression in lesional skin correlates with reduced melanocyte survival, suggesting JAK1/2 as therapeutic targets.[41]Tregs, which normally suppress autoreactive T cells, are impaired in vitiligo, with reduced numbers and function.[42]

Relapses are common (40% within one year) and are linked to CD8+ tissue-resident memory T cells (Trm) maintained by IL-15 signaling. High CD122 levels in Trm cells were observed and targeting IL-15 with anti-CD122 antibodies reversed

disease in mice, offering a potential treatment. [43] Autoimmune/ autoinflammatory conditions associated with vitiligo are enlisted in table 2

Table 2: Conditions associated with vitiligo [19,44]

Alopecia areata
Pernicious anemia
Rheumatoid arthritis
Atopic dermatitis
Dermatomyositis
Type 1 Diabetes Mellitus
Addison's disease
Crohn's disease
Graves' disease
Systemic scleroderma
Multiple sclerosis
Systemic lupus erythematosus
Psoriasis
Ulcerative colitis
Sjogren's Syndrome
Hashimoto's Thyroiditis

IV. Neural theory

The neural theory of vitiligo suggests neuroimmunological influences, as lesion distribution aligns with dermatomes in segmental. vitiligo and symmetrically in nonsegmental cases.[45] Nerve injury and psychological stress are also linked to vitiligo onset[46] Neuropeptide Y. is elevated in perilesional and lesional skin, while substance P correlates with disease stability.[47] Extreme stress activates the hypothalamic-pituitary-adrenal axis. (HPA), increasing catecholamines, neutrophilia, NK cells, and proinflammatory cytokines like IL-6, which may promote autoimmune and autoinflammatory diseases.[48]

CLINICAL FEATURES OF VITILIGO

Vitiligo appears as irregular patches that are chalky-white or milky, usually round or oval with scalloped edges. The affected areas can average in size, from just a few millimeters to several centimeters, impacting both the skin and/or mucous membranes. While the condition is generally without symptoms, some individuals may experience itching or a burning sensation. Vitiligo progresses slowly and can have periods of improvement and flare-ups, often triggered by specific events.[49] The condition tends to affect areas with hyperpigmentation such as the face, groin, underarms, areolas, and genital areas. It can also appear in spots subject to frequent friction, including the ankles, elbows, and knees, because of Koebner's phenomenon.[50] Extensive vitiligo presents with symmetrical lesions and may have a dermatomal or mucous membrane distribution.[49]

Koebner's phenomenon (KP)., also called as the isomorphic response, is characterized by the appearance of new lesions of a skin condition at sites of mechanical injury. In contrast to other conditions like psoriasis, KP in vitiligo is more closely linked to everyday activities, particularly those involving chronic friction.[51]

CLASSIFICATION OF VITILIGO

Table 3 Types of vitiligo [51]

Type	Subtype
Vitiligo	Acrofacial, focal, mucosal (>1 mucosal site), generalized, universal, mixed (associated with segmental vitiligo), and rare variants
Segmental/ undetermined/unclassified vitiligo	Unisegmental and multisegmental

Focal vitiligo- Isolated, tiny, depigmented patches (10–15 cm2) with no unilateral segmental distribution are the hallmark of focal vitiligo, which does not worsen for at least two years.

Mucosal vitiligo- This form is characterized by depigmented patches on the mucosal membrane of the oral cavity and genitalia

Vitiligo acrofacialis affects the hands, feet, and head.

In South Asia, frequently **lip-tip vitiligo** is noticed with distal finger, toe, and facial orifice involvement and is challenging to cure.

Complete or almost entire skin depigmentation, including or excluding body hair (>80%), is known as **universal vitiligo.**

Segmental vitiligo - unilateral patch of depigmentation, often with a linear or block-like pattern. Early onset, and quick stabilization of vitiligo are characteristics of this

variant of vitiligo. This is less frequently associated with autoimmune disorders and family history than other forms of vitiligo.

Generalized vitiligo/ vitiligo vulgaris - The typical kind of vitiligo is generalized vitiligo. Depigmented macules and patches are the hallmarks of vitiligo vulgaris. The distribution of this variant is symmetrical.

Mixed: it refers to the concurrent presence of both non-segmental. and segmental vitiligo. The segmental form usually comes before NSV.

Rare types include follicular, mild, and punctata vitiligo.[51]

Morphological variants

Trichrome vitiligo : a narrow to wide area of intermediate hue between the surrounding normal skin and the vitiligo macule. One subtype of unstable vitiligo, which is thought to be this form's variant, is cockade-like vitiligo. [52]

Quadri chrome Vitiligo: This variation, seen in darker skin types, includes a fourth color in vitiligo lesions. Perifollicular or marginal hyperpigmentation, which denotes repigmentation, is a prominent characteristic.[53]

Penta-chrome Vitiligo: A rare variation that is most frequently observed in people with darker skin and is characterized by a series of white., tan, brown., and blue-gray hyperpigmentation alongside normal skin.[54]

Blue vitiligo -Macules of vitiligo that appear in regions of post-inflammatory hypermelanosis especially in AIDS patient.[55]

Inflammatory vitiligo - The vitiligo macule has an erythematous, elevated border that is frequently itchy and/or burning. Aggressive treatment may cause these symptoms.[56]

DIFFERENTIAL DIAGNOSIS

Many dermatoses resemble vitiligo, a few are listed below[49,57,58]

Table 4: Differential diagnosis of vitiligo

○ **Congenital**
• Nevus depigmentosus - serrated margins of the lesion.
• Nevus anemicus- pale lesion, which on diascopy becomes unnoticeable.
• Tuberous sclerosis (Ash leaf spots)- Lanceolate shape more commonly seen over the trunk.
• Piebaldism - Patterned depigmented patches, white forelock seen in 85% of cases.
• Waardenburg syndrome: Since birth depigmented patches seen, with a white forelock. Most common associated features are heterochromia. iridis, dystopia. canthorum, and congenital sensorineural deafness.
• Hypomelanosis of Ito: Blaschko's lines have hypopigmented, linear whorls and streaks.
• Incontinentia pigmenti: (fourth stage) history of previous lesions will be present, Linear, atrophic, hypopigmented streaks are seen with loss of hair and sweat pores over it.
○ **Acquired**
▪ **Inflammatory**
• Pityriasis alba- Hypopigmented macules/patches with fine scaling, mostly seen over face.

- Lichen striatus: linear hypopigmented shiny papules.

- Lichen sclerosus et atrophicus (LSEA): Porcelain-white atrophic macules. In case of genital lesions, atrophy, and resorption of genital structure are seen in girls and phimosis is seen in boys.

- Postinflammatory hypopigmentation- history pertaining to the past lesion will be present.

▪ **Infections**

- Pityriasis versicolor : hypopigmented macules with furfaraceous scales, most commonly seen in seborrheic distribution.

- Leprosy - hypopigmented patches with sensation loss and peripheral nerve involvement seen.

- Post-Kala azar dermal leishmaniasis -symmetric hypopigmented macules which are widespread, associated nodules may be seen.

- Pinta: mostly seen in endemic areas and family members are also affected. Widespread slate-blue hyperpigmentation initially which is replaced by depigmented macules are seen.

▪ **Miscellaneous**

- Polymorphous light eruption: Pruritic, scaly, hypopigmented, papule/macule/patch seen in photo exposed areas with a history of photo exacerbation seen.

- Contact depigmentation: only over the contact areas patterned depigmentation seen (footwear, diaper).

- Topical steroid abuse history of pre-existing dermatoses and chronic abuse of steroid.

DISEASE ACTIVITY

Before considering any surgical treatment or assessing the advancement of vitiligo, it is crucial to evaluate the stability of disease. Clinically, this can be determined through three key indicators:

- Patient history
- Koebner phenomenon
- Test grafting[59]

Falabella[60] has outlined some benchmark guidelines to assess the activity of the disease, that includes:

- No lesions have progressed over the last two years (this time frame may be shorter in unilateral vitiligo, while stability is usually maintained after several years in bilateral vitiligo).
- During the same period, no new lesions appeared.
- Lack of recent Koebner phenomenon, as determined by experimental testing or patient history.
- Repigmentation of depigmented areas can occur spontaneously or as a result of medical therapy.
- A successful outcome of the mini-grafting test.
- At the donor site, Koebnerization is not taking place.[61]

There is currently no agreement on a duration of stability, and many authors have suggested that it might range from four months to three years.[62] The IADVL taskforce recommends one year of inactivity to perform vitiligo surgery.[63]

Histologically, spongiosis, basal-layer vacuolization, and epidermal lymphocytes in the epidermis, as well as dermal lymphocytes and melanophages in the dermis, are indicative of active vitiligo.[64] Natural regulatory T cells are less common in the lesions, which are primarily populated by CD4+ and CD8+ T cells. [65]

A valid and trustworthy method for determining the stability. of the disease is the Vitiligo Signs of Activity Score (VSAS), which assesses characteristics including hypochromic areas/borders, the Koebner phenomenon., and confetti-like. Depigmentation.[66]

Laboratory indicators that are showing promise as diagnostic tools for disease stability include CXCL9 and S100B protein, which may be linked to vitiligo activity.[67] For an in-depth examination of disease stability, a combination of patient self-assessment, clinical evaluation criteria (such Vitiligo Area Scoring Index (VASI) or Vitiligo European Task Force (VETF), and digital imaging of individual skin lesions during the past 12 months is recommended.[68]

Vitiligo Disease Activity Score (VIDA).

Based on the patient's observation of the disease's progression over time, vitiligo activity is measured using a six-point rating system. The appearance of new lesions or the enlargement of old ones are characteristics of active vitiligo. The scoring system is as follow

+4: Activity lasting 6 weeks or less
+3: Activity lasting 6 weeks to 3 months
+2: Activity lasting 3 to 6 months
+1: Activity lasting 6 to 12 months
0: Stable for 1 year or more
-1: Stable with spontaneous repigmentation for 1 year or more

A lower VIDA score reflects less disease activity.[69]

DIAGNOSIS

Vitiligo is often diagnosed based on clinical characteristics; however, a Wood's light examination, dermoscopy, biopsy can occasionally be useful in distinguishing it from other depigmented or hypopigmented conditions.[70]

WOOD'S LIGHT

Wood's light works by emitting long-wave ultraviolet radiation, also known as black light, which is produced by mercury arc under high pressure fitted with nickel oxide at 9% and barium silicate filter, otherwise known as the "Wood's filter." It filters out all light rays except light belonging to wavelength between 320-400 nm. Normal skin has very little to no fluorescence and is mostly as a result of elastin. components, aromatic amino. acids, and melanin precursors or products.[71]

Less or no epidermal melanin can be seen in lesions that are depigmented or hypopigmented. The light-induced autofluorescence of dermal collagen can then be observed through this window. Other hypopigmentary conditions can be ruled out

with the use of a simple bedside gadget, a Wood's lamp light examination. Under Wood's light, the edges of hypopigmented or depigmented areas are more distinct because the visible emission from vitiligo skin abruptly stops. Autofluorescence causes the lesions to display bright blue-white.[72] (figure 2)

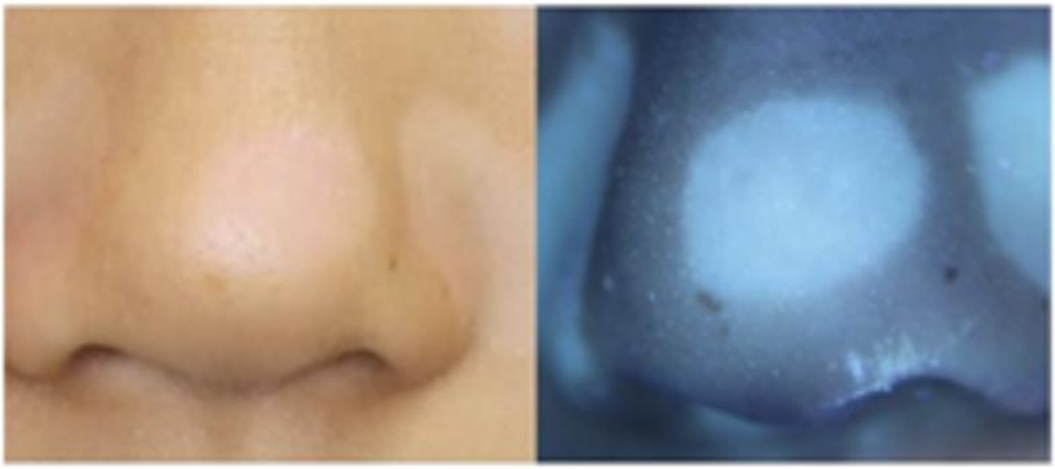

Figure.1- Woods lamp examination of vitiligo patch [72]

DERMOSCOPY

Dermoscopy is essential for evaluating treatment response, identifying lesion stability prior to surgery, and distinguishing developing vitiligo from other hypopigmentary illnesses.[73] It assists in determining important dermoscopic characteristics and diagnosing various phases of vitiligo:

- Developing vitiligo lesions: These exhibit a diffuse white glow, perilesional hypopigmentation, a decreased or nonexistent pigmentary network, and a reversed pigmentary network, which can be mistaken for other hypopigmentary disorders.[74]

- Lesions that are fully evolved usually exhibit a diffuse white glow, leukotrichia, and the total lack of a pigmentary network.

- Perifollicular pigmentation is seen in repigmenting lesions.[75]

- Dermoscopy can reveal patterns like a polka. dot or confetti-like pattern, trichrome. pattern, comet-tail. pattern, star burst/feathery. pattern, and a tapioca or sago grain. pattern in normal skin next to the lesion if the vitiligo is unstable or progressing. Early indicators of impending vitiligo include perifollicular depigmentation and developing leukotrichia in areas that have not changed .[76]

- Dermoscopy reveals ameboid or petaloid patterns in stable vitiligo. [77] Clinical judgments are aided by these dermoscopic characteristics, particularly when it comes to surgical procedures.

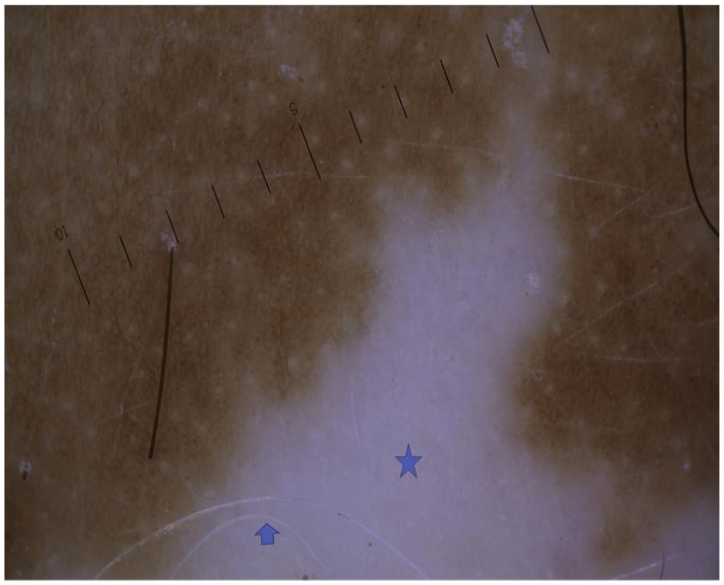

Figure.2- Completely evolved vitiligo showing absence of pigment network in dermoscopy. [74]

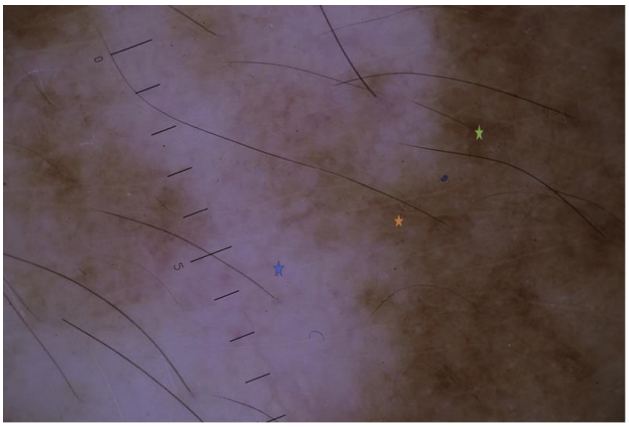

Figure.3- Progressive vitiligo showing trichome pattern in dermoscopy. [74]

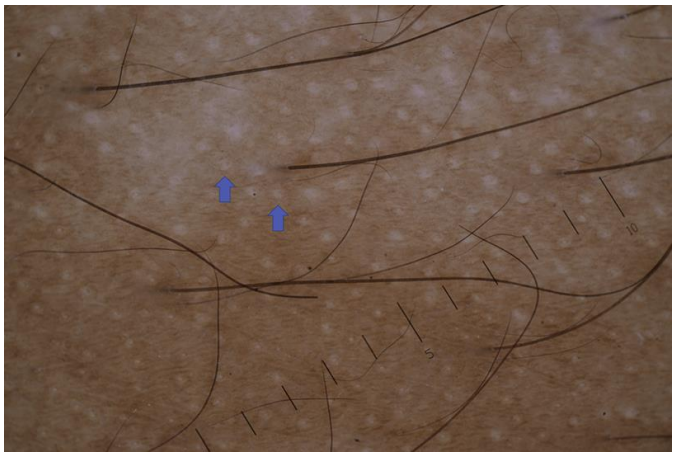

Figure.4- Sago grain or tapioca appearance in dermoscopy. [74]

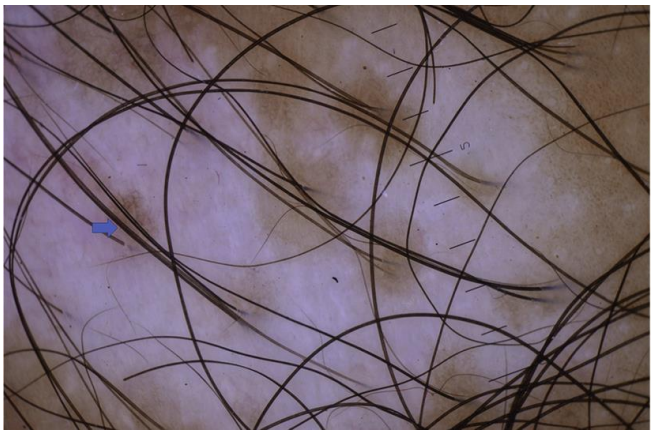

Figure.5- Repigmenting vitiligo showing perifollicular pigmentation. [74]

HISTOPATHOLOGY

The primary histopathological characteristic of vitiligo is the ansence of functional melanocytes in the basal layer of the epidermis . Fontana-Masson stains can be used to confirm this absence. Additionally, immunohistochemistry with melanocyte-specific markers like HMB-45. and Melan-A., as well as electron microscopy, can be employed for further confirmation.[78]

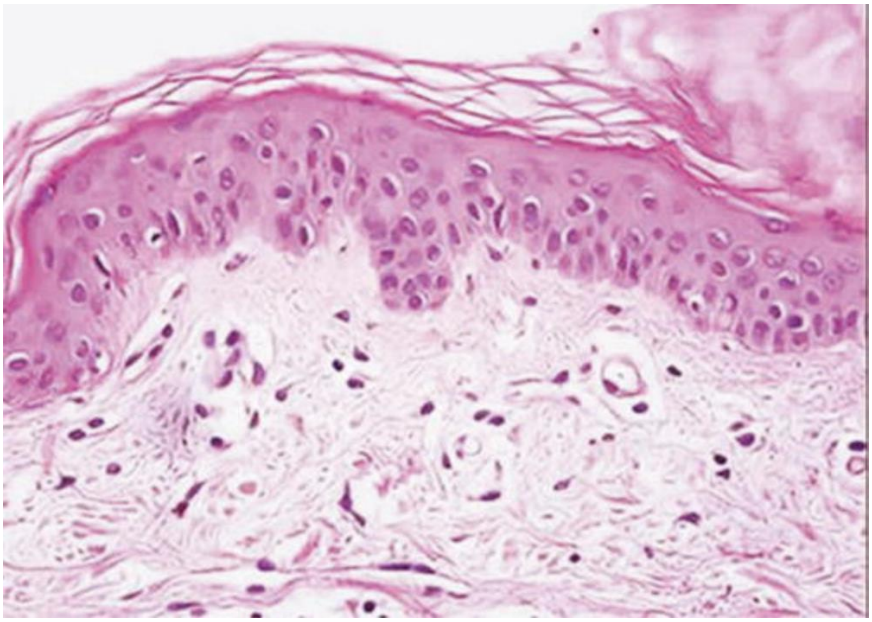

Figure 6- Absence of basal melanocytes (H&E, X400).[78]

Additional features commonly associated with vitiligo include spongiosis, intraepidermal and dermal lymphocytic infiltration, basal cell vacuolation, and dermal melanophages. A histopathology scoring system for vitiligo has been developed based on these parameters, with each of the mentioned features receiving a score of one. The final score is then calculated to assess the extent of these histopathological changes.[79]

SEVERITY OF VITILIGO

Vitiligo Area Scoring. Index (VASI) and Vitiligo European Task Force. (VETF) tools were developed for more accurate disease severity and evaluating treatment.

Vitiligo Area Scoring Index (VASI): [80]

VASI, derived from the Psoriasis Area Severity Index. (PASI), measures repigmentation using a quantitative parametric score. The body is divided into five regions (hands, upper extremities, trunk, .lower extremities, and feet), with each region assessed for the extent of depigmentation using hand units. One hand unit equals approximately 1% of total body surface area. Residual depigmentation is scored in increments of .0%, 10%, 25%, 50%, 75%, 90%, or 100%. The total VASI score is calculated by summing contributions from all body sites.

Vitiligo European Task Force (VETF): [80]

Analysis of extent, stage, and advancement (spreading) are all combined in the VETF system. The "rule of nines"—which is also used in atopic dermatitis—is used to assess the extent. The pigmentation of vitiligo patches, which is rated from 0 to 3, determines the staging.:

- Stage 0: Normal pigmentation
- Stage 1: Incomplete depigmentation
- Stage 2: Complete depigmentation with minor hair whitening (<30%)
- Stage 3: Complete depigmentation with significant hair whitening (>30%)

Spreading assesses disease progression, rated as +1. (progressive), 0 (stable), or -1 (regressive.), using both Wood's lamp and electric light in a dark room, including magnification to assess vellus hairs.

Together, these tools offer a standardized approach to evaluating vitiligo severity, treatment response, and disease progression.[80]

TREATMENT

A complete clinical history, including lesion categorization, illness extent, skin phototype, age of onset, and any triggering circumstances, is necessary for managing vitiligo.. It's also important to assess whether the disease is stable or progressing rapidly, as this impacts treatment decisions.[80]

I. Management of Non segmental vitiligo. (NSV):

The management of non-segmental vitiligo. (NSV) is guided by several treatment protocols, with key recommendations from three major guidelines:

1) 2012 European Dermatology Forum (EDF) Consensus[81]

- **Limited NSV (<2-3% BSA**):

Potent topical corticosteroids (TCS) once daily for up to 3 months.

Topical calcineurin inhibitors (TCI) twice daily for 6 months for head/neck regions.

Narrowband UVB (nbUVB) therapy or excimer laser as second-line options.

- **Generalized NSV**:

First-line: nbUVB for 1-2 years.

Second-line: Oral Psoralen plus UV-A (PUVA).

Third-line: Surgical techniques for visible areas.

For **rapidly progressing disease**: Weekend oral dexamethasone minipulse therapy (2.5 mg daily for 3-6 months).

Grafting is recommended as a third line of treatment for patients with **stable disease**, no repigmentation, and a negative .Koebner phenomenon in nonresponding areas, particularly those with a strong aesthetic impact.

Depigmentation therapy (hydroquinone monobenzyl. ether or 4-methoxyphenol. or with Q-switched ruby laser): For widespread >50% vitiligo or visible areas with positive Koebner phenomenon.

The updated European Dermatology Forum /European Academy of Dermatology and Venerology [82,83] guidelines highlight a shared decision-making approach with three treatment goals: stabilization., repigmentation, and depigmentation.

- For active NSV. (within the past 6 months):

For stabilization and repigmentation, topical therapies like strong once-daily TCS or twice-daily TCI are advised, in addition to phototherapy (targeted if required). If the disease is advancing quickly, systemic therapies may be tried (for up to 6

months). Treatment for TCI may be continued for up to a year or more if it is beneficial.

- For stable NSV (within the past 6 months)

TCS/TCI maintenance treatment or clinical follow-up at least twice. a week for six months is recommended for stability of stable NSV (within the last six months). This recommendation is different from the one from 2012.TCS/TCI. and phototherapy (targeted if necessary) are advised for repigmentation in stable NSV. Only instances who have been stable for at least a year and have not improved with previous therapies are given consideration for surgery**.**

2) 2021 British Association of Dermatologists Guidelines[84]

First-line: Potent TCS or TCI for localized vitiligo, reassessed every 3-6 months.

Second-line: nbUVB therapy, with TCS/TCI for extensive or progressive disease.

Third-line: Excimer laser or CO2 laser with 5-fluorouracil for resistant cases, especially on hands/feet.

Systemic corticosteroids: For rapidly progressing disease, oral betamethasone. (0.1 mg/kg twice weekly for 3 months).

Surgical treatment: For stable, unresponsive vitiligo with significant distress.

3) 2022 S1 German Guidelines[85]

For <3% BSA involvement: Potent TCS/TCI or targeted light therapy (308-nm excimer laser recommended).

For >3% BSA:

Chronic forms: nbUVB with TCS/TCI.

Rapidly progressing forms: Systemic corticosteroids (e.g., betamethasone, 5 mg twice weekly for 3-6 months).

Surgical treatment: For stable, unresponsive vitiligo.

Depigmentation therapy : Only in rare cases of extensive vitiligo.

Topical ruxolitinib 1.5% (TR): Approved by FDA (July 2022) and EMA (February 2023), effective for localized NSV, particularly on the face. TR shows good results in combination with phototherapy but larger studies are needed (over 75% improvement in facial VASI at 24 weeks).

II. Management of Segmental Vitiligo (SV):

1. **European Dermatology Forum. (EDF) and European Academy of Dermatology and Venereology. (EADV) Guidelines**[81–83]

- **Active Segmental Vitiligo (SV)** (within the last 12 months):

Topical Treatments:

Potent topical corticosteroids (TCS) once daily or twice-daily tacrolimus (TCI) are recommended for both stabilization and repigmentation.

Phototherapy (targeted, if needed) can be used alongside these topical treatments.

Systemic Corticosteroids (CS):

While systemic corticosteroids (oral) may be considered for rapidly progressing disease, they are not included in the treatment algorithm for SV in the EDF/EADV guidelines.

- Stable Segmental Vitiligo (SV) (for at least 12 months):

Stabilization Goal:

If stabilization is the goal, clinical follow-up is sufficient. No further treatment is necessary unless disease progression is noted.

Repigmentation Goal:

If repigmentation is desired and topical treatments/phototherapy have not been effective, surgical techniques (e.g., grafting) are recommended for resistant cases.

Depigmentation:

Although depigmentation is not specifically mentioned for SV, it should be taken into consideration in cases of positive Koebner phenomenon or widespread or highly noticeable resistant cases (such as on the hands or face).

Maintenance Treatment:

SV does not require maintenance treatment with TCS/TCI after the disease stabilizes, unlike NSV.

2. **British Association of Dermatologists (BAD) Guidelines (2021)**[84]

- **For Active SV:**

  - First-Line Treatments:

Potent TCS once daily is the first-line option for SV (avoiding the periocular area).

For facial involvement or photo-exposed areas, 0.1% tacrolimus (TCI) twice daily can be used as an alternative.

Intermittent regimens of TCS (potent/very potent) or in areas with thinner skin TCI is recommended

  - Second-Line Treatments:

NbUVB therapy is recommended for SV and NSV as a second-line treatment. It can be used in combinatiom with potent TCS or TCI.

Other second-line options are excimer laser or light plus TCI, but their availability may be limited.

Systemic Corticosteroids:

For rapidly progressing disease, systemic corticosteroids (e.g., betamethasone 0.1 mg/kg oral weekly twice) are used in combination with NbUVB therapy.

Surgical Treatment:

For stable and resistant cases of vitiligo, such as when patients are subjectively distressed by the esthetic impact, surgical treatments such cellular grafting are reserved.

Depigmentation:

Depigmentation is generally only considered for extensive disease (greater than 50% skin involvement) and highly visible areas (like the face or hands), especially when other treatments have failed.

3. **S1 German Guidelines (2023)**[85]

For Chronic SV:

To stabilize the condition and perhaps cause repigmentation, strong TCS/TCI. or focused light therapy is advised, either separately or in combination.

For Acute SV.:

A combination of systemic corticosteroids (e.g., oral betamethasone) with potent TCS/TCI and targeted light therapy is recommended to control rapidly progressing disease.

Surgical Therapy:

For stable and unresponsive SV, surgical treatment (such as cellular grafting) is advised.

Depigmentation:

Only in extremely rare instances with subtotal vitiligo, after every other treatment option has been tried, is depigmentation advised. For SV, it isn't specified explicitly.

Supportive Care:

As with other guidelines, UV protection, dermatocosmetics, camouflage, and psychosocial support (e.g., psychotherapy or self-help groups) are also emphasized for managing SV.

4. **Key Differences Between SV and NSV in the Guidelines**[81]

- Temporal Cut-offs:

In SV, topical treatments like TCS/TCI are used based on a 12-month activity window (active disease within the last year), whereas in NSV, the activity window is 6 months.

- Maintenance Treatment:

SV does not require maintenance treatment once the disease is stable. In contrast, NSV requires maintenance treatment with TCS/TCI. (at least twice a week. for 6 months) to prevent recurrence.

- Surgical Interventions:

Surgical treatments in SV are recommended for resistant cases that have been stable for at least a year, while NSV may consider surgery earlier in resistant cases.

- Systemic Corticosteroids:

Systemic corticosteroids are an option for rapidly progressing disease in both SV. and NSV. but are more commonly used in the context of NSV in practice.

- Depigmentation:

Depigmentation is rarely considered in SV, except in highly visible or widespread cases, whereas it is a more commonly considered option in NSV for extensive or resistant cases, particularly with a positive Koebner phenomenon

Even with the wide range of available treatments, vitiligo is still a serious dermatological issue. Despite the lack of symptoms, it can significantly impact an individual's quality of life. Significant psychosocial manifestations, such as low self-esteem and depression, may occur.[86]

MICRONEEDLING

Microneedling (MN), also called as collagen induction therapy, involves using sterilized microneedles to puncture the skin and stimulate regeneration. The concept originated in 1995 with "subcision," developed by Orentreich and Orentreich for scar treatment.[87] In 2006, Dr. Desmond Fernandes. created the first modern MN device, the Dermaroller®.[88]

Microneedling (MN) devices, including the Dermaroller and Dermapen®, are widely used for various skin treatments and are US FDA registered. The Dermaroller. is a hand-held device with 192 solid steel microneedles arranged in 24 circular arrays, used in multiple directions on the skin surface. Medical models like the CIT 8™. and MF 8™. offer needle heights of 500 micrometre and 1,500 micrometre. Home-use

models, such as the Beauty Mouse®, have a larger number of needles (480) for broader skin areas.[89]

The Dermapen. is a spring-loaded, electrically powered device that delivers microneedles in a stamping motion across the skin[90] There are also advanced microneedling technologies that combine mechanical MN with other modalities for specific treatments, including fractional radiofrequency microneedling (FRFM), DermaFrac™ (combining microdermabrasion, LED light, and serum infusion), LED microneedling, and microneedle drug delivery systems.[89]

MN is a low-cost, minimally invasive procedure for treating various aesthetic and dermatologic conditions.[91] The treatment relies on inducing physical distress to the skin, creating micro-conduits through the epidermis and into the dermis. This triggers a wound healing response, stimulating collagen and elastin production via growth factors such as TGF-alpha., TGF-beta, and PDGF, which are released by platelets and neutrophils.[92]

Over time, MN has expanded beyond cosmetic uses, with clinical trials supporting its effectiveness for treating conditions like actinic keratoses, pigmentation disorders, hyperhidrosis, striae, and even hair loss.[89] In hair restoration, MN is thought to stimulate dermal papilla stem cells, increase blood flow, and activate growth factors.[93] Additionally, it helps treat scars by breaking down superficial collagen and promoting collagen synthesis just beneath the epidermis.[94]

In vitiligo, microneedling results in a subclinical -inflammation of the epidermal layer, which promotes the migration of keratinocytes, melanocytes and repigmentation. It also facilitates the effective transplantation of melanocytes from pigmented to non-pigmented areas and improves topical medication penetration into

the skin. It may be used as a stand-alone treatment for vitiligo or in conjunction with therapeutic injection.[95]

5 FLUOROURACIL (5FU)

5-fluorouracil (5-FU) is a well-established topical chemotherapeutic agent used primarily for dermatological conditions involving keratinocyte hyperproliferation, such as skin cancer, actinic keratosis, and keratoacanthoma. It has been in clinical use for over 45 years, offering efficacy in treating various skin disorders.[96] 5-Fluorouracil (5-FU) is a pyrimidine. analog that is composed of a pyrimidine. and furan ring.[97]

Pharmacokinetics of 5FU

Because fluorouracil's (FU) bioavailability varies greatly and is impacted by saturable first-pass hepatic metabolism, oral administration of FU causes unpredictable plasma concentrations. When given subcutaneously, absorption through undamaged skin is less than 10%, and bioavailability rises with increasing doses. Prolonged infusion is a more effective way to administer FU because of its short plasma half-life of about 12 minutes, which means that its cytotoxic effects are most noticeable during the S-phase. of the cell cycle. FU dose and steady-state plasma levels after continuous venous infusion (CVI) are consistently correlated in studies, with clearance staying constant at roughly 2L/min/m^2.[98]

Metabolism of 5FU

5-FU is mostly activated by being converted to fluorouridine monophosphate. (FUMP). Phosphoribosyl pyrophosphate. (PRPP) is the cofactor for orotate phosphoribosyltransferase (OPRT), which can do this directly. Alternatively,

fluorouridine (FUR) can do this indirectly through the sequential actions of uridine phosphorylase. (UP) and uridine kinase. (UK). After being created, FUMP undergoes further phosphorylation to produce fluorouridine diphosphate (FUDP), which can either be transformed by ribonucleotide reductase (RR) into fluorodeoxyuridine diphosphate (FdUDP) or the active metabolite fluorouridine triphosphate (FUTP). The active form, fluorodeoxyuridine monophosphate. (FdUMP), can be obtained by dephosphorylating FdUDP or by further phosphorylating it to fluorodeoxyuridine triphosphate. (FdUTP). Thymidine phosphorylase. (TP) is another mechanism for 5-FU activation. It transforms 5-FU into fluorodeoxyuridine (FUDR), which is subsequently phosphorylated by thymidine kinase (TK) into FdUMP.[99]

Catabolism of 5 FU

The liver, intestinal mucosa, and other organs contain the enzyme dihydropyrimidine dehydrogenase (DPD), which converts 5-FU to 5,6-dihydro-5-fluorouracil. (DHFU). Urine contains the final products of this process, α-fluoro-β-ureido-propionic acid and α-fluoro-β-alanine. (FBAL). According to studies, 60–90% of 5-FU that is given is eliminated as FBAL in a 24-hour period .[100] Majority of patients tolerate 5-FU adequately, individuals who lack DPD are more likely to experience severe toxicities following conventional 5-FU dosages, such as diarrhea, mucositis, neurotoxicity, and even death.[101]

FBAL, a key 5-FU catabolite, has been implicated in neurotoxicity and cardiotoxicity. In animal studies, FBAL was found to be more toxic than monofluoroacetic acid, leading to neurological damage similar to that seen in patients[102] FBAL has also been linked to cardiotoxicity through the formation of fluoroacetate, which inhibits aconitase in the heart. [103] Additionally, FBAL

contributes to hand-foot syndrome (HFS) in patients undergoing 5-FU-based chemotherapy., with higher incidence observed in those receiving continuous 5-FU infusion compared to bolus infusion.[104]

Mechanism of action of topical 5FU

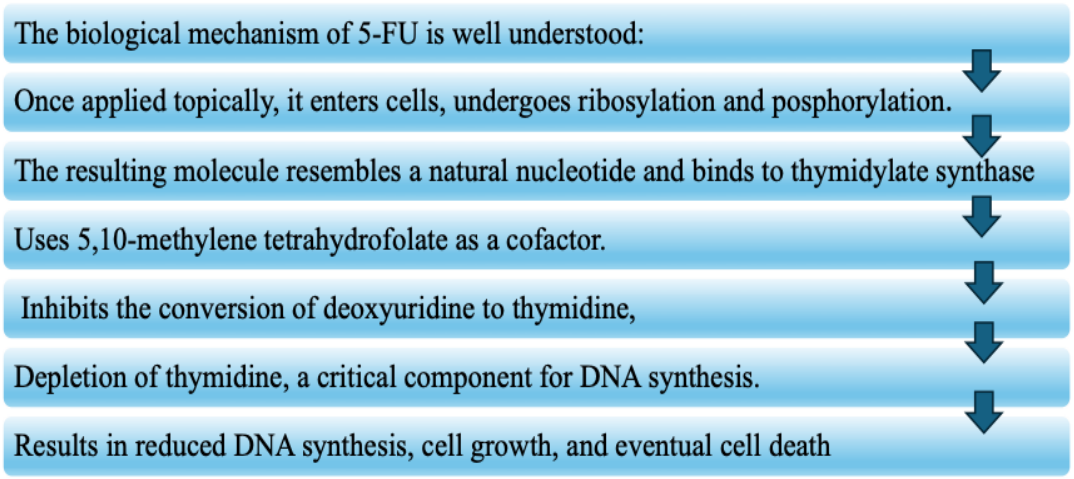

Figure.7 -Depicts the mechanism of action of topical 5FU[97]

5-FU is particularly effective in targeting rapidly proliferating cells, which makes it most cytotoxic to pathological lesions with high rates of cell division. Normal, non-proliferating skin cells are less affected due to the limited penetration of 5-FU into healthy skin .

Formulations

5-FU is normally given without further dilution and is available at a dosage of 50 mg/mL. It is available in different concentrations for topical use, such as 0.5%, 1%, 4%, and 5% for creams and 2% and 5% for solutions. The most popular is the 0.5% cream, as it causes the least irritation and promotes better patient compliance .[98]

Table 5- Dermatological applications of 5 FU [97]

FDA-labeled indications	**Non-FDA-labeled indications**
Actinic keratosis	Keratoacanthoma
Superficial basal cell carcinoma	Psoriatic nail dystrophies
	Squamous cell carcinoma in situ
	Vitiligo
	Warts

Topical 5-fluorouracil (5-FU) has been explored as a treatment for vitiligo, particularly when combined with procedures like dermabrasion or microneedling. Its mechanism of action in promoting repigmentation involves several key processes:

1. Selective Cytotoxicity: 5-FU is an antimetabolite that interferes with DNA synthesis, exhibiting greater cytotoxic effects on rapidly dividing keratinocytes than on melanocytes. This selective action leads to the destruction of keratinocytes while sparing melanocytes, which can then proliferate and migrate to repopulate depigmented areas.

2. Inflammatory Response and Delayed Wound Healing: When applied after epidermal ablation techniques such as dermabrasion or laser therapy, 5-FU induces a robust inflammatory response and delays re-epithelialization. This prolonged inflammatory milieu releases various mediators and enzymes, including metalloproteinases, which create a favorable environment for melanocyte activation, proliferation, and migration into depigmented regions.

3. Stimulation of Melanocyte Migration: The combination of epidermal injury and 5-FU application enlarges intercellular spaces within the epidermis, facilitating the movement of melanocytes from adjacent pigmented areas or hair follicles into vitiliginous patches. This migration is essential for initiating repigmentation.[105]

Research indicates that 5-FU cream absorption can be improved by procedures such dermabrasion or fractional CO2/erbium: YAG laser. Additionally, 5-FU cream plus microneedling shown to be more successful for vitiligo than 5-FU cream alone.[106] Another therapy option for vitiligo is intralesional 5-FU injection. [107] While intralesional 5-FU injections are administered every two weeks, 5-FU cream should be applied once daily. [97]

Safety

In addition to other typical anticancer medication side effects, myelosuppression and mucositis are the most major side effects of systemic 5-FU. 5-FU, however, has not been linked to any notable systemic side effects when used topically to dermatological diseases.

Common local side effects include irritation., pain, hyperpigmentation., ulceration, and inflammatory reactions, though these are generally temporary. While both topical and intralesional 5-FU can cause similar side effects, intralesional 5-FU is more likely to lead to pain, hyperpigmentation., and blister formation. Serious complications such as superficial necrosis, local infection, and wound dehiscence. are rare.

Topical 5-FU is widely accepted for treating various dermatologic conditions, and in cases where patients cannot tolerate the burning sensation associated with intralesional injections, topical options may be used instead. Injection-related pain is typically manageable with local anesthesia and cold air.[108]

TACROLIMUS

Vitiligo can now be treated with tacrolimus, a macrolide immunosuppressant derived from the fungus Streptomyces tsukubaensis.[109] Tacrolimus, approved in the US in 1994, is used to prevent organ rejection in liver, heart, small bowel, and kidney transplants, and is being now studied for autoimmune diseases. It is highly lipophilic, soluble in several solvents but insoluble in water, and unstable in alkaline conditions, which makes formulating it into an ideal dosage form challenging.[110]

Pharmacokinetics

Tacrolimus's low absorptivity, low plasma levels, and interference from metabolites and other medications make it difficult to assess the drug's concentration in biological fluids using different techniques. Tacrolimus cannot be distinguished from its metabolites using enzyme-linked immunosorbent tests, which are the basis for the majority of pharmacokinetic studies. About 25% of the oral dose of tacrolimus is accessible, and its absorption rate varies. Peak concentrations are obtained in 0.5 to 6 hours. With a blood-to-plasma. ratio of 15, it is strongly linked to red blood cells and mostly binds to plasma's albumin and α1-acid glycoprotein. Tacrolimus has a mean half-life of 12 hours and a total body clearance of .0.06 L/h/kg. It is completely digested before being eliminated. It is eliminated more slowly when liver damage occurs and when it interacts with other medications. [110]

Metabolism of tacrolimus

The liver is the primary site of metabolism for tacrolimus, which is mainly removed from the body as several metabolites. But the metabolism of tacrolimus is also influenced by the intestines. Human plasma, bile, and liver microsomes from different species have been found to have at least 15 tacrolimus metabolites. Other species' livers process tacrolimus more slowly than those of humans and baboons.

Tacrolimus metabolism depends on cytochrome P450 enzymes, especially CYP3A4, with hydroxylation and demethylation serving as the main routes. 13-O-demethyl-tacrolimus is the most prevalent metabolite found in patient blood and human liver microsomes. Several dihydrodiols and hydroxy derivatives are examples of additional metabolites. The most common metabolites in blood from recipients of liver and kidney transplants are demethyl and demethylhydroxy, which greatly affect tacrolimus's area under the curve (AUC).

In mouse lymphocyte responses, 13-O-demethyl-tacrolimus has been found to be about a tenth as active as tacrolimus, although having a similar immunosuppressive effect. Nevertheless, more research is necessary to determine how tacrolimus metabolites may contribute to its immunological and toxic effects. [111]

Excretion

In the urine of liver transplant. recipients, < 1% of the intravenous dose of tacrolimus is eliminated unaltered, with renal clearance making up < 1% of the total body clearance. The urine also contains trace amounts of tacrolimus conjugate. In a similar vein, bile contains fewer than 1% of tacrolimus or its cross-reacting

metabolites that are unaltered. According to research on animals, the main pathway for tacrolimus metabolites is biliary excretion.[112]

Mechanism of actions

In order to suppress calcineurin, tacrolimus binds to the .cytosolic 12kDa FK506 binding protein. (FK-BP) and forms a complex. This inhibition suppresses the expression of various proinflammatory T-cell cytokines, such as granulocyte-stimulating factors, IL-2, IL-3, IL-4, IL-5, IFN-γ, and TNF-α, by preventing the .phosphorylation of NFAT (nuclear factor of activated T-cells).

Studies have shown that tacrolimus increases the expression of IL-10, an anti-inflammatory cytokine, in vitiligo lesions. Elevated IL-10 levels contribute to the suppression of the inflammatory response, creating a more favourable environment for melanocyte survival and function.[113]

Melanocyte behaviour is affected by topical tacrolimus. This effect is associated with increased activity and expression of tyrosinase, a key enzyme in melanin synthesis, and upregulation of c-KIT, a receptor important for melanocyte function.[114] Tacrolimus-treated keratinocyte supernatant dramatically increased melanocyte proliferation, according to a study by Lan et al. Furthermore, they noticed that the supernatant had higher levels of matrix metallopeptidase-9 activity and stem-cell factor, indicating that tacrolimus had a beneficial effect on melanocyte migration and growth in vitro.[114]

Tacrolimus is more effectively delivered, absorbed, and penetrated into vitiliginous tissues when applied following microneedling. By reducing the length of

therapy and hastening repigmentation, this combination may produce better results. It is a simple method that can be used in addition to tacrolimus therapy.[115]

Table 6-Dermatological applications of tacrolimus[116]

FDA approved	Off label
Atopic dermatitis	Papulosquamous disorders
• Psoriasis	
• Lichen planus	
Pigmentary disorders	
• Vitiligo	
Bullous dermatoses	
• Pemphigus vulgaris/foliaceous	
• Bullous pemphigoid	
• Cicatricial pemphigoid	
Others	
• Pyoderma gangrenosum	
Graft versus host disease	
• Lichen striatus	
• Zoon's balanitis	
• Lichen nitidus	
• Lichen sclerosus et atrophicus (LSetA)	
• Ichthyosis linearis circumflexa	

Oral tacrolimus are explored in psoriasis(0.15mg/kg BD), Behcet's disease (0.1mg/kg/day), pyoderma gangrenosum (0.15 to 0.2 mg/kg/day), Graft. versus host disease, cutaneous t cell lymphoma. (0.15mg/kg/day) with significant improvement.[117]

Contraindications

Absolute

- Hypersensitivity to tacrolimus or any of the ingredients in the ointment
- Children under two years old

Relative

- An active skin illness (at the place to be treated). [116]

Safety

- Tacrolimus can also lead to hyperglycemia and new-onset diabetes, especially in liver transplant recipients
- neurologic side effects (tremors, paresthesia, and insomnia)
- cardiovascular (hypertension, chest pain),
- gastrointestinal (nausea, diarrhea, liver dysfunction),
- pulmonary (dyspnea, pleural effusion),
- hematological (anemia, leukocytosis),
- metabolic (hyperglycemia, hyperlipidemia),
- urogenital (renal dysfunction, infections),
- and cutaneous (rash, pruritus) side effects.[117]

  Topical tacrolimus usually results only in transient burning sensation , purpura and irritation over the application site.[112]

MATERIALS AND METHODS

Source of Data.: Patients aged 18 years to 50 years, of either gender having clinically stable vitiligo at KLE's Dr. Prabhakar. Kore Hospital And Medical Research Centre, Nehru Nagar, Belagavi 590010.

Study Design.: open label, non-randomized , interventional study

Ethical clearance.: Clearance was taken from the Ethical committee of the institute.

Study Period: 1 year (1st april 2023 to 31st march 2024)

Sample Size: The sample size was calculated using G-Power under F tests for ANOVA :

Reapeated measures, within factors considering effect size = 0.25 as per the study by Matharoo P et al.,[118]

alpha = 0.05,

beta =0.10 ,

power of test = 0.90 ,

number of groups = 3,

number of measurements =6 correlation among repeated measures,

Nonsphericity correction = 1 ,

the sample size was 24 for each group.

Patients having more than three patches of vitiligo were recruited, in whom each of the three patches was randomly allocated to either 5 fluorouracil with microneedling or tacrolimus with microneedling group or microneedling alone group.

INCLUSION CRITERIA:

- All patients. aged from 18 to 50 years with localized & stable vitiligo for a year willing to come for regular follow up
- No history of topical application for 2 weeks, oral medication for 4 weeks, phototherapy and excimer laser for 8 weeks when enrolled in the study
- Patients with 3 or more stable vitiligo patches

EXCLUSION CRITERIA

- Pregnant or lactating women
- Presence of Bleeding /coagulation disorders
- Past history of keloidal tendency
- Patients with active Koebner's phenomenon, infections
- Patients with known allergy to 5-fluorouracil, tacrolimus
- Immunosuppressed patients
- Any concomitant skin conditions over the vitiligo patch
- Mucosal and palmoplantar vitiligo

DATA COLLECTION PROCEDURE:

- After screening, all patients with clinically stable vitiligo (no new lesion for a year) were chosen.
- Before starting treatment, detailed history, clinical examination of patients was done.
- The patients were informed about the treatment given and possible side effects like pain, erythema, ulceration, hyperpigmentation which would occur.
- Informed written consent was taken from each patient recruited.
- Data was collected by a single examiner and recorded in case proforma.
- Three vitiligo patches were randomly selected in a single patient and divided to group A, group B, group C and noted in schematic human diagram.
- Digital photographs of the patches was taken using identical camera settings, patient positioning and room lighting at baseline and at each sessions.
- Topical anaesthetic cream eutectic. mixture of local anaesthetic was applied 30 to 45 mins before procedure to anaesthetize the area.
- Under strict aseptic conditions, microneedling of the vitiligo patches was done using Dr.pen ultima A6.
- The needle penetration depth was kept at 0.25 to 0.5mm over face and 1-2mm over the body and microneedling was done in horizontal and vertical direction until pinpoint bleeding points appear.
- It is followed by the application of 5fluorouracil 5% cream in group A, 0.1%

tacrolimus ointment in group B and bland emollient in group C.

- Occlusive dressing was done and kept for a day.
- This procedure was repeated every 4 weeks for a maximum of 4 sessions (4 months). Digital photographs were taken at each session.
- Patients were advised to apply 5fluorouracil 5% cream in Group A, 0.1% tacrolimus ointment in group B, bland emollient in group C once daily in between sessions and until follow-up visit at the end of 5^{th} month.
- After 5 months, the digital photographs were compared with baseline.
- Patient's assessment of improvement was recorded by Visual Analog Scale.
- Physician's assessment of improvement was also be recorded by using Physician. Global Assessment Scale.
- The efficacy of the treatment was calculated based on percent of repigmentation.
- The safety of the treatment was assessed by the side effects associated with the treatment

STATISTICAL ANALYSIS:

Data was entered into Microsoft Excel. and statistical analysis was carried out in SPSS software version 17.0. Qualitative variables like age categories, gender, site of the lesion and leukotrichia were presented as frequency and percentages. Bar diagram and pie charts were used for graphical representation of data.

Comparison of drug efficacy, patient satisfaction and patient safety across the three groups were done using Chi square test. In each group, comparison of drug efficacy, patient satisfaction and patient safety pre and post intervention was done with Chi square test. A p value of less than .0.05 was considered as statistically significant.

RESULTS

Table-7: Age distribution

Age groups (years)	Number	Percentage
≤30	6	25.0
31-40	12	50.0
>40	6	25.0
Total	24	100.0

The study consists of a total of 24 participants. Among them, 6 (25.0%) were aged ≤30 years, 12 (50.0%) were between 31 and 40 years, and 6 (25.0%) were older than 40 years. The largest proportion of participants fell within the 31–40 age group, representing half of the sample. The ≤30 and >40 age groups had an equal distribution, each accounting for one-quarter of the participants. The mean (SD) age of the participants was 36.6 (11.7) years

Figure 8: Age distribution

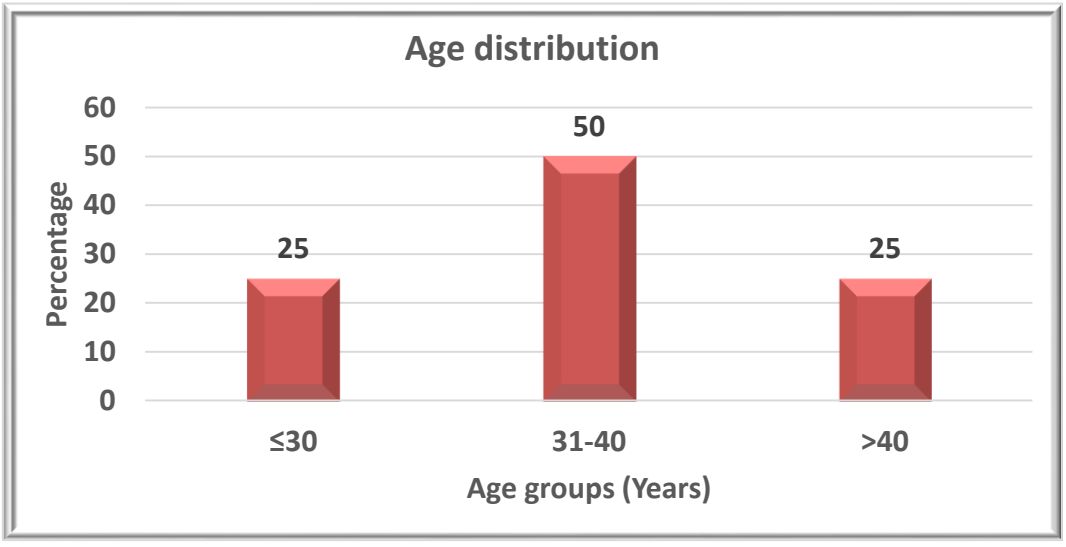

Table-8: **Gender distribution**

Gender	Number	Percentage
Male	9	37.5
Female	15	62.5
Total	24	100.0

Out of 24 participants recruited, 9 (37.5%) were male, while 15 (62.5%) were female. The majority of participants were female, making up nearly two-thirds of the sample.

Figure -9: **Gender distribution**

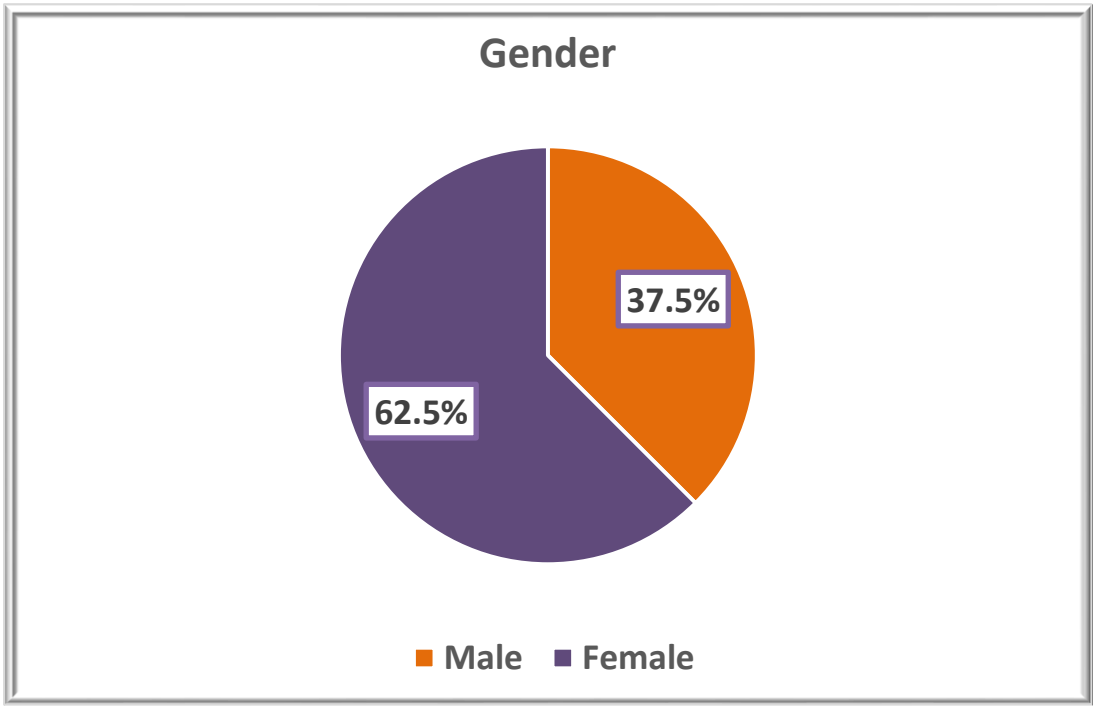

Table 9- **Distribution.of patients based on duration of vitiligo**

Duration	Number	Percentage
< 5 years	14	58
>5 years	10	42
Total	24	100.0

About 14 (58%) participants had a disease duration of less than 5 years, while 10 (42%) participants had a disease duration. of more than 5 years.

Figure 10- **Distribution.of patients based on duration of vitiligo**

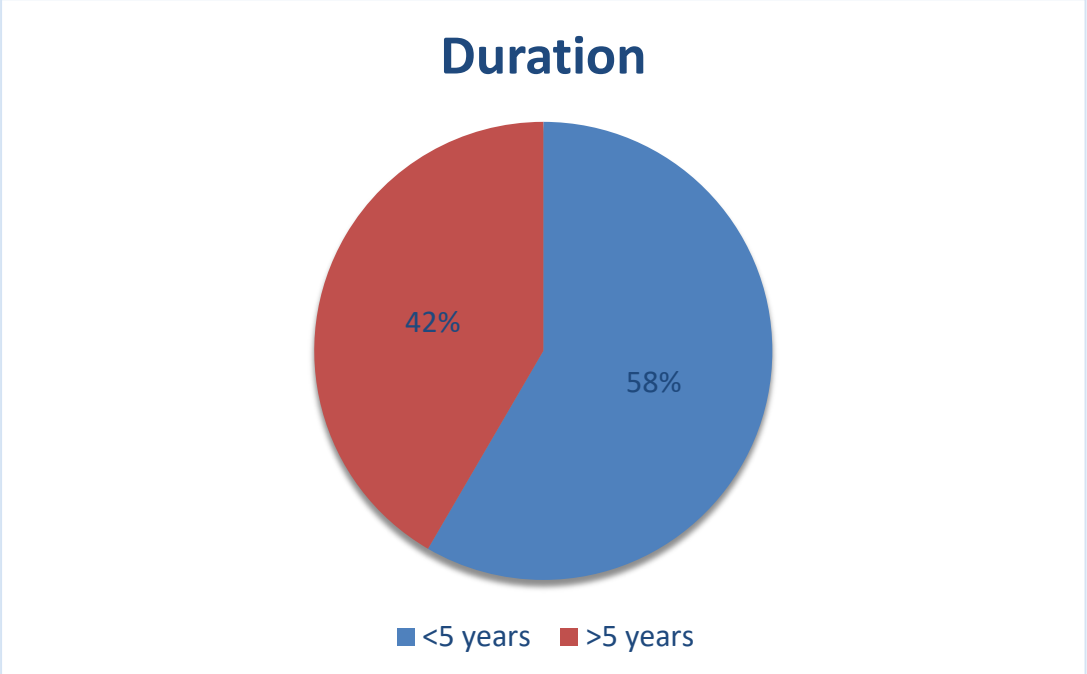

Table-10: **Distribution.of patients based on co-morbid conditions**

Co morbid conditions	Number	Percentage
NIL	18	75.0
DM	2	8.3
HTN	1	4.2
Hypothyroidism	3	12.5

Among the 24 participants, 18 (75.0%) had no comorbid conditions. Diabetes mellitus (DM) was present in 2 (8.3%) participants, hypertension (HTN) in 1 (4.2%), and hypothyroidism in 3 (12.5%).

Figure-11: **Distribution.of patients based on co-morbid conditions**

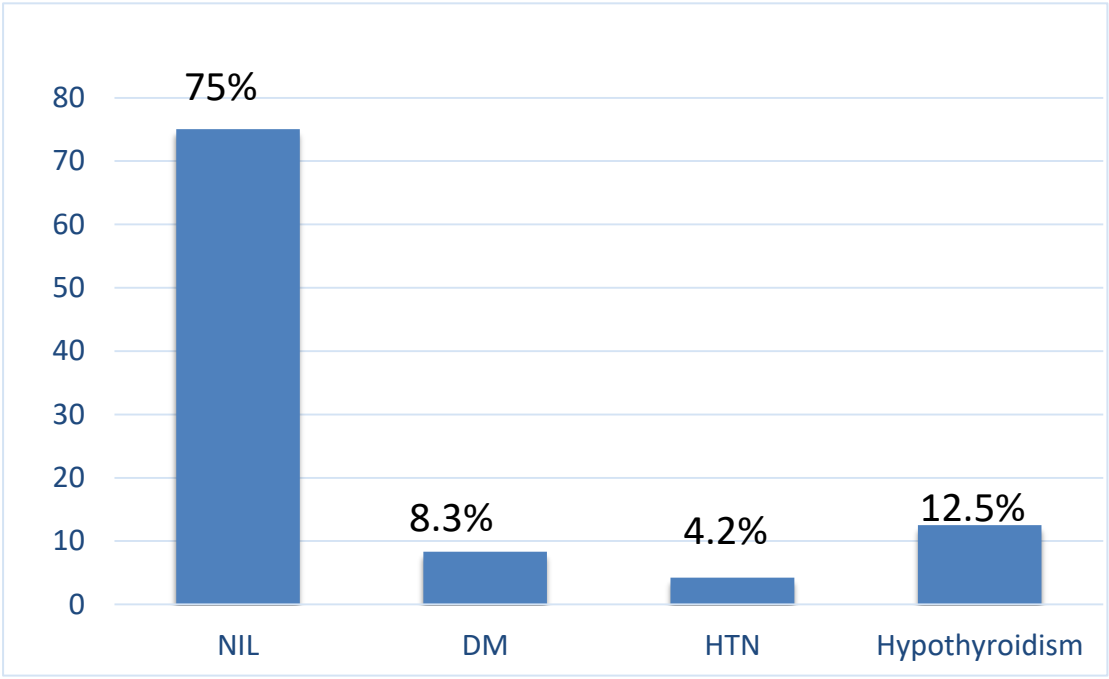

Table -11: **Distribution.of patients based on family history**

Family history	Number	Percentage
Yes	1	4.2
No	23	95.8

A positive family history was reported in 1 (4.2%) participant, while 23 (95.8%) had no family history of vitiligo.

Figure -12: Distribution.of patients based on family history

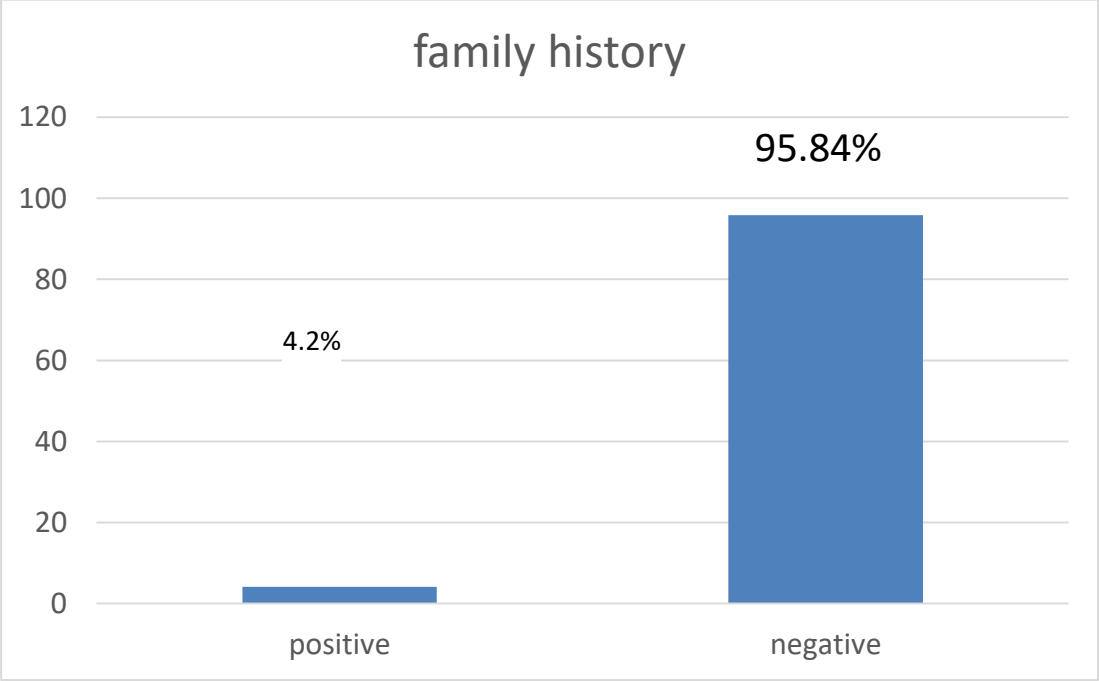

Table-12: **Distribution.of vitiligo patches based on body regions**

Body regions	Number	Percentage
Head and Neck	12	16.7
Trunk	22	30.6
Extremities	38	52.8
Total	72	100

About 12 patients (16.7%) had vitiligo patches located on the head and neck, 22 (30.6%) on the trunk, and 38 (52.8%) on the extremities. Most lesions were found on the extremities, accounting for more than half of the cases, followed by the trunk and then the head and neck region.

Figure 13- **Distribution.of vitiligo patches based on body regions**

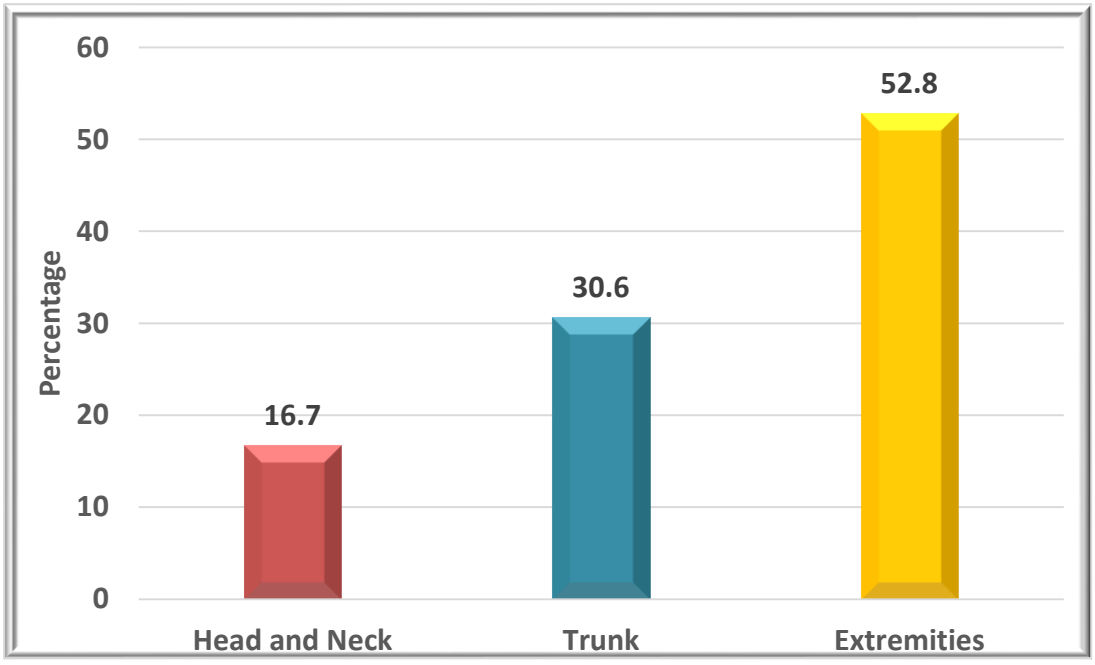

Table-13: **Distribution.of vitiligo patches based on site of lesion.**

Site of the lesion	Number	Percentage
Abdomen	4	5.6
Thigh	12	16.7
Leg	24	33.3
Back	3	4.2
Hands	10	13.9
Neck	7	9.7
Trunk	1	1.4
Breast	2	2.8
Occipital & Popliteal region	2	2.8
Eyelid	3	4.2
Post auricular	1	1.4
Chest	3	4.2

A total of 72 lesions were analyzed, with the most common sites being the leg 24 (33.3%), thigh 12 (16.7%), and hands 10 (13.9%). Other sites included the neck 7 (9.7%), abdomen 4 (5.6%), back 3 (4.2%), chest 3 (4.2%), eyelid 3 (4.2%), breast 2 (2.8%), occipital and popliteal region 2 (2.8%), trunk 1 (1.4%), and post-auricular region 1 (1.4%).

The efficacy of the treatment was measured using Global Physician Assessment scale, and its grading is as follows;

Grade 0 Repigmentation & response absent

Grade 1 <25% repigmentation & poor response

Grade 2 25 to 50% repigmentation & good response

Grade 3 50 to 75% repigmentation & very good response

Grade 4 >75% repigmentation & excellent response

Patient satisfaction was measured using Visual Anologue Scale as follows

Score 0 -not satisfied

Score 1 -slightly satisfied

Score 2 -very satisfied

Score 3 -extremely satisfied

Table 14: **Comparison of physician assessment scale in microneedling group with 5-Flourouracil before and after follow up**

Pre intervention Grades	**Global physician assessment scale (Post)**								**Total**	**P-value**
	Grade I		**Grade II**		**Grade III**		**Grade IV**			
	n	%	n	%	n	%	n	%		
Grade I	2	8.7	6	26.1	10	43.5	5	21.7	23	**0.37**
Grade III	0	0	0	0	0	0	1	100	1	
Total	2	8.3	6	25	10	41.7	6	25	24	

Pre-intervention grades were compared with post-intervention among 24 participants. For those graded as Grade I pre-intervention (n = 23), 2 (8.7%) were post-intervention Grade I, 6 (26.1%) were Grade II, 10 (43.5%) were Grade III, and 5 (21.7%) were Grade IV. For those graded as Grade III pre-intervention (n = 1), 0 (0%) remained Grade I, Grade II, or Grade III post-intervention, while 1 (100%) was graded Grade IV post-intervention. There was a difference in grades were observed in this group, however it was not statistically significant with the p value of 0.37.

Table 15: **Comparison of physician assessment scale in microneedling group with tacrolimus 0.1% before and after follow up**

Pre intervention Grades	**Global physician assessment scale (Post)**										**Total**	**P-value**
	Grade 0		**Grade I**		**Grade II**		**Grade III**		**Grade IV**			
	n	%	n	%	n	%	n	%	n	%		
Grade 0	1	20	4	80	0	0	0	0	0	0	5	**0.12**
Grade I	0	0	4	22.2	10	55.6	3	16.7	1	5.6	18	
Grade II	0	0	0	0	1	100	0	0	0	0	1	
	1	4.2	8	33.3	11	45.8	3	12.5	1	4.2	24	

For those graded as Grade 0 pre-intervention (n = 5), 1 (20.0%) remained Grade 0 post-intervention, 4 (80.0%) progressed to Grade I, and none were graded as Grade II, III, or IV. For those graded as Grade I pre-intervention (n = 18), 4 (22.2%) were graded Grade I, 10 (55.6%) progressed to Grade II, 3 (16.7%) progressed to Grade III, and 1 (5.6%) progressed to Grade IV post-intervention. For those graded as Grade II pre-intervention (n = 1), 1 (100%) progressed to Grade II post-intervention. The p-value for the comparison between pre- and post-intervention grades was 0.12, indicating no statistically significant difference in the distribution of grades post-intervention.

Table 16: **Comparison of physician assessment scale in microneedling alone before and after follow up**

Pre grades	Global physician assessment scale (Post)						Total	P-value
	Grade 0		Grade I		Grade II			
	n	%	n	%	n	%		
Grade 0	6	42.9	7	50	1	7.1	14	**0.04**
Grade I	0	0	7	70	3	30	10	
Total	6	25	14	58.3	4	16.7	24	

In the microneedling group, those graded as Grade 0 in the pre-intervention (n = 14), 6 (42.9%) remained Grade 0 post-intervention, 7 (50.0%) progressed to Grade I, and 1 (7.1%) progressed to Grade II. For those graded as Grade I pre-intervention (n = 10), 7 (70.0%) were graded Grade I post-intervention, and 3 (30.0%) progressed to Grade II. The p-value for the comparison between pre- and post-intervention grades was 0.04, indicating statistically significant difference in the distribution of grades post-intervention.

Table 17: **Comparison of patient satisfaction in microneedling with 5-fluorouracil group before and after follow up**

Pre intervention grades	**Visual analogue scale-Patient satisfaction (Post)**								**Total**	**P-value**
	Grade 0		**Grade I**		**Grade II**		**Grade III**			
	n	%	n	%	n	%	n	%		
Grade 0	1	33.3	1	33.3	0	0	1	33.3	3	**0.06**
Grade I	0	0	3	15.8	13	68.4	3	15.8	19	
Grade II	0	0	0	0	0	0	1	100	1	
Grade III	0	0	0	0	0	0	1	100	1	
Total	1	4.2	4	16.7	13	54.2	6	25	24	

The table shows the pre and post comparison of patient satisfaction in microneedling with 5-fluorouracil group. For those graded as Grade 0 pre-intervention (n = 3), 1 (33.3%) remained Grade 0 post-intervention, 1 (33.3%) progressed to Grade I, and 1 (33.3%) progressed to Grade III. For those graded as Grade I pre-intervention (n = 19), 3 (15.8%) were graded Grade I post-intervention, 13 (68.4%) progressed to Grade II, and 3 (15.8%) progressed to Grade III. For those graded as Grade II pre-intervention (n = 1), 1 (100%) progressed to Grade III post-intervention. For those graded as Grade III pre-intervention (n = 1), 1 (100%) progressed to Grade III post-intervention. There was no statistically significant difference across the groups with respect to patient satisfaction (Visual Analogue Scale), as indicated by a p-value of 0.06. This suggests that while there may be a difference in patient satisfaction among the groups, the evidence is not strong enough to rule out the possibility that the observed difference is due to chance.

Table 18: **Comparison of patient satisfaction in microneedling with tacrolimus 0.1% group before and after follow up**

Pre intervention grades	**Visual analogue scale-Patient satisfaction (Post)**								**Total**	**P-value**
	Grade 0		**Grade I**		**Grade II**		**Grade III**			
	n	%	n	%	n	%	n	%		
Grade 0	1	12.5	7	87.5	0	0	0	0	8	**0.04**
Grade I	0	0	5	35.7	7	50	2	14.3	14	
Grade II	0	0	0	0	2	100	0	0	2	
Total	1	4.2	12	50	9	37.5	2	8.3	24	

Among 8 participants in Grade o in the pre intervention, 1 (12.5%) remained Grade 0 post-intervention, and 7 (87.5%) progressed to Grade I. No participants progressed to Grade II or Grade III. For those graded as Grade I pre-intervention (n = 14), 5 (35.7%) were graded Grade I post-intervention, 7 (50.0%) progressed to Grade II, and 2 (14.3%) progressed to Grade III. For those graded as Grade II pre-intervention (n = 2), both participants (100.0%) progressed to Grade II post-intervention. The difference in patient satisfaction across the groups was significant with the p-value of 0.04

Table 19: **Comparison of patient satisfaction in microneedling alone before and after follow up**

Pre grades	**Visual analogue scale-Patient satisfaction (Post)**						**Total**	**P-value**
	Grade 0		**Grade I**		**Grade II**			
	n	%	n	%	n	%		
Grade 0	9	60	5	33.3	1	6.7	15	**0.004**
Grade I	0	0	7	87.5	1	12.5	8	
Grade II	0	0	0	0	1	100	1	
Total	9	37.5	12	50	3	12.5	24	

For those graded as Grade 0 pre-intervention (n = 15), 9 (60.0%) remained Grade 0 post-intervention, 5 (33.3%) progressed to Grade I, and 1 (6.7%) progressed to Grade II. For those graded as Grade I pre-intervention (n = 8), 7 (87.5%) were graded Grade I post-intervention, and 1 (12.5%) progressed to Grade II. For those graded as Grade II pre-intervention (n = 1), 1 (100.0%) remained Grade II post-intervention. The difference in patient satisfaction across the groups was significant with the p-value of 0.04.

Table 20: **Comparison of drug efficacy across the groups during follow up**

Groups	Global physician assessment scale										Total	P-value
	Grade 0		Grade I		Grade II		Grade III		Grade IV			
	n	%	n	%	n	%	n	%	n	%		
Microneedling with 5-Flouroura cil 5%	0	0	2	8.3	6	25	10	41.7	6	25	24	**<0.001**
Microneedling with tacrolimus 0.1%	1	4.2	8	33.3	11	45.8	3	12.5	1	4.2	24	
Microneedling alone	6	25	14	58.3	4	16.7	0	0	0	0	24	
Total	7	9.7	24	33.3	21	29.2	13	18.1	7	9.7	72	

The Global Physician Assessment Scale was used to evaluate the drug efficacy across three groups. In the microneedling with 5-fluorouracil group (n = 24), 0 (0%) were graded as Grade 0, 2 (8.3%) as Grade I, 6 (25.0%) as Grade II, 10 (41.7%) as Grade III, and 6 (25.0%) as Grade IV. In the microneedling with tacrolimus 0.1% group (n = 24), 1 (4.2%) were graded as Grade 0, 8 (33.3%) as Grade I, 11 (45.8%) as Grade II, 3 (12.5%) as Grade III, and 1 (4.2%) as Grade IV. In the microneedling-alone group (n = 24), 6 (25.0%) were graded as Grade 0, 14 (58.3%) as Grade I, 4 (16.7%) as Grade II, and none were graded as Grade III or IV. Improvement was comparatively higher in microneedling with 5-flourouracil group when compared to others and this was significant with the p-value of <0.001.

Table 21: **Comparison of patient satisfaction across the groups during follow up**

	Patient satisfaction (Visual analogue scale)								**Total**	**P-value**
	Grade 0		**Grade I**		**Grade II**		**Grade III**			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil 5%	1	4.2	4	16.7	13	54.2	6	25	24	**<0.001**
Microneedling with tacrolimus 0.1%	1	4.2	12	50	9	37.5	2	8.3	24	
Microneedling alone	9	37.5	12	50	3	12.5	0	0	24	
Total	11	15.3	28	38.9	25	34.7	8	11.1	72	

Patient satisfaction was assessed using the Visual Analogue Scale across three treatment groups. In the microneedling with 5-fluorouracil group (n = 24), 1 (4.2%) reported Grade 0 satisfaction, 4 (16.7%) reported Grade I, 13 (54.2%) reported Grade II, and 6 (25.0%) reported Grade III. In the microneedling with tacrolimus 0.1% group (n = 24), 1 (4.2%) reported Grade 0 satisfaction, 12 (50.0%) reported Grade I, 9 (37.5%) reported Grade II, and 2 (8.3%) reported Grade III. In the microneedling-alone group (n = 24), 9 (37.5%) reported Grade 0 satisfaction, 12 (50.0%) reported Grade I, 3 (12.5%) reported Grade II, and none reported Grade III.

Overall, better satisfaction was observed in microneedling with 5-flurouracil group and the p-value for the comparison among groups was <0.001, indicating a statistically significant difference in patient satisfaction across the treatment groups.

Table 22- **Drug safety (side effects)**

Side effects	Number	Percentage
NIL	63	87.5
Erythema	5	6.9
Hyperpigmentation	2	2.8
Ulceration	3	4.2
Pain	3	4.2

About 63 (87.5%) had no reported side effects. Erythema was observed in 5 (6.9%) cases, hyperpigmentation in 2 (2.8%), ulceration in 3 (4.2%), and pain in 3 (4.2%)

Figure 14- **Drug safety (side effects)**

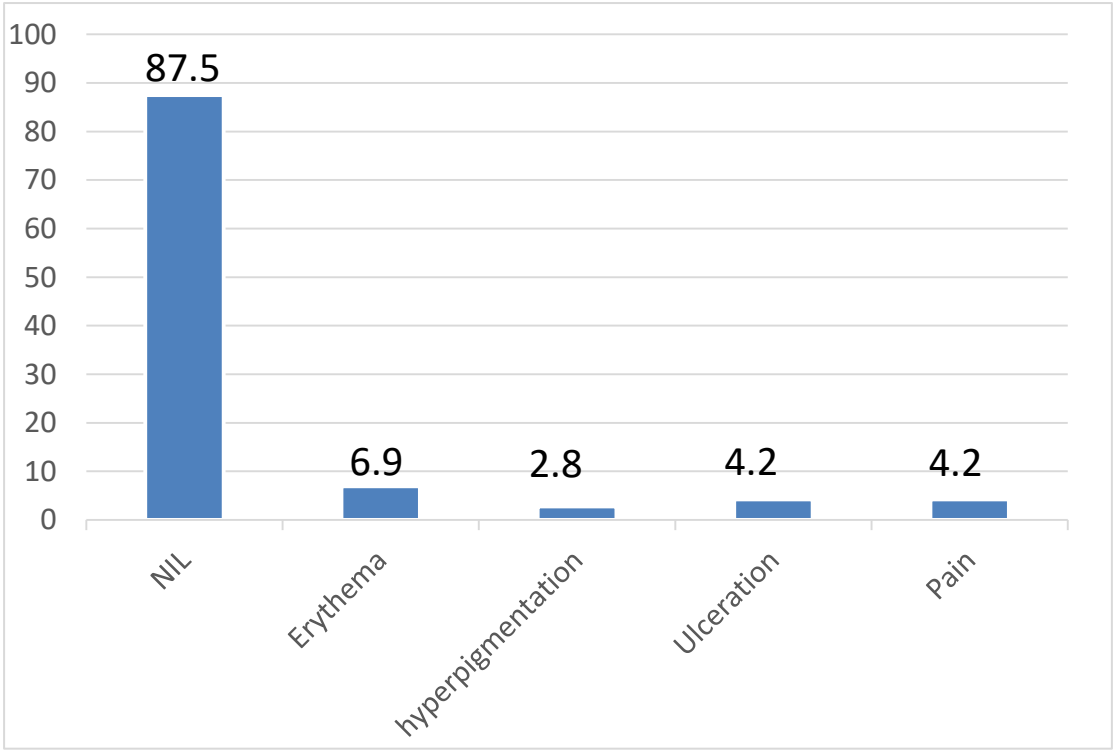

Figure 15- **Distribution of drug safety (side effects) across groups**

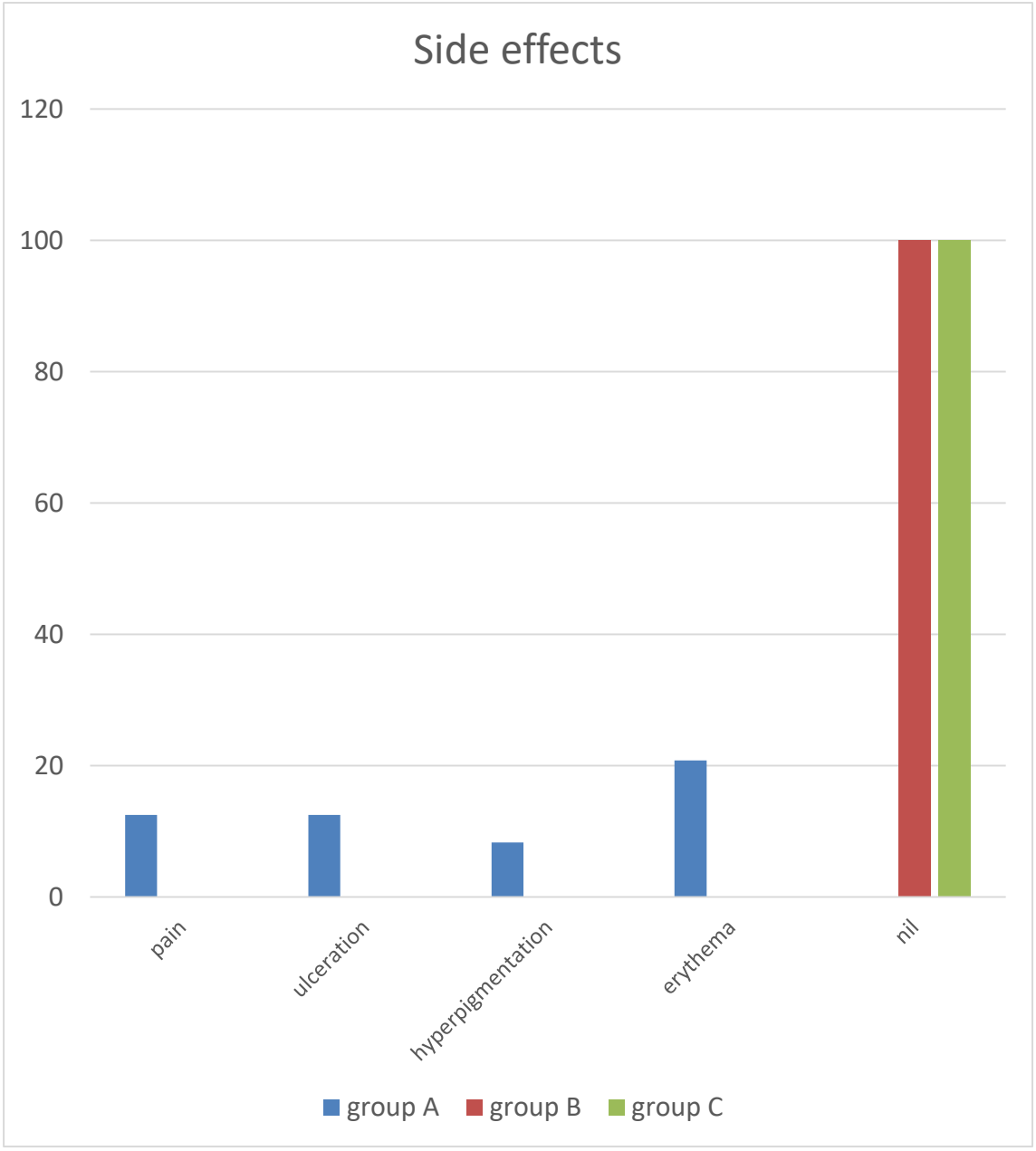

Table 23: **Comparison of drug safety across the groups during follow up**

Groups	Side effects				Total	P-value
	Absent		Present			
	n	%	n	%		
Microneedling with 5-Flourouracil 5%	16	66.7	8	33.3	24	**0.001**
Microneedling with tacrolimus 0.1%	23	95.8	1	4.2	24	
Microneedling alone	24	100	0	0	24	
Total	63	87.5	9	12.5	72	

In the microneedling with 5-fluorouracil group, 16 (66.7%) participants had no side effects, while 8 (33.3%) experienced side effects. In the microneedling with tacrolimus 0.1% group (n = 24), 23 (95.8%) had no side effects, and 1 (4.2%) experienced side effects. In the microneedling-alone group (n = 24), all 24 (100.0%) participants had no side effects. The p-value for the comparison among groups was 0.001, indicating a statistically significant difference in the occurrence of side effects across the treatment groups.

Table-24 : **Leukotrichia**

Leukotrichia	Number	Percentage
Present	2	8.3
Absent	22	91.7

Leukotrichia was present in 2 (8.3%) participants, while 22 (91.7%) did not have this condition.

Figure-16 : **Leukotrichia**

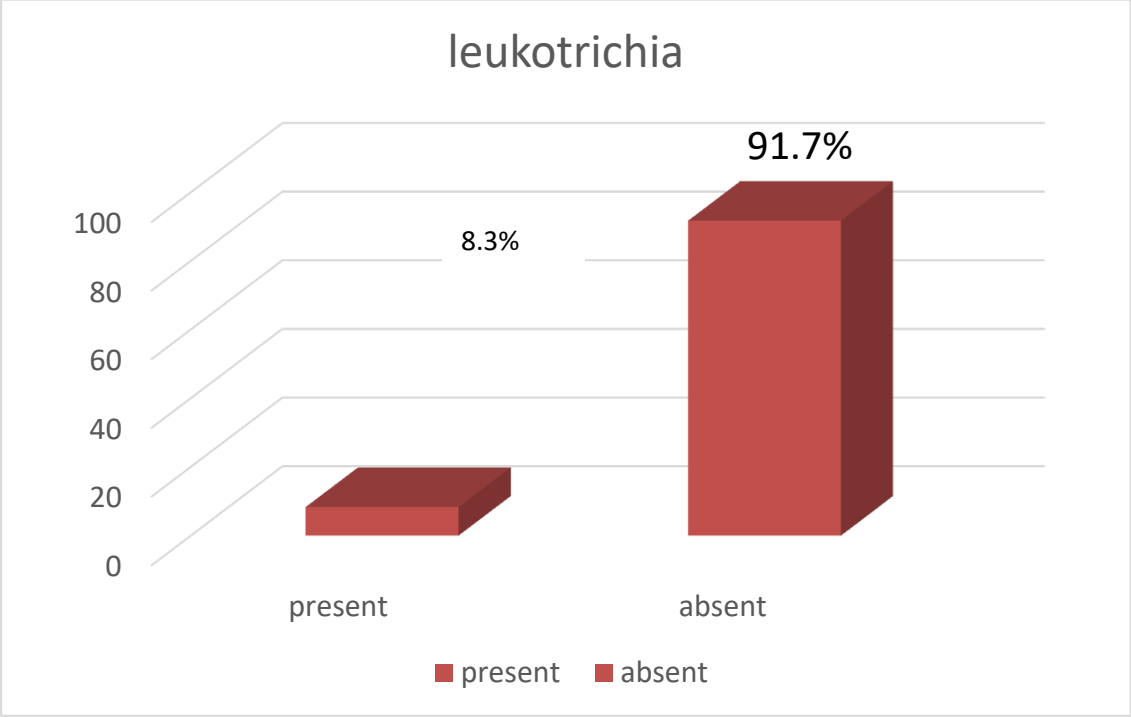

Table 25 : **Comparison of leukotrichia across the groups**

Groups	Leukotrichia				Total	P-value
	Present		Absent			
	n	%	n	%		
Microneedling with 5-Flourouracil	2	8.3	22	91.7	24	**1.0**
Microneedling with tacrolimus 0.1%	2	8.3	22	91.7	24	
Microneedling alone	2	8.3	22	91.7	24	
Total	6	8.3	66	91.7	72	

In the microneedling with 5-fluorouracil group (n = 24), 2 (8.3%) had leukotrichia, while 22 (91.7%) did not. Similarly, in the microneedling with tacrolimus 0.1% group (n = 24), 2 (8.3%) had leukotrichia, and 22 (91.7%) did not. The same distribution was observed in the microneedling-alone group (n = 24), with 2 (8.3%) having leukotrichia and 22 (91.7%) not having it. There was no significant difference in the occurrence of leukotrichia across the groups (p=1.0)

Table-26: **Association of drug efficacy (Global physician assessment scale) between the groups in patients with leukotrichia**

Groups	At Follow up						Total	P-value
	Grade 0		Grade I		Grade II			
	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	1	50	1	50	2	0.44
Microneedling with tacrolimus 0.1%	1	50	0	0	1	50	2	
Microneedling alone	2	100	0	0	0	0	2	
Total	3	50	1	16.7	2	33.3	6	

In patients with leukotrichia, for those treated with microneedling with 5-Fluorouracil (n = 2), 0 (0%) had a Grade 0 response, 1 (50%) had a Grade I response, and 1 (50%) had a Grade II response at follow-up. For patients treated with microneedling with tacrolimus 0.1% (n = 2), 1 (50%) had a Grade 0 response, 0 (0%) had a Grade I response, and 1 (50%) had a Grade II response. For patients treated with microneedling alone (n = 2), 2 (100%) had a Grade 0 response, and no patients had Grade I or Grade II responses. There was no significant difference in drug efficacy (Global Physician Assessment Scale) between the groups in patients with leukotrichia, as indicated by a p-value of 0.44

Table-27: **Association of drug efficacy (Global physician assessment scale) between the groups in patients without leukotrichia**

Groups	At follow up										Total	P-value
	Grade 0		Grade I		Grade II		Grade III		Grade IV			
	n	%	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	1	4.5	5	22.7	10	45.5	6	27.3	22	**<0.001**
Microneedling with tacrolimus 0.1%	0	0	8	36.4	10	45.5	3	13.6	1	4.5	22	
Microneedling alone	4	18.2	14	63.6	4	18.2	0	0	0	0	22	
Total	4	6.1	23	34.8	19	28.8	13	19.7	7	10.6	66	

In patients without leukotrichia, for those treated with microneedling with 5-Fluorouracil (n = 22), 0 (0%) had a Grade 0 response, 1 (4.5%) had a Grade I response, 5 (22.7%) had a Grade II response, 10 (45.5%) had a Grade III response, and 6 (27.3%) had a Grade IV response at follow-up. For patients treated with microneedling with tacrolimus 0.1% (n = 22), 0 (0%) had a Grade 0 response, 8 (36.4%) had a Grade I response, 10 (45.5%) had a Grade II response, 3 (13.6%) had a Grade III response, and 1 (4.5%) had a Grade IV response. For patients treated with microneedling alone (n = 22), 4 (18.2%) had a Grade 0 response, 14 (63.6%) had a Grade I response, 4 (18.2%) had a Grade II response, and no patients had Grade III or Grade IV responses. There was a significant difference in drug efficacy (Global Physician Assessment Scale) between the groups in patients without leukotrichia, as indicated by a p-value of <0.001. This implies that the absence of leukotrichia may be associated with a better response or better satisfaction with treatment outcomes.

Table-28: **Association of patient satisfaction between the groups in patients with leukotrichia**

Groups	At Follow up				Total	P-value
	Grade 0		Grade I			
	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	2	100	2	0.05
Microneedling with tacrolimus 0.1%	0	0	2	100	2	
Microneedling alone	2	100	0	0	2	
Total	2	33.3	4	66.7	6	

In patients with leukotrichia, for those treated with microneedling with 5-Fluorouracil (n = 2), 0 (0%) had a Grade 0 response, and 2 (100%) had a Grade I response at follow-up. For patients treated with microneedling with tacrolimus 0.1% (n = 2), 0 (0%) had a Grade 0 response, and 2 (100%) had a Grade I response. For patients treated with microneedling alone (n = 2), 2 (100%) had a Grade 0 response, and no patients had a Grade I response. There was a significant difference in patient satisfaction (Visual Analogue Scale) between the groups in patients with leukotrichia, as indicated by a p-value of 0.05. This implies that the presence of leukotrichia may be associated with a poorer response or lower satisfaction with treatment outcomes.

Table-29: **Association of patient satisfaction between the groups in patients without leukotrichia**

Groups	At Follow up								Total	P-value
	Grade 0		Grade I		Grade II		Grade III			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	1	4.5	2	9.1	13	59.1	6	27.3	22	<0.001
Microneedling with tacrolimus 0.1%	1	4.5	10	45.5	9	40.9	2	9.1	22	
Microneedling alone	7	31.8	12	54.5	3	13.6	0	0	22	
Total	9	13.6	24	36.4	25	37.9	8	12.1	66	

In patients without leukotrichia, for those treated with microneedling with 5-Fluorouracil (n = 22), 1 (4.5%) had a Grade 0 response, 2 (9.1%) had a Grade I response, 13 (59.1%) had a Grade II response, and 6 (27.3%) had a Grade III response at follow-up. For patients treated with microneedling with tacrolimus 0.1% (n = 22), 1 (4.5%) had a Grade 0 response, 10 (45.5%) had a Grade I response, 9 (40.9%) had a Grade II response, and 2 (9.1%) had a Grade III response. For patients treated with microneedling alone (n = 22), 7 (31.8%) had a Grade 0 response, 12 (54.5%) had a Grade I. response, 3 (13.6%) had a Grade II response, and no patients had a Grade III response. There was a significant difference in patient satisfaction (Visual Analogue Scale) between the groups in patients without leukotrichia, as indicated by a p-value of <0.001. This implies that the presence of leukotrichia may be associated with a poorer response or lower satisfaction with treatment outcomes.

Table-30**: Association of drug efficacy (Global physician assessment scale) between the groups and Head and neck lesions**

Groups	At Follow up								Total	P-value
	Grade 0		Grade I		Grade III		Grade IV			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	2	33.3	3	50	1	16.7	6	0.44
Microneedling with tacrolimus 0.1%	2	40	2	40	1	20	0	0	5	
Microneedling alone	0	0	1	100	0	0	0	0	1	
Total	2	16.7	5	41.7	4	33.3	1	8.3	12	

In head and neck lesions during the follow up, 2 (33.3%) in grade I and 3 (50%) in grade II and the remaining 16.7% were in grade III in microneedling with 5-flourouracil group. But only 20% were in grade III and no one was in Grade IV in Microneedling with 0.1% tacrolimus group. All the patients in microneedling group were in grade I in the post intervention period. The proportion of participants in higher grade was higher in microneedling with 5-flourouracil when compared to the other two groups. This implies that microneedling with 5-flourouracil had better outcome in head and neck region.

Table-31: **Association of drug efficacy (Global physician assessment scale) between the groups and lesions in the trunk**

Groups	At Follow up								Total	P-value
	Grade 0		Grade I		Grade III		Grade IV			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	2	40	0	0	3	60	5	**0.02**
Microneedling with tacrolimus 0.1%	4	40	4	40	2	20	0	0	10	
Microneedling alone	4	57.1	3	42.9	0	0	0	0	7	
Total	8	36.4	9	40.9	2	9.1	3	13.6	22	

The drug efficacy in the lesions in the trunk, 2 (40%) were in Grade I and 3 (60%) were in grade IV in microneedling with 5-flourouracil group. But 40% in Grade 0 and 40% in Grade I in microneedling with tacrolimus 0.1%. The remaining 20% were in Grade III. In microneedling group, 4 (57.1%) in grade 0 and 3 (42.9%) in grade I. There was a significant differences in improvement in global physician assessment scale across the groups (p=0.02). This implies that microneedling with 5-flourouracil had better outcome in trunk region.

Table-32: **Association of drug efficacy (Global physician assessment scale) between the groups and lesions in the extremities**

Groups	At Follow up										Total	P-value
	Grade 0		Grade I		Grade II		Grade III		Grade IV			
	n	%	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	2	15.4	2	15.4	7	53.8	2	15.4	13	**<0.001**
Microneedling with tacrolimus 0.1%	1	11.1	2	22.2	5	55.6	0	0	1	11.1	9	
Microneedling alone	6	37.5	10	62.5	0	0	0	0	0	0	16	
Total	7	18.4	14	36.8	7	18.4	7	18.4	3	7.9	38	

For patients with extremity lesions treated with microneedling with 5-Fluorouracil, 2 (15.4%) were graded as Grade I post-intervention, 2 (15.4%) were graded as Grade II, 7 (53.8%) were graded as Grade III, and 2 (15.4%) were graded as Grade IV. No participants remained in Grade 0. For those treated with microneedling with tacrolimus, 1 (11.1%) was graded as Grade 0 post-intervention, 2 (22.2%) were graded as Grade I, 5 (55.6%) were graded as Grade II, and 1 (11.1%) was graded as Grade IV. No participants progressed to Grade III. For those treated with microneedling alone (n = 16), 6 (37.5%) were graded as Grade 0 post-intervention, and 10 (62.5%) were graded as Grade I. No participants progressed to Grade II, III, or IV. There was a significant difference in drug efficacy (Global Physician Assessment Scale) across the treatment groups for extremity lesions, as indicated by a p-value of <0.001. This implies that microneedling with 5-flourouracil had better outcome in extremities.

Table-33: **Association of patient satisfaction (Visual analogue scale) between the groups and site of the head and neck lesions**

Groups	At Follow up								Total	P-value
	Score 0		Score 1		Score 2		Score 3			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	1	16.7	1	16.7	3	50	1	16.7	6	0.98
Microneedling with tacrolimus 0.1%	1	20	1	20	2	40	1	20	5	
Microneedling alone	0	0	0	0	1	100	0	0	1	
Total	2	16.7	2	16.7	6	50	2	16.7	12	

For patients with head and neck lesions treated with microneedling with 5-Fluorouracil, 1 (16.7%) reported a patient satisfaction score of 0, 1 (16.7%) reported a score of 1, 3 (50.0%) reported a score of 2, and 1 (16.7%) reported a score of 3 post-intervention. In those treated with microneedling with tacrolimus 0.1%, 1 (20.0%) reported a score of 0, 1 (20.0%) reported a score of 1, 2 (40.0%) reported a score of 2, and 1 (20.0%) reported a score of 3 post-intervention. For those treated with microneedling alone, 1 (100.0%) reported a score of 2 post-intervention. No participants reported grades of 0, 1, or 3. There was no significant difference in patient satisfaction (Visual Analogue Scale) across the treatment groups for head and neck lesions, as indicated by a p-value of 0.98.

Table-34: Association of patient satisfaction (Visual analogue scale) between the groups and site of the trunk lesions

Groups	At Follow up								Total	P-value
	Score 0		Score 1		Score 2		Score 3			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	1	20	2	40	2	40	5	**0.04**
Microneedling with tacrolimus 0.1%	0	0	7	70	3	30	0	0	10	
Microneedling alone	2	28.6	3	42.9	2	28.6	0	0	7	
Total	2	9.1	11	50	7	31.8	2	9.1	22	

For patients with trunk lesions treated with microneedling with 5-Fluorouracil (n = 5), 1 (20.0%) reported a patient satisfaction score of 1, 2 (40.0%) reported a score of 2, and 2 (40.0%) reported a score of 3 post-intervention. No participants reported a score of 0. For those treated with microneedling with tacrolimus 0.1% (n = 10), 7 (70.0%) reported a score of 1, and 3 (30.0%) reported a score of 2. No participants reported scores of 0 or 3. For those treated with microneedling alone (n = 7), 2 (28.6%) reported a score of 0, 3 (42.9%) reported a score of 1, and 2 (28.6%) reported a score of 2. No participants reported a score of 3. There was a significant difference in patient satisfaction (Visual Analogue Scale) across the treatment groups for trunk lesions, as indicated by a p-value of 0.04.

Table-35: Association of patient satisfaction (Visual analogue scale) between the groups and site of the extremity lesions

Groups	At Follow up								Total	P-value
	Score 0		Score 1		Score 2		Score 3			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	2	15.4	8	61.5	3	23.1	13	**<0.001**
Microneedling with tacrolimus 0.1%	0	0	4	44.4	4	44.4	1	11.1	9	
Microneedling alone	7	43.8	9	56.3	0	0	0	0	16	
Total	7	18.4	15	39.5	12	31.6	4	10.5	38	

For patients with extremity lesions treated with microneedling with 5-Fluorouracil (n = 13), 2 (15.4%) reported a patient satisfaction score of 1, 8 (61.5%) reported a score of 2, and 3 (23.1%) reported a score of 3 post-intervention. No participants reported a score of 0. For those treated with microneedling with tacrolimus 0.1% (n = 9), 4 (44.4%) reported a score of 1, 4 (44.4%) reported a score of 2, and 1 (11.1%) reported a score of 3. No participants reported a score of 0. For those treated with microneedling alone (n = 16), 7 (43.8%) reported a score of 0, and 9 (56.3%) reported a score of 1. No participants reported scores of 2 or 3. There was a significant difference in patient satisfaction (Visual Analogue Scale) across the treatment groups for extremity lesions, as indicated by a p-value of <0.001. This implies that microneedling with 5-flourouracil had better patient satisfaction in extremities

Table-36: **Association of drug efficacy (Global physician assessment scale) between the groups and duration of the lesion < 5 years**

Groups	Duration <5 years At follow up										Total	P-value
	Grade 0		Grade I		Grade II		Grade III		Grade IV			
	n	%	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	1	5.9	4	23.5	8	47.1	4	23.5	17	**<0.001**
Microneedling with tacrolimus 0.1%	1	5.9	6	35.3	8	47.1	1	5.9	1	5.9	17	
Microneedling alone	6	35.3	9	52.9	2	11.8	0	0	0	0	17	
Total	7	13.7	16	31.4	14	27.5	9	17.6	5	9.8	51	

For patients with a disease duration of less than five years treated with microneedling with 5-Fluorouracil, 1 (5.9%) had a Grade I response, 4 (23.5%) had a Grade II response, 8 (47.1%) had a Grade III response, and 4 (23.5%) had a Grade IV response at follow-up. No patients achieved a Grade 0 response. And patients who were treated with microneedling with tacrolimus 0.1% (n = 17), 1 (5.9%) had a Grade 0 response, 6 (35.3%) had a Grade I response, 8 (47.1%) had a Grade II response, 1 (5.9%) had a Grade III response, and 1 (5.9%) had a Grade IV response. For patients treated with microneedling alone (n = 17), 6 (35.3%) had a Grade 0 response, 9 (52.9%) had a Grade I response, and 2 (11.8%) had a Grade II response. No patients had a Grade III or Grade IV response. There was a significant good difference in treatment response (Global Physician Assessment Scale) across the groups for patients with a disease duration of less than five years, as indicated by a p-value of <0.001. This implies that patient with disease duration less than 5 years had better clinical improvement, with microneedling with 5 fluorouracil group showing best results.

Table-37 **Association of drug efficacy (Global physician assessment scale) between the groups and duration of the lesion > 5 years**

Groups	Duration >5 years At follow up								Total	P-value
	Grade I		Grade II		Grade III		Grade IV			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	1	14.3	2	28.6	2	28.6	2	28.6	7	0.15
Microneedling with tacrolimus 0.1%	2	28.6	3	42.9	2	28.6	0	0	7	
Microneedling alone	5	71.4	2	28.6	0	0	0	0	7	
Total	8	38.1	7	33.3	4	19	2	9.5	21	

For patients with a disease duration of more than five years treated with microneedling with 5-Fluorouracil (n = 7), 1 (14.3%) had a Grade I response, 2 (28.6%) had a Grade II response, 2 (28.6%) had a Grade III response, and 2 (28.6%) had a Grade IV response at follow-up. For those treated with microneedling with tacrolimus 0.1% (n = 7), 2 (28.6%) had a Grade I response, 3 (42.9%) had a Grade II response, and 2 (28.6%) had a Grade III response. No patients had a Grade IV response. For patients treated with microneedling alone (n = 7), 5 (71.4%) had a Grade I response, and 2 (28.6%) had a Grade II response. No patients had a Grade III or Grade IV response. There was no significant difference in treatment response (Global Physician Assessment Scale) across the groups for patients with a disease duration of more than five years, as indicated by a p-value of 0.15.

Table-38: **Association of patient satisfaction (Visual analogue scale) between the groups and duration of the lesion < 5 years**

Groups	Duration <5 years – At follow up								Total	P-value
	Grade 0		Grade I		Grade II		Grade III			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	1	5.9	2	11.8	9	52.9	5	29.4	17	**0.001**
Microneedling with tacrolimus 0.1%	1	5.9	9	52.9	5	29.4	2	11.8	17	
Microneedling alone	8	47.1	6	35.3	3	17.6	0	0	17	
Total	10	19.6	17	33.3	17	33.3	7	13.7	51	

For patients with a disease duration of less than five years treated with microneedling with 5-Fluorouracil (n = 17), 1 (5.9%) had a Grade 0 satisfaction score, 2 (11.8%) had a Grade I score, 9 (52.9%) had a Grade II score, and 5 (29.4%) had a Grade III score at follow-up. For those treated with microneedling with tacrolimus 0.1% (n = 17), 1 (5.9%) had a Grade 0 satisfaction score, 9 (52.9%) had a Grade I score, 5 (29.4%) had a Grade II score, and 2 (11.8%) had a Grade III score. For patients treated with microneedling alone (n = 17), 8 (47.1%) had a Grade 0 satisfaction score, 6 (35.3%) had a Grade I score, and 3 (17.6%) had a Grade II score. No patients had a Grade III satisfaction score. There was a significant difference in patient satisfaction (Visual Analogue Scale) across the groups for patients with a disease duration of less than five years, as indicated by a p-value of 0.001. This implies that patient with disease duration less than 5 years had better patient satisfaction, with microneedling with 5 fluorouracil group showing best results.

Table-39 **Association of patient satisfaction (Visual analogue scale) between the groups and duration of the lesion > 5 years**

Groups	Duration >5 years – At follow up								Total	P-value
	Grade 0		Grade I		Grade II		Grade III			
	n	%	n	%	n	%	n	%		
Microneedling with 5-Flourouracil	0	0	2	28.6	4	57.1	1	14.3	7	0.11
Microneedling with tacrolimus 0.1%	0	0	3	42.9	4	57.1	0	0	7	
Microneedling alone	1	14.3	6	85.7	0	0	0	0	7	
Total	1	4.8	11	52.4	8	38.1	1	4.8	21	

For patients with a disease duration of more than five years treated with microneedling with 5-Fluorouracil (n = 7), 0 (0%) had a Grade 0 satisfaction score, 2 (28.6%) had a Grade I score, 4 (57.1%) had a Grade II score, and 1 (14.3%) had a Grade III score at follow-up. For those treated with microneedling with tacrolimus 0.1% (n = 7), 0 (0%) had a Grade 0 satisfaction score, 3 (42.9%) had a Grade I score, 4 (57.1%) had a Grade II score, and 0 (0%) had a Grade III score. For patients treated with microneedling alone (n = 7), 1 (14.3%) had a Grade 0 satisfaction score, 6 (85.7%) had a Grade I score, and no patients had Grade II or Grade III scores. There was no significant difference in patient satisfaction (Visual Analogue Scale) across the groups for patients with a disease duration of more than five years, as indicated by a p-value of 0.11. This implies that patient with disease duration more than 5 years had lesser patient satisfaction.

DISCUSSION

The open label, non-randomized, interventional study was conducted to compare the efficacy and safety of microneedling with 5 fluorouracil., microneedling with tacrolimus. and microneedling alone in a stable vitiligo patient for a period of one year. The uniqueness of our study lies in the fact that all three modalities were compared together in a single study involving the same patients. The efficacy was measured by means of Physician's Global Assessment Grading, patient's satisfaction by Visual Analog scale and safety by the side effect profile of the drugs.

A total of 24 participants completed the study. Majority (50%) were in the 31 to 40-year age group, which aligns with the findings of Chhabra S et .al,[86] who reported that most participants fell within the 20 to 40 age range. Similarly, Ahmed AA et al.[119]found that 60.9% of their participants were over 20 years of age, while Matharoo P et al.[118] reported a higher percentage (80%) of participants below 30 years. Mina M et al.[120] study showed 52% less than 20 years and 48% more than 20 years.

Regarding gender distribution, two-thirds (62.5%) of the participants were female, while 37.5% were male. This was consistent with findings by Matharoo P et al[118] who observed 63.3% females and 36.7% males, as well as Chhabra S et al,[86] who reported a female-to-male ratio of 1.55:1. Additionally, Ahmed AA et al.[119] found 57% of participants were female and 43% were male, Mina M et al.[120] found 60% of participants.were female and 40% were male. All these studies demonstrated a female predominance among their participants. The female predominance may be due to higher cosmetic concerns, greater healthcare-seeking behaviour, hormonal influences, and the psychosocial impact of the disease.

In the current study, a total of 72 patches were analyzed, with 52.8% located on the extremities, 30.6% on the trunk, and 16.7% on the head and neck. In comparison, Matharoo P et al.[118] found that 78.2% of patches were on the extremities, 13% on the trunk, and 8.7% on the face. Chhabra S et al.[86] reported that 41.9% of patches were on the trunk/abdomen, 35.66% on the limbs, and 22.37% were distributed acrally. In Mina M et al.[120] study the distribution of vitiligo patch was 40% in acral parts (hands and feet), 32% in leg, 16% in elbows and 12% in knees.

In our study, 4.2% of participants had a family history of vitiligo, while 95.8% did not. In comparison, Matharoo P et al.[118] reported a positive family history in 6.7% and a negative history in 93.3%. In contrast, Ahmed AA et al.[119] found that 34.8% of participants had a positive family history, with 65.2% having no family history. Chhabra S et al.[86] observed a positive family history in 6.8% of participants, while 93.2% had no such history and Mina M et al. found positive history in 16% and negative in 84%. Variations in family history of vitiligo across studies may be due to genetic differences, ethinicity, sample size, recall bias, and environmental factors.

In our study, 42% of participants had vitiligo for more than 5 years, while 58% had it for less than 5 years. In Ahmed AA et al.'s study,[119] 56.5% had a duration of more than 5 years, and 43.5% had a duration of less than 5 years. Similarly, Matharoo P et al.[118] reported that 46.7% of participants had vitiligo for more than 4 years, while 53.3% had it for less than 4 years and Mina M et al.[120] showed 52% participants with vitiligo duration less than 5 year and 48% more than 5 years.

The microneedling with 5-fluorouracil group (n = 24) demonstrated the highest efficacy, with 25.0% (6 patients) achieving Grade IV improvement (>75%)

and 41.7% (10 patients) achieving Grade III improvement (50-75%), resulting in a total of 66.7% of patients experiencing significant improvement. This suggests that the combination of microneedling with 5-fluorouracil is highly effective in enhancing repigmentation in vitiligo patients compared to other treatment groups.

In the microneedling with tacrolimus 0.1% group (n = 24), only 4.2% (1 patient) achieved Grade IV improvement (>75%), while 12.5% (3 patients) reached Grade III improvement (50-75%), and 45.8% (11 patients) showed moderate improvement (Grade II, 25-50%). This indicates a lower overall efficacy compared to the 5-fluorouracil group, with only 16.7% of patients achieving significant improvement (Grade III or IV), suggesting that while tacrolimus has some benefit, it is less effective in producing substantial repigmentation.

In the microneedling-alone group (n = 24), 25.0% (6 patients) showed no improvement, while 58.3% (14 patients) had minimal improvement (Grade I, <25%) and 16.7% (4 patients) had moderate improvement (Grade II, 25-50%). Notably, none of the patients achieved Grade III or IV improvement, indicating that microneedling alone had the least efficacy among the three groups. The significant difference in treatment response across groups ($p < 0.001$) highlights the superior effectiveness of microneedling with 5-fluorouracil compared to the other interventions.

In the microneedling with 5-fluorouracil (5-FU) group, Mina M et al.[120] reported a higher percentage (48%) of patients achieving >75% improvement compared to 25% in the current study. However, the combined percentage of patients with at least 50% improvement was similar (66.7% in the current study vs. 52% in Mina M et al.).

In the microneedling with tacrolimus 0.1% group, Mina M et al.[120] found a higher percentage (16%) of patients achieving >75% improvement, whereas only 4.2% showed similar improvement in the current study. Additionally, 24% of patients in Mina M et al. had 50-75% improvement, compared to only 12.5% in the current study.

The differences in outcomes between the two studies could be attributed to variations in patient demographics, lesion duration and location, treatment protocols, or adherence to therapy. Despite these variations, both studies consistently indicate that tacrolimus is less effective than 5-FU, with most patients achieving only moderate improvement (25-50%) rather than significant repigmentation.

In the microneedling with 5-fluorouracil (5-FU) group, Matharoo P et al.[118] reported a higher percentage (46.7%) of patients achieving excellent improvement (>75%) compared to 25% in the current study. However, the percentage of patients with good improvement (50-75%) was higher in the current study (41.7%) compared to 30% in Matharoo P et al.

In the microneedling with tacrolimus 0.1% group, Matharoo P et al.[118] showed better efficacy, with 16.7% of patients achieving excellent improvement (>75%) compared to only 4.2% in the current study. Similarly, 46.6% of patients had good improvement (50-75%) in Matharoo P et al., while only 12.5% showed similar improvement in the current study.

The differences in results between the two studies may be due to variations in sample size (n=30 vs. n=24), patient selection criteria, treatment protocols, lesion characteristics, or follow-up duration. Despite these differences, both studies

consistently show that microneedling with 5-FU is more effective than tacrolimus, with a greater proportion of patients achieving significant repigmentation.

In the microneedling with 5-fluorouracil (5-FU) group, Chhabra S et al.[86] reported a higher percentage (48.6%) of patients achieving Grade IV improvement (>75%) compared to 25.0% in the current study. However, the percentage of patients with Grade III improvement (50-75%) was slightly lower in Chhabra S et al. (37.5%) compared to 41.7% in the current study. Additionally, Grade II responses were much lower (11.1%) in Chhabra S et al. compared to 33.3% in the current study, indicating a wider distribution of treatment responses between the two studies.

In the microneedling-alone group, Chhabra S et al.[86] reported 16.9% of patients achieving Grade IV (>75%) improvement and 28.1% achieving Grade III (50-75%), whereas the current study showed no cases of Grade III or IV improvement. Instead, the current study had a higher percentage of patients with minimal improvement (Grade I, 58.3%) or no improvement (25.0%), suggesting a less favorable response to microneedling alone compared to the Chhabra S et al. study.

The differences in results between the two studies may be due to variations in sample size (n=72 vs. n=24), patient demographics, vitiligo lesion characteristics, treatment protocols, or follow-up duration. Despite these discrepancies, both studies reinforce the superior efficacy of microneedling with 5-FU compared to microneedling alone.

In the microneedling with tacrolimus 0.1% group, Ahmed AA et al.[119] reported a higher percentage of patients achieving Grade IV improvement (8.7%) compared to 4.2% in the current study. Additionally, Grade II responses were slightly higher in Ahmed AA et al. (52.2%) compared to 45.8% in the current study.

However, the current study reported a higher percentage of Grade III improvement (12.5% vs. 8.7%), indicating some variability in treatment response. Despite these differences, both studies consistently show that most patients fall into Grade II or Grade I categories, reflecting moderate improvement with microneedling and tacrolimus.

In the microneedling-alone group, Ahmed AA et al.[119] reported better outcomes, with 13.0% of patients achieving Grade IV (>75%) improvement and another 13.0% achieving Grade III (50-75%), whereas the current study reported no cases of Grade III or IV improvement. Instead, a larger percentage of patients in the current study experienced minimal improvement (Grade I, 58.3%) or no improvement (25.0%), suggesting a less favorable response to microneedling alone in the current study compared to Ahmed AA et al.

The observed differences between the two studies may be due to variations in sample size (n=23 vs. n=24), patient selection, vitiligo lesion characteristics, treatment duration, or methodology. Nonetheless, both studies confirm that microneedling with tacrolimus shows moderate efficacy, while microneedling alone produces the least favorable results, reinforcing the need for combination therapies for better repigmentation outcomes.

A key highlight of current study is that no previous research has assessed patient satisfaction scores in this context. Patient satisfaction, assessed using the Visual Analogue. Scale, was highest in the microneedling with 5-fluorouracil group, where 25.0% reported Grade III. satisfaction and 54.2% reported Grade II, indicating greater overall satisfaction. In the microneedling with tacrolimus group, most patients reported Grade I (50.0%) or Grade II (37.5%) satisfaction, with only 8.3% achieving

Grade III. The microneedling-alone group had the lowest satisfaction, with 37.5% reporting Grade 0 and none achieving Grade III. The p-value was <0.001, indicating a statistically significant difference in satisfaction levels across the treatment groups, with microneedling with 5-fluorouracil showing the most favourable outcomes.

In the microneedling with 5-fluorouracil (5-FU) group, treatment response varied across lesion sites, with the best improvement observed in trunk lesions, where 60% of patients achieved Grade IV (>75% improvement). In extremity lesions, 53.8% of patients reached Grade III (50-75% improvement) and 15.4% achieved Grade IV, indicating strong efficacy in these areas as well. However, in head and neck lesions, only 16.7% of patients reached Grade III, suggesting comparatively lower effectiveness in this region.

In the microneedling with tacrolimus 0.1% group, the best response was seen in trunk lesions, where 20% of patients reached Grade IV improvement. In head and neck lesions, 20% of patients achieved Grade III (50-75% improvement), but no higher responses were noted. In extremity lesions, treatment response peaked at Grade II (25-50% improvement), with no cases of Grade III or IV improvement, suggesting a more moderate effect of tacrolimus compared to 5-FU, especially in distal areas.

In the microneedling-alone group, treatment response was the lowest across all lesion sites. In head and neck lesions, no patients progressed beyond Grade I (<25% improvement), indicating minimal repigmentation. Similarly, in trunk and extremity lesions, patients remained in the lower grades, with no cases of Grade III or IV improvement, reinforcing the limited efficacy of microneedling as a monotherapy. The significant p-values ($p=0.02$ for trunk lesions, $p<0.001$ for extremity lesions)

further highlight the superior effectiveness of microneedling with 5-FU compared to other treatment approaches.

Matharoo P et al.[118] study and current study confirm that 5-fluorouracil is the most effective treatment, especially for trunk and extremity lesions, with higher Grade III–IV responses. The Matharoo P et al. [118] study aligns, showing higher "excellent" responses in 5-fluorouracil for limbs (42.85%) and trunk (28.57%), while though tacrolimus performed better for limb lesions (60% excellent) but was ineffective for acral areas.

Mina M et al.[120] study and current study support that 5-fluorouracil outperforms tacrolimus in most areas. However, the Mina M et al. suggests tacrolimus had better efficacy in certain limb sites (legs: 75% excellent), whereas current study emphasized 5-fluorouracil's dominance, especially for trunk and extremities. The p-values in both studies indicate statistical significance in treatment differences ($p=0.02$ and $p<0.001$ in current study, $p=0.050$ in the Mina M et al. for tacrolimus). The discrepancy between the Mina M et al. study and the current study regarding tacrolimus efficacy in limb lesions may be due to differences in patient characteristics, lesion stability, treatment protocols, statistical variability ($p=0.050$ vs. $p<0.001$), and environmental or adherence factors, while both studies consistently support 5-fluorouracil as the superior treatment overall.

On comparing current study with Chhabra S et al.[86] study, Group A (microneedling with 5-fluorouracil) had a significantly higher number of patches showing excellent response, particularly in the trunk, followed by the limbs, with little to no improvement in acro-facial areas than Group B (microneedling alone). However, both analyses consistently highlight the superior efficacy of 5-FU,

particularly for trunk and limb lesions, with acro-facial areas showing the least response across all groups.

The current study indicate that patients with a disease duration of less than five years demonstrated better clinical improvement, with microneedling combined with 5-fluorouracil yielding the highest response rates, as reflected in the significant p-value (<0.001). This suggests that earlier intervention enhances treatment efficacy, possibly due to higher melanocyte reservoir availability and better wound healing response. In contrast, among patients with disease duration exceeding five years, the treatment response was more modest across all groups, with no significant differences between them (p=0.15), indicating that chronic vitiligo lesions may have a reduced capacity for repigmentation.

The current study suggest that patients without leukotrichia responded significantly better to treatment, particularly with microneedling combined with 5-fluorouracil, as evidenced by a higher proportion of Grade III and IV improvements and a statistically significant p-value (<0.001). This aligns with the understanding that leukotrichia, which indicates complete melanocyte loss in hair follicles, is a negative prognostic factor for repigmentation, as follicular melanocytes are a key reservoir for repigmentation in vitiligo. In contrast, patients with leukotrichia exhibited poor response rates across all treatment groups, with no significant difference in efficacy (p=0.44), reinforcing the notion that repigmentation is more challenging in these cases due to irreversible melanocyte depletion.

Other key highlights of our study were the relationship between vitiligo duration and treatment outcomes, the impact of leukotrichia on intervention results, and a detailed comparison of pre- and post-intervention outcomes within each

treatment group. These parameters were uniquely analysed in our study and were not comparatively studied in previous research.

In current study, ulceration and ecchymosis were the most common side effects in the microneedling with 5-FU group, affecting 33.3% of participants. This finding is consistent with Matharoo P et al,[118] where 23.7% of lesions developed ulceration and 6.7% had ecchymosis, as well as Mina M et al,[120] which reported ulceration in 4% of cases. The mechanical trauma from microneedling combined with the cytotoxic effects of 5-FU likely contributes to ulceration. Notably, no ulceration was observed in the tacrolimus or microneedling-alone groups in the current study, further supporting the higher risk of tissue damage with 5-FU. However, Ahmed AA et al.[119] reported 17.39% ulceration in their tacrolimus-treated group, which contradicts our findings and may be due to differences in microneedling intensity or patient skin sensitivity.

Hyperpigmentation was observed in 16% of patients in the 5-FU-treated group in the Mina M et al.[120] study, whereas the current study did not explicitly report this side effect. This suggests that post-inflammatory hyperpigmentation may vary based on patient skin type or treatment protocols. Interestingly, Ahmed AA et al.[119] noted 47.82% hyperpigmentation in the tacrolimus group, which is significantly higher than other studies. This discrepancy may stem from regional skin differences, genetic predisposition, or variations in post-procedural care.

The Ahmed AA et al.[119] study reported pain in 78.26% of tacrolimus-treated patients and 86.9% in the microneedling-alone group, alongside erythema in 65.21% and 73.9%, respectively. In contrast, the current study found no reported side effects in the microneedling-alone group, highlighting a major discrepancy in patient-reported discomfort. The higher incidence in Ahmed AA et al. could be due to variations in needle depth, session intensity, or individual pain thresholds. The lower

pain and erythema rates in our tacrolimus group suggest that it remains a better-tolerated option compared to 5-FU while still being more effective than microneedling alone.

Ahmed AA et al.[119] noted 43.47% of patients in the tacrolimus group experienced itching, which was not observed in the current study, suggesting possible formulation differences or patient sensitivity variations. Mina M et al.[120] reported 12% inflammation in the 5-FU group, consistent with the increased inflammatory response caused by the cytotoxic nature of 5-FU. This contrasts with our study, where side effects in the 5-FU group were higher (33.3%) but were not individually categorized, making direct comparisons difficult.

Overall, 5-FU remains the most effective but least tolerated, while tacrolimus provides a safer alternative with mild side effects, and microneedling alone is the safest but least effective option.

The discrepancies between our study findings and those of others can be attributed to variations in several key factors like variations in treatment protocols, duration between treatment, lesion sites, or patient characteristics between the two studies. These differences may lead to distinct outcomes and interpretations across studies, highlighting the importance of considering contextual. factors when comparing research finding.

CONCLUSION

The current study aimed. to evaluate and compare the efficacy., patient satisfaction, and safety. of microneedling (Mn) alone, Mn with 5-Fluorouracil (5-FU), and Mn with tacrolimus in the treatment of vitiligo. 24 patients with a total of 72 patches of vitiligo were recruited. The results demonstrated that the patches where microneedling, particularly when combined with 5-FU, showed significant improvement in repigmentation when compared to results of microneedling with tacrolimus in the vitiligo patch and microneedling alone in the other subset of vitiligo patches. In each group 24 patches were studied.

Efficacy of Treatment Modalities:

- Microneedling with 5-FU achieved the highest repigmentation rates, with a significant number of patients reaching Grade III or IV on the Global Physician Assessment Scale.
- Microneedling with tacrolimus also showed positive outcomes but was less effective than Mn with 5-FU.
- Microneedling alone had the least improvement, with most patients remaining at Grade I or II.

Patient Satisfaction:

- Patients treated with Microneedling with 5-FU reported the highest satisfaction scores on the Visual Analogue Scale, indicating improved cosmetic outcomes and faster results.

- Microneedling with tacrolimus also resulted in notable improvement, but patient-reported outcomes were lower than those for Mn with 5-FU.

- Microneedling alone had the lowest satisfaction scores, with fewer patients experiencing noticeable repigmentation.

Safety and Side Effects:

- The majority of patients tolerated all three treatment approaches well.

- The highest incidence of mild side effects (erythema, hyperpigmentation, ulceration, and pain) was observed in the microneedling with 5-FU group. However, these effects were temporary and did not significantly impact treatment continuation.

- Microneedling with tacrolimus had fewer side effects, and microneedling alone had no reported adverse events, confirming the safety of microneedling as a standalone procedure.

Treatment Response Based on Lesion Location:

- The best response was observed. in the head and neck. region, followed by the trunk and extremities..

- Extremities, being the most resistant site, still showed notable improvement in microneedling with 5-FU, suggesting its potential for difficult-to-treat vitiligo cases.

Treatment Response Based on duration :

- Response in patients with disease duration <5 years was better than those with >5 years, highlighting the importance of early intervention in stable lesions.

STRENGTHS

- Microneedling, an emerging treatment modality, was integrated into our study as a novel approach for vitiligo management.
- To our knowledge, no previous studies have directly compared the efficacy. of microneedling. with 5-Fluorouracil, microneedling with tacrolimus., and microneedling alone, making this research the first of its kind.
- Our study uniquely evaluated vitiligo improvement using both a physician assessment scale and patient satisfaction scores, distinguishing it as the first to incorporate this dual assessment approach.
- We also analyzed the relationship between vitiligo duration and treatment outcomes, the impact of leukotrichia on intervention results, and a detailed comparison of pre- and post-intervention effects within each treatment group.
- This comprehensive methodology sets our study apart from previous research, which has not employed such an extensive assessment framework.

LIMITATIONS

- The sample size used in our study was relatively small, which would have limited how representative our results are in context to the general population; increasing the sample size would improve the data' robustness and generalizability.
- Our ability to evaluate potential long-term impacts or changes in treatment outcomes was hampered by the short follow-up time.

SUMMARY

This study evaluates the efficacy. and safety. of microneedling alone, microneedling with 5-fluorouracil (5-FU), and microneedling with tacrolimus. in stable vitiligo patients. Each patient who had atleast three vitiligo patches were assigned to one of the three treatment groups. Microneedling was performed under aseptic conditions, followed by the application of 5-FU. (Group A), tacrolimus (Group B), or an emollient (Group C). The procedure was repeated every four weeks for four sessions, with daily topical application of the same topical drug used with microneedling between sessions. Efficacy was assessed by Global Physician Assessment Scale, while safety was evaluated based on side effects. Digital photographs and clinical assessments was used to compare baseline and post-treatment results after five months. The study's uniqueness lies in evaluating all three treatment modalities within the same patient cohort.

A total of 24 participants completed the study, with most falling within the 31–40-year age group and a majority (62.5%) being female. A total of 72 vitiligo patches were analyzed, predominantly located on the extremities (52.8%), followed by the trunk (30.6%) and head/neck (16.7%). The study also assessed variables like family history, disease duration, and lesion distribution, comparing findings with previous literature.

Microneedling with 5-FU demonstrated the highest efficacy, with 66.7% of patients achieving more than 50% repigmentation, significantly outperforming microneedling with tacrolimus (16.7%) and microneedling alone, which showed no notable improvement. Statistical analysis confirmed the superiority of microneedling with 5-FU ($p < 0.001$). Patient satisfaction was also highest in the microneedling with

5-FU group, where 25% of patients reported Grade III satisfaction and 54.2% reported Grade II, while satisfaction levels were lower for microneedling with tacrolimus and the lowest for Mn alone ($p < 0.001$). In terms of lesion response, repigmentation was most significant in the trunk and extremities, with microneedling with 5-FU proving particularly effective in resistant areas. However, safety analysis revealed that the microneedling with 5-FU group had the highest incidence of side effects (33.3%), including erythema and ulceration, whereas microneedling with tacrolimus had only 4.2% adverse effects. Microneedling alone had no reported side effects, confirming its superior safety but lower efficacy ($p = 0.001$).

Microneedling, especially in combination with 5-FU, proves to be a promising treatment for vitiligo, offering improved repigmentation outcomes compared to Mn with tacrolimus and Mn alone. While Mn alone remains a safe and minimally invasive option, its efficacy is significantly enhanced when used with adjunctive therapies. The study reinforces the role of microneedling as an effective technique in vitiligo treatment, particularly for localized and resistant lesions, and paves the way for further research to optimize its application in clinical practice.

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ANNEXURES

ANNEXURE – I - INFORMED CONSENT FORM

Title of the study: A comparative interventional study to evaluate the safety and efficacy of microneedling combined with 5-fluorouracil 5% cream versus microneedling combined with tacrolimus 0.1% ointment versus microneedling alone in clinically stable vitiligo patients

Name of Student/Principal Investigator:

Name of Guide/Co Investigators:

Introduction: Vitiligo is a chronic acquired condition that causes hypopigmented or depigmented macules and patches on the skin and mucous membranes, as a result of autoimmune aggression against melanocytes

Explanation of procedure: After screening, if you fit in the inclusion criteria you will be chosen. Before starting treatment, detailed history, clinical examination will be done .You will be informed about the treatment given and possible side effects like pain, erythema, burning, hyperpigmentation which might occur. Informed written consent will be taken. Data will be collected by a single examiner and recorded in case proforma. Three vitiligo patches will be randomly selected in you and divided to group A, group B, group C. Digital photographs of the patches will be taken at baseline and at each sessions. Topical anaesthetic cream eutectic mixture of local anaesthetic will be applied 30 to 45 mins before procedure to anaesthetize the area. Under strict aseptic conditions, microneedling of the vitiligo patches will be made using Dr.pen ultima A6. The needle penetration depth will be 0.25 to 0.5mm over face and 1-2mm over the body done in horizontal and vertical direction until pinpoint

bleeding points appear. It will be followed by the application of 5fluorouracil 5% cream in group A, 0.1% tacrolimus ointment in group B.Occlusive dressing are done and kept for a day. This procedure will be repeated every 4 weeks for a maximum of 4 sessions (4 months). Digital photographs will be taken at each session. You are advised to apply 5fluorouracil 5% cream in Group A patch, 0.1% tacrolimus ointment in group B patch, bland emmolients in group C patch once daily in between sessions. You will be followed up for 3 months. After 7 months, the digital photographs will be compared with baseline. The cost of microneedling will be done by me.

Withdrawal from participation in the study: Participation in this study in voluntary. You will be free to decide whether to participate in this study or continue participation once enrolled. In case you decide to withdraw your participation, you are free to do so. However, please convey the decision to the principal investigator.

Possible benefits from participating in the study: You will/will not get any benefits by participating in this study. The data gathered will help population at large.

Possible risks from participating in the study: There are no risks involved in participating in this study.

Privacy and confidentiality: The information collected from you will be coded, to prevent any person to identify you. Your identity will never be revealed. The data collected from you will be kept confidential and only processed or aggregated data will be used for publication.

Financial incentives: You will not receive any payment for participating in this study.

Authorization for publication of aggregated data: Results obtained after processing of the aggregated data will be published for scientific purpose and or presented to scientific groups. However, your identity will never be revealed.

Questions:If you have any question or complaints with regard to your right as study participant you may contact Dr Harsha Hegde, Chairperson, Ethical committee of JNMC, 0831-2473777 Extension 4052.

Legal rights: By signing this consent form, we are not waving any of your legal rights

CONSENT STATEMENT

I am making a voluntary decision to participate in the study "A comparative interventional study to evaluate the efficacy and safety of microneedling combined with 5-fluorouracil 5% cream versus microneedling combined with tacrolimus 0.1% ointment versus microneedling alone in clinically stable vitiligo patients". My signature below indicates that I have decided to participate and I have read the information provided above or the information provided above has been read to me in the language that I understand best. I was given the opportunity to ask questions and that they have been answered to my satisfaction.

Name of the participant:

Signature or left thumb impression of the participant:

Name of the witness:

Signature or left thumb impression of the witness:

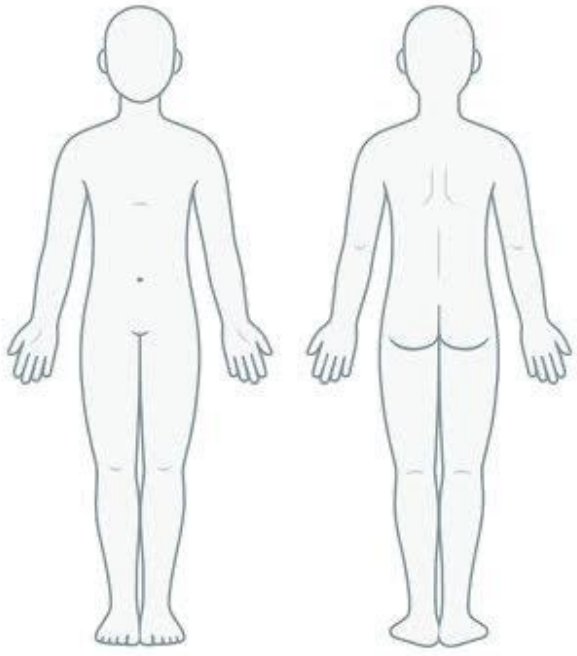
Name of the investigator:

Signature of the investigator:

ANNEXURE II - PROFORMA

	Demographic details	
DATE		
NAME		
OP NUMBER		
IP NUMBER		
AGE		
SEX	**FEMALE**	**MALE**
ADDRESS WITH PHONE NUMBER		
MARITAL STATUS	**MARRIED**	**UNMARRIED**
OCCUPATION	**EMPLOYED**	**UNEMPLOYED**
CHIEF COMPLAINTS		
DISEASE DURATION	**YEARS**	**MONTHS**
H/O TREATMENT		
SYSTEMIC	**PRESENT**	**ABSENT**
MENTION (IF ANY)		
TOPICAL	**PRESENT**	**ABSENT**
MENTION (IF ANY)		

PHOTOTHERAPY	**PRESENT**	**ABSENT**
MENTION (IF ANY)		
FAMILY HISTORY	**PRESENT**	**ABSENT**
GENERAL EXAMINATION		
PR	**bpm**	
BP	**mm/hg**	
WEIGHT	**Kg**	
HEIGHT	**cm**	
BMI		
LEUKOTRICHIA	**PRESENT**	**ABSENT**
SITE OF LESION		



PHYSICIAN'S GLOBAL ASSESSMENT GRADING

GRADE	**GROUP A**	**GROUP B**	**GROUP C**
1st sitting Date			
2nd sitting **Date**			
3rd sitting **Date**			
4th sitting **Date**			
5thmonth(follow up) **Date**			

PHYSICIAN'S GLOBAL ASSESSMENT GRADING

G0 Repigmentation & response absent

G1 <25% repigmentation & poor response

G2 25 to 50% repigmentation & good response

G3 50 to 75% repigmentation & very good response

G4 >75% repigmentation & excellent response

VISUAL ANALOG SCALE (PATIENT SATISFACTION)

SCORE	1st sitting Date			2nd sitting Date			3rd sitting Date			4th sitting Date			5thmonth(followup) Date		
	A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
0															
1															
2															
3															

VISUAL ANALOG SCALE

0-NOT SATISFIED

1-SLIGHTLY SATISFIED

2-VERY SATISFIED

3-EXTREMELY SATISFIED

SIDE EFFECTS ASSESSMENT

	GROUP A	**GROUP B**	**GROUP C**
Nil			
Pain			
Erythema			
Ulceration			
Hyperpigmentation			

ANNEXURE III - PHOTOGRAPHS

Case 1- 35 years female patient with 1.5 years of vitiligo

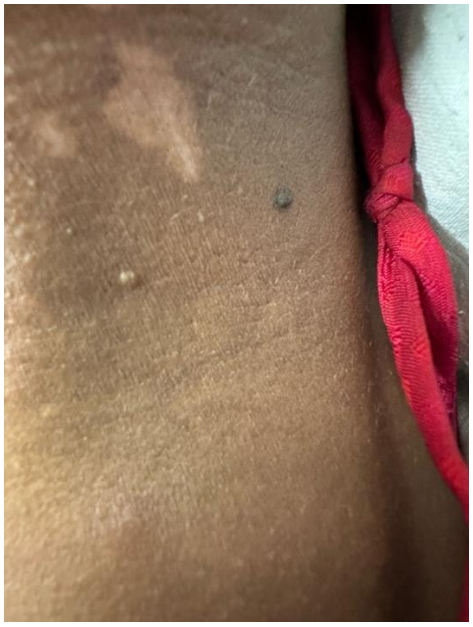


Figure 17a – Baseline picture before microneedling with 5FU

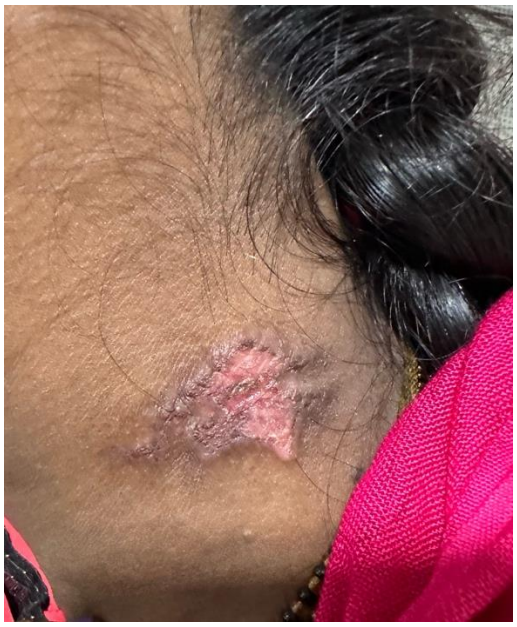


Figure 17b- Ulceration after 1 sitting of 5FU

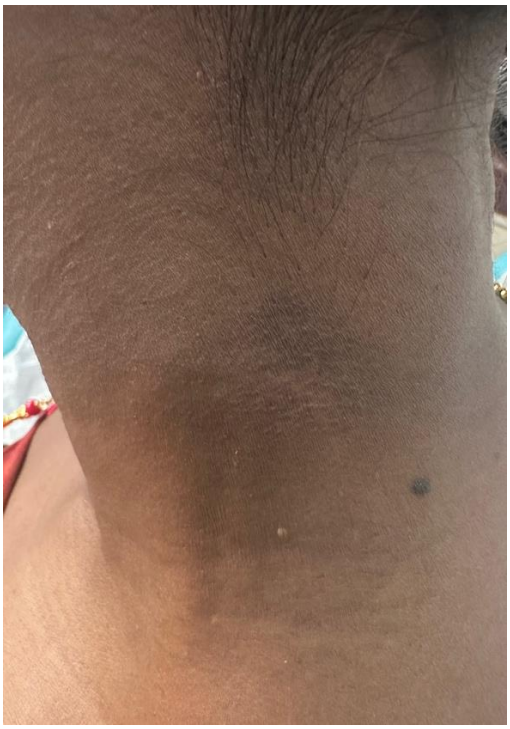


Figure 17c- Post treatment after microneedling with 5FU (follow-up)

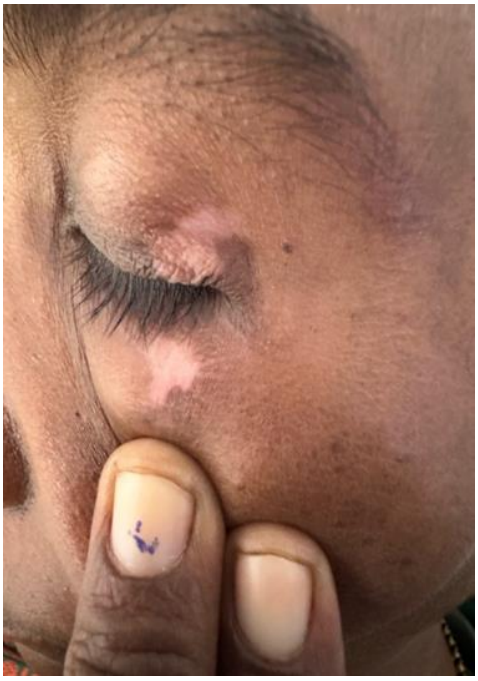


Figure 17d- Baseline image- vitiligo patch above eye- microneedling alone, vitiligo patch below eye- microneedling with tacrolimus

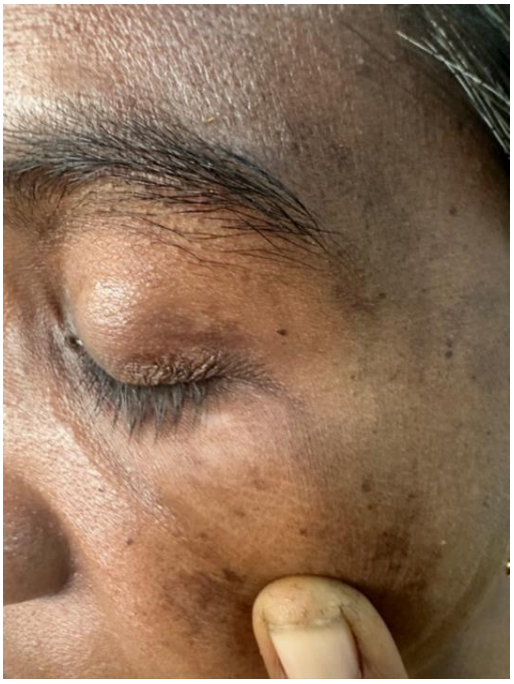


Figure 17e- Post treatment (follow-up)- vitiligo patch above eye- microneedling alone, vitiligo patch below eye- microneedling with tacrolimus

Case 2- 26 years female patient with 3 years of vitiligo

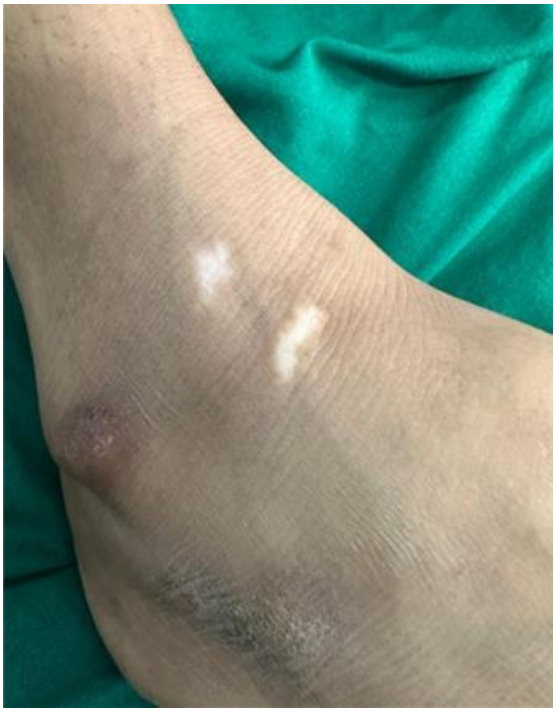


Figure 18a – Baseline image – above vitiligo patch – microneedling with 5FU, below vitiligo patch- microneedling with microneedling alone

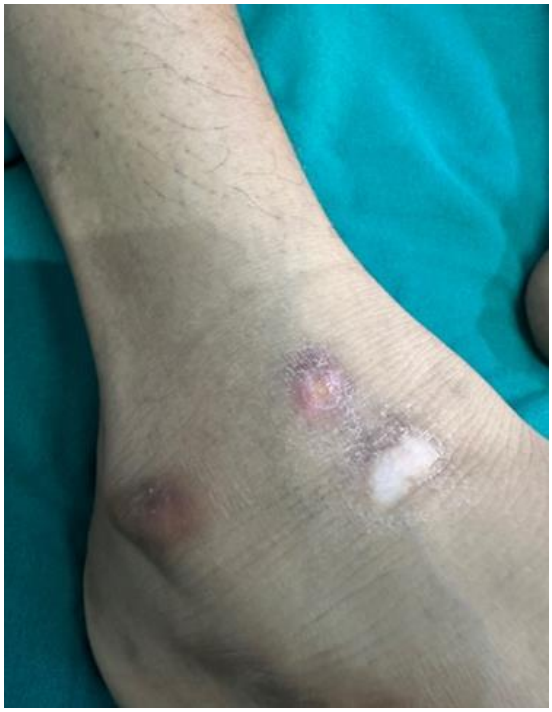


Figure 18b- After 2 sittings of microneedling with 5FU and microneedling alone

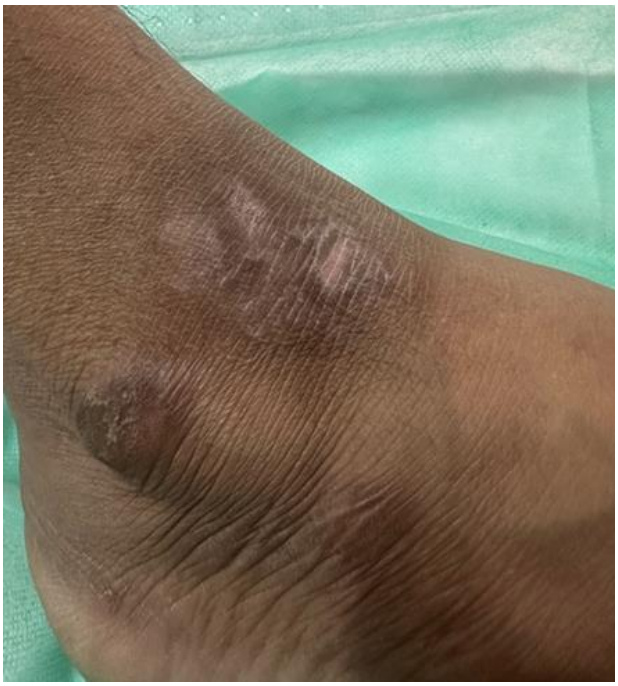


Figure 18c- Post treatment (follow-up)- above vitiligo patch – microneedling with 5FU, below vitiligo patch- microneedling with microneedling alone

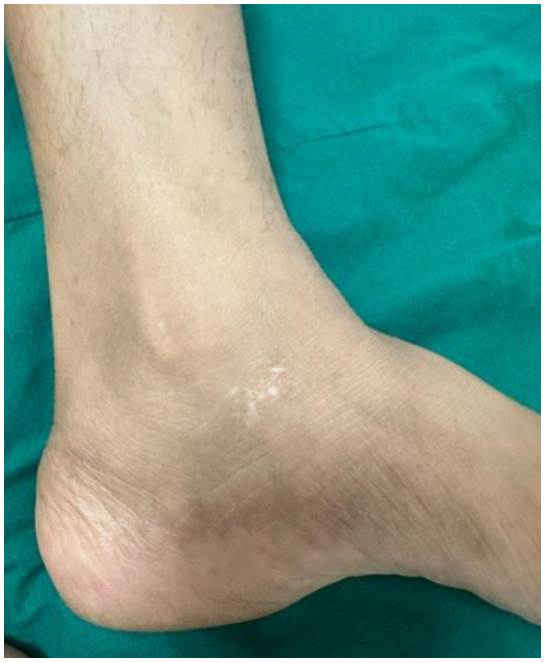


Figure 18d- Baseline image before microneedling with tacrolimus

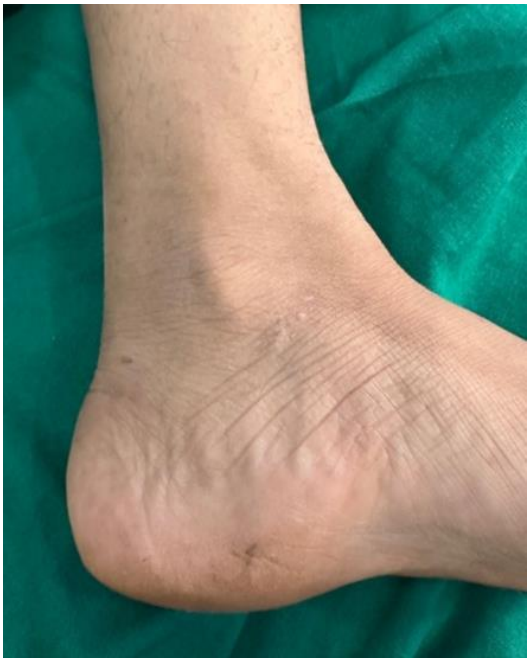


Figure 18e- Post treatment (follow-up) after microneedling with tacrolimus

Case 3- 29 years old female patient with 2.5 years of vitiligo

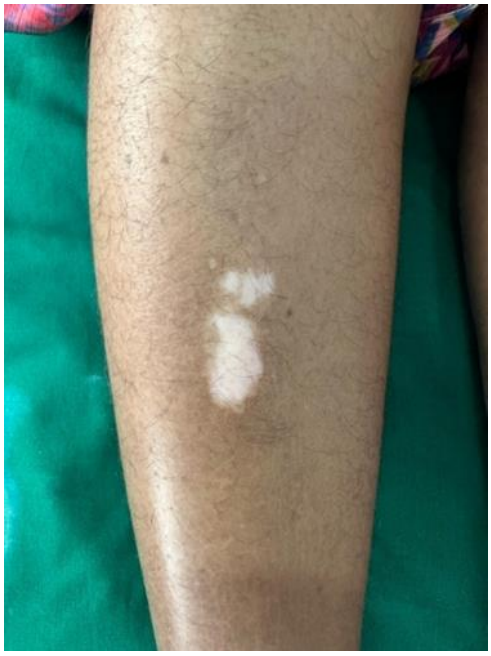


Figure 19a- Baseline image before microneedling with 5FU

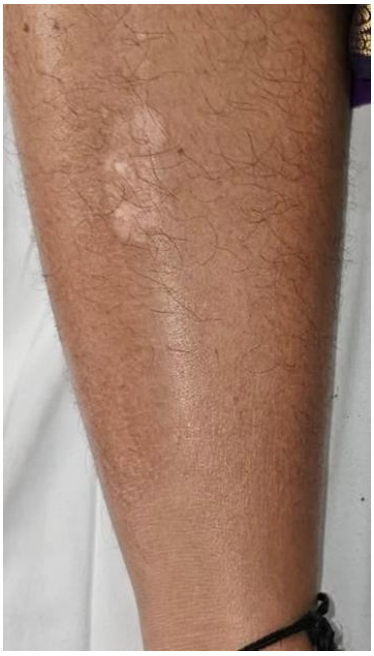


Figure 19b-Post treatment (follow-up) after microneedling with 5FU

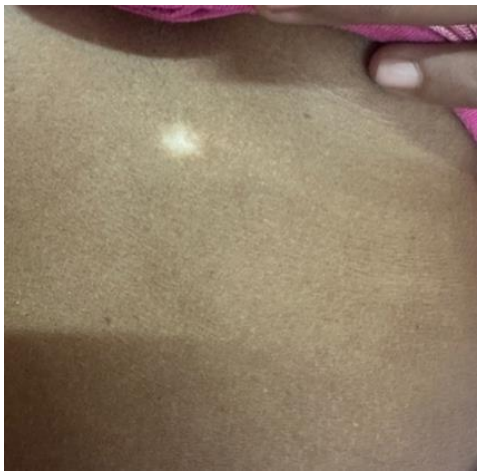


Figure 19c- Baseline image before microneedling with tacrolimus

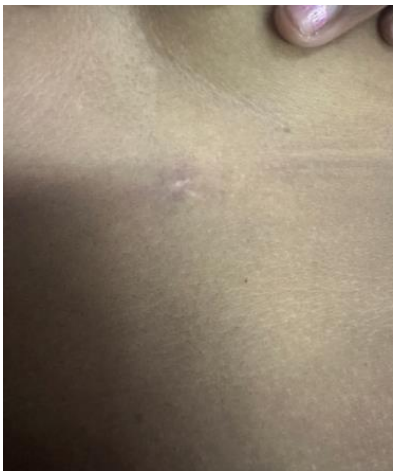


Figure 19d- Post treatment (follow-up) after microneedling with tacrolimus

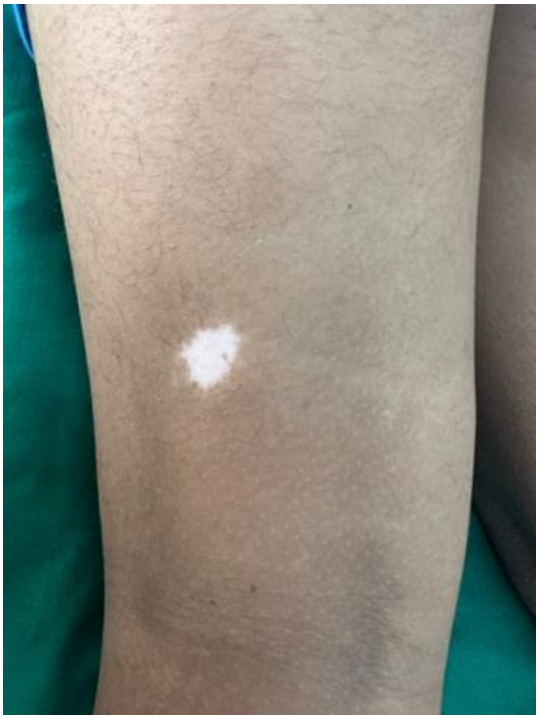


Figure 19e- Baseline image before microneedling alone

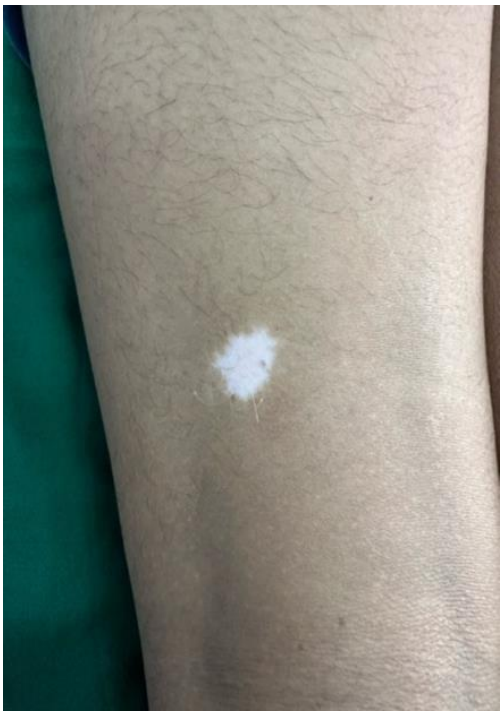


Figure 19f- Post treatment (follow-up) after microneedling alone

ANNEXURE IV

KEY TO MASTER CHART

F- Female

M- Male

HTN –Hypertension

DM- Diabetes mellitus

A-microneedling with 5 fluorouracil

B-microneedling with tacrolimus

C- microneedling alone

G1- Grade 1

G2- Grade 2

G3- Grade 3

G4- Grade 4

ANNEXURE V- MASTER CHART

sl no	name	op number	age	sex	duration of vitiligo	history of treatment	family history	comorbidities	group	site of lesion	leukotrichia	global physician assessment scale					visual analogue scale					side effects
												baseline	2nd sitting	3rd sitting	4th sitting	followup	baseline	2nd sitting	3rd sitting	4th sitting	followup	
1	Basangowda	6764580	21	M	3 years	NO	no	no	A	left ankle	absent	G1	G2	2	G3	G3	1	2	2	3	3	nil
									B	right ankle	absent	G1	G1	G1	G2	G2	1	1	2	2	2	nil
									C	back-right thigh	present	GO	GO	GO	G0	G0	0	0	0	0	0	nil
2	Maruti	6027915	39	M	2 years	NO	NO	HTN	A	left leg	absent	G1	G1	G2	G3	G3	1	1	2	2	3	nil
									B	right leg	absent	G1	G1	G1	G1	G2	1	1	1	2	2	erythema
									C	lateral-right leg	absent	G0	G0	G1	G1	G1	0	0	1	1	1	nil
3	Pooja	7091720	22	F	2.5 years	Topical	NO	no	A	right neck	absent	G1	G1	G2	G2	G3	1	1	1	1	2	ulceration, pain
									B	right breast	absent	G2	G2	G2	G2	G2	2	2	2	2	2	nil
									C	left breast	absent	G1	G1	G1	G1	G1	2	2	2	2	2	nil
4	Rajashree	7091981	37	F	1.5 years	NO	NO	no	A	left neck	absent	G3	G3	G3	G4	G4	3	3	3	3	3	ulceration, hyperpigmentation, pain
									B	left lower eyelid	absent	G1	G1	G2	G3	G3	1	1	2	3	3	nil
									C	left upper eyelid	absent	G1	G1	G1	G1	G2	1	1	1	2	2	nil
5	Aminsab	7227064	20	M	3 years	Topical	no	no	A	right thigh	present	G1	G1	G1	G1	G1	1	1	1	1	1	nil
						oral			B	right thigh	present	G0	G0	G0	G0	G0	1	1	1	1	1	nil
									C	left thigh	present	G0	G0	G0	G0	G0	0	0	0	0	0	nil
6	Archana	7186086	31	F	2 years	NO	no	no	A	back	absent	G1	G3	G3	G3	G4	1	3	3	3	3	hyperpigmentation
									B	back	absent	G1	G2	G2	G2	G2	1	2	2	2	2	nil

									C	right thigh	absent	G0	G1	G1	G1	G1	1	1	1	1	1	nil
7	sumithra	7188723	35	F	2 years	oral	no	hypothyroidism	A	right leg	absent	G1	G3	G3	G4	G4	2	3	3	3	3	nil
						topical			B	left leg	absent	G1	G2	G2	G2	G2	2	2	2	2	2	nil
									C	left ankle	absent	G1	G1	G1	G1	G1	1	1	1	1	1	nil
8	sunil	4578910	45	M	8 years	Phototherapy	NO	DM	A	right ankle	absent	G1	G1	G2	G2	G2	1	1	1	2	2	nil
						Oral			B	left ankle	absent	G1	G1	G1	G2	G2	1	1	1	2	2	nil
						topical			C	right thigh	absent	G1	G1	G1	G1	G1	1	1	1	1	1	nil
9	sheela	4835766	67	F	5 years	topical	NO	no	A	right leg	absent	G1	G1	G2	G3	G3	1	2	2	2	2	nil
									B	right ankle	absent	G1	G1	G2	G2	G2	1	1	1	1	1	nil
									C	left ankle	absent	G1	G1	G1	G1	G1	1	1	1	1	1	nil
10	Shailaja	859571	42	F	6 years	phototherapy	YES	no	A	chest	absent	G1	G2	G3	G3	G4	1	1	2	2	2	nil
						oral			B	right occipital	absent	G1	G2	G2	G2	G2	1	1	1	2	2	nil
						topical			C	abdomen	absent	G0	G2	G2	G2	G2	0	1	1	1	1	nil
11	Sushma	7354887	56	F	1.5 years	NO	NO	hypothyroidism	A	right popliteal	absent	G1	G2	G3	G4	G4	1	2	2	2	2	nil
									B	left ankle	absent	G0	G1	G1	G1	G1	0	1	1	1	1	nil
									C	left thigh	absent	G0	G0	G0	G0	G0	0	0	0	0	0	nil
12	Jagadeesha	7269141	34	M	2 years	NO	NO	no	A	left elbow	absent	G1	G1	G2	G2	G2	1	1	1	1	1	nil
									B	right elbow	absent	G1	G1	G1	G1	G1	1	1	1	1	1	nil
									C	right elbow	absent	G0	G0	G1	G1	G1	0	0	0	0	0	nil
13	Netravathi	6559132	22	F	2 years	NO	NO	no	A	neck	absent	G1	G1	G2	G2	G2	0	0	0	0	0	nil
									B	postauricular	absent	G0	G0	G1	G1	G1	0	0	0	0	0	nil
									C	right thigh	absent	G0	G0	G0	G0	G0	0	0	0	0	0	nil
14	Shahbaz	5529573	36	M	6 years	phototherapy	NO	no	A	right thigh	absent	G1	G1	G2	G3	G3	1	1	1	1	2	nil
						oral			B	abdomen	absent	G1	G1	G1	G2	G3	0	1	1	1	1	nil
						topical			C	back	absent	G0	G1	G1	G1	G1	0	0	0	0	0	nil
15	shivaleela	4176451	40	F	8 years	phototherapy	NO	no	A	right ankle	absent	G1	G2	G2	G2	G3	1	2	2	2	2	nil
						oral			B	neck	absent	G1	G1	G2	G2	G2	1	1	1	2	2	nil
						topical			C	right leg	absent	G1	G1	G1	G1	G1	1	1	1	1	1	nil
16	surekha	7277333	39	F	2.5 years	oral	NO	no	A	right ankle	absent	G1	G2	G2	G2	G2	1	1	2	2	2	erythema
						topical			B	left ankle	absent	G1	G1	G3	G4	G4	1	2	3	3	3	nil
									C	right ankle	absent	G1	G1	G1	G1	G1	1	1	1	1	1	nil
17	Jayashree	7390226	51	F	5 years	oral	NO	no	A	left side of neck	absent	G1	G1	G2	G3	G3	1	1	2	2	2	ulceration, pain
						topical			B	right thigh	absent	G1	G1	G1	G1	G1	1	1	1	1	1	nil
									C	left thigh	absent	G0	G0	G0	G0	G0	0	0	0	0	0	nil
18	Prabhavathi	4160373	40	F	8 years	phototherapy	NO	DM	A	right- nape of neck	present	G1	G1	G1	G2	G2	1	1	1	1	1	erythema

						oral			B	chest	absent	G1	G1	G2	G3	G3	1	1	1	2	2	nil
						topical			C	abdomen	absent	G1	G1	G2	G2	G2	1	1	1	1	1	nil
19	Gurunath	3390069	39	M	4 years	Phototherapy	NO	no	A	left knee	absent	G1	G2	G3	G3	G3	1	1	2	2	2	erythema
						oral			B	trunk	absent	G1	G2	G2	G2	G2	0	1	1	1	1	NIL
						topical			C	right knee	absent	G1	G1	G1	G1	G1	0	0	0	0	0	NIL
20	Achyut	7720298	20	M	4 years	Phototherapy	NO	no	A	right hand	absent	G1	G1	G2	G2	G2	1	1	2	2	2	erythema
						topical			B	left hand	absent	G1	G1	G1	G1	G1	1	1	1	1	1	nil
									C	right thigh	absent	G0	G0	G0	G0	G0	0	0	0	0	0	nil
21	Hanumesh	7022178	32	M	3 years	NO	NO	no	A	left leg	absent	G1	G2	G2	G2	G3	1	1	1	2	2	nil
									B	left arm	present	G1	G1	G1	G2	G2	0	0	1	1	1	nil
									C	right knee	absent	G0	G0	G0	G1	G1	0	0	0	1	1	nil
22	Shabhana	7511354	45	F	7 years	phototherapy	no	no	A	right ankle	absent	G1	G1	G1	G1	G1	0	0	0	1	1	nil
						oral			B	right shoulder	absent	G0	G0	G1	G1	G1	0	0	0	0	1	nil
						topical			C	left arm	absent	G0	G0	G1	G1	G1	0	0	0	0	1	nil
23	sangeeta	7477614	25	F	5 years	no	no	no	A	right jaw	absent	G1	G1	G2	G3	G3	1	1	2	2	2	nil
									B	right shoulder	absent	G0	G0	G1	G1	G1	0	0	0	1	1	nil
									C	chest	absent	G1	G1	G2	G2	G2	0	0	1	1	2	nil
24	Shridevi	7186053	40	F	8 years	phototherapy	NO	hypothyroidism	A	abdomen	absent	G1	G1	G3	G4	G4	0	1	1	2	3	nil
						oral			B	left postauricle	absent	G1	G1	G1	G1	G1	0	0	1	1	1	nil
						topical			C	left ankle	absent	G0	G0	G1	G1	G1	0	0	1	1	1	nil