“AN OPEN LABEL NON-RANDOMIZED SPLIT FACE INTERVENTIONAL STUDY TO COMPARE THE EFFICACY AND SAFETY OF MICRONEEDLING WITH AZELAIC ACID 10% GEL VERSUS MICRONEEDLING WITH TRANEXAMIC ACID 5% GEL IN THE TREATMENT OF MELASMA.”

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DOCTOR OF MEDICINE (M.D)

In

DEPARTMENT OF DERMATOLOGY, VENEREOLOGY AND LEPROSY

DEPARTMENT OF DERMATOLOGY,
VENEREOLOGY AND LEPROSY
J. N. MEDICAL COLLEGE
BELAGAVI - 590010. KARNATAKA

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Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"AN OPEN LABEL NON-RANDOMIZED SPLIT FACE INTERVENTIONAL STUDY TO COMPARE THE EFFICACY AND SAFETY OF MICRONEEDLING WITH AZELAIC ACID 10% GEL VERSUS MICRONEEDLING WITH TRANEXAMIC ACID 5% GEL IN THE TREATMENT OF MELASMA"**, is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee.

(Dr. Smita Sonoli)
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ABSTRACT

Background: Melasma is an acquired disorder of pigmentation characterized by symmetrically distributed hypermelanotic patches on the face. Various options for its management have been evaluated. Microneedling is one such treatment, often used in conjunction with topical modalities. In this study, we assessed the efficacy and safety of microneedling combined with topical azelaic acid and tranexamic acid in 41 patients.

Aims and objectives: To compare the efficacy and safety of microneedling with azelaic acid 10% gel versus microneedling with tranexamic acid 5% gel in the treatment of melasma and to assess and compare the Melasma Quality of Life (MelasQoL) score at the beginning and end of the study.

Materials and methods: Microneedling was done using the Dr. Pen Ultima A6 on bilateral sides of the face over the melasma lesions. Subsequently, 10% azelaic acid gel was applied topically to the right side of the face, and 5% tranexamic acid gel to the left side, immediately after the procedure and then nightly for the next three months. Microneedling was repeated monthly for the following two more months, and the Hemi-mMASI score for each side was assessed at each visit. Immediate and delayed side effects were documented, and the MelasQoL score was also assessed.

Results: Forty-one patients of both sexes, aged 18 to 60 years, were recruited for the study. The cohort included six males and 35 females. After three months of treatment, the Hemi-mMASI scores for microneedling with azelaic acid and microneedling with tranexamic acid showed significant improvement ($p < 0.05$), with tranexamic acid demonstrating better results. Side effects were significantly more frequent in the azelaic acid group.

Conclusion: Microneedling was found to be more effective when combined with tranexamic acid than with azelaic acid, with fewer side effects.

Key words- Melasma, Microneedling, Tranexamic acid, Azelaic Acid.

LIST OF ABBREVIATIONS USED

S. NO.	ABBREVIATION	FULL FORM
1.	ACTH	Adrenocorticotropic Hormone
2.	ADIPOQ	Adiponectin
3.	ADMH	Acquired Dermal Macular Hyperpigmentation
4.	AHA	Alpha Hydroxy Acid
5.	ALOX-15B	Arachidonate 15-Lipoxygenase Type B
6.	ANGPTL1	Angiopoietin-Like 1
7.	AWAT-1	Acyl-CoA Wax Alcohol Acyltransferase 1
8.	AZA	Azelaic Acid
9.	BM	Basement Membrane
10.	COCs	Combined Oral Contraceptives
11.	DHT	Dihydrotestosterone
12.	DNA	Deoxyribonucleic Acid
13.	ER2	Estrogen Receptor Beta
14.	ERK1-2	Extracellular Signal-Regulated Kinases 1 and 2
15.	FABP-4	Fatty Acid-Binding Protein 4
16.	GA	Glycolic Acid
17.	GDA	Guanine Deaminase
18.	hMASI	Hemi-Melasma Area and Severity Index

19.	HPSE	Heparanase
20.	HQ	Hydroquinone
21.	INA	Intermediate Filament Protein
22.	IPL	Intense Pulsed Light
23.	KA	Kojic Acid
24.	KGF	Keratinocyte Growth Factor
25.	LGR-5	Leucine-Rich Repeat-Containing G-Protein Receptor 5
26.	LH	Luteinizing Hormone
27.	MASI	Melasma Area and Severity Index
28.	MC1R	Melanocortin Type-1 Receptor
29.	MelasQoL	Melasma Quality of Life
30.	MITF	Microphthalmia-Associated Transcription Factor
31.	MLANA	Melan-A
32.	mMASI	Modified Melasma Area and Severity Index
33.	MMP-2	Matrix Metalloproteinase 2
34.	MN	Microneedling
35.	NADH	Nicotinamide Adenine Dinucleotide
36.	NADPH	Nicotinamide Adenine Dinucleotide Phosphate
37.	NQO1	NAD(P)H Quinone Dehydrogenase 1
38.	PCOS	Polycystic Ovary Syndrome
39.	PDZK-1	PDZ Domain-Containing Protein 1

40.	Perilipin-1	Perilipin 1
41.	PG	Prostaglandin
42.	PIH	Post-Inflammatory Hyperpigmentation
43.	PKA	Protein Kinase A
44.	PPAR-GC1A	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha
45.	PPAR-α	Peroxisome Proliferator-Activated Receptor Alpha
46.	PR	Progesterone Receptor
47.	PTG-IS	Prostaglandin I Synthase
48.	PTG-S1	Prostaglandin S1
49.	QOL	Quality of Life
50.	RCM	Reflectance Confocal Microscopy
51.	ROS	Reactive Oxygen Species
52.	SC	Stratum Corneum
53.	SCCA1	Squamous Cell Carcinoma Antigen 1
54.	sFRP-2	Secreted Frizzled-Related Protein 2
55.	SOD-2	Superoxide Dismutase 2
56.	SPF	Sun Protection Factor
57.	TC	Triple Combination
58.	TEWL	Trans-Epidermal Water Loss
59.	TXA	Tranexamic Acid

60.	TYR	Tyrosinase
61.	TYRP-1	Tyrosinase-Related Protein 1
62.	UV	Ultraviolet
63.	UVR	Ultraviolet Radiation
64.	VEGF	Vascular Endothelial Growth Factor
65.	WIF-1	Wnt Inhibitory Factor 1
66.	WNT-3A	Wingless-Type MMTV Integration Site Family, Member 3A
67.	Wnt-5A	Wingless-Type MMTV Integration Site Family Member 5A

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INTRODUCTION

Melasma is an acquired disorder of pigmentation affecting millions worldwide. It manifests as symmetric hypermelanosis, presenting as non-uniform light brown to grayish-brown macules and patches.[1]

It mainly affects the facial regions, including the brow, nose, malar area, perioral area, and chin. In rarer cases, it also can develop on other body surfaces, such as the extensor surface of the forearms.[2,3]

It predominantly occurs in women having III to V skin type as classified by Fitzpatrick, and who reside in areas with high ultraviolet (UV) index, significantly affecting their quality of life.[4]

An array of options for melasma treatment has been studied, but they often yield unsatisfactory outcomes, with incomplete clearance of pigment and an increased likelihood of recurrence.[5]

Microneedling serves as an effective adjuvant to various topical treatments that are available for melasma. Its action is by enhancement of transcutaneous absorption of the topical agents through microchannels formed by the microneedles. It stimulates fibroblast proliferation, which promotes neocollagenesis, neoelastogenesis, and epidermal thickening. It also facilitates the transdermal removal of melanin.[6]

Azelaic acid (AZA), which is also called nonanedioic acid or 1,7-heptanedicarboxylic acid, is an organic molecule which occurs naturally. It is a highly potent topical therapy for acne vulgaris and several cutaneous hyperpigmentation disorders, including melasma. AZA functions by inhibiting tyrosinase competitively and reducing the activity of reactive oxygen species.[7]

Tranexamic acid (TXA) is an antifibrinolytic agent. It can be utilized in oral form, topical application, and intradermal injections for treating melasma. It suppresses melanin production by blocking the action of the tyrosinase enzyme in melanocytes.[8]

Topical AZA alone has been studied before, but there are no studies to date, to our knowledge, that evaluate both the effectiveness and safety of AZA in melasma when combined with microneedling. Additionally, this study will help compare the effectiveness and adverse effects of AZA gel versus TXA gel when used in combination with microneedling for melasma management.

AIMS AND OBJECTIVES

PRIMARY OBJECTIVE:

To compare the efficacy of microneedling with azelaic acid 10 % gel versus microneedling with tranexamic acid 5% gel in the treatment of melasma.

SECONDARY OBJECTIVE:

1. To compare the safety of microneedling with azelaic acid 10 % gel versus microneedling with tranexamic acid 5% gel in the treatment of melasma.
2. To assess and compare Melasma Quality of Life (MelasQoL) score at the beginning and at end of the study.

REVIEW OF LITERATURE

MELASMA:

The name 'melasma' comes from the Greek word 'melas,' which translates to 'black,' signifying its characteristic dark pigmentation. Other terms, such as "mask of pregnancy" and "chloasma" (Latin: chlóos and Greek: cloazein, meaning "greenish"), have been utilized interchangeably.[9]

BACKGROUND:

Descriptions in the early medical literature dates back to the writings of the Hippocrates (470–360 B.C.). Dr. G. Pernet first introduced the concept of skin-localized melanosis in 1910. He detailed microscopic skin sections showing epithelial degeneration, melanocytes, and slow-developing melanosis cutis.[9,10]

In the early 1900's, Dr. Castellani identified chloasma bronzeum and symmetricum among residents of Ceylon (now Sri Lanka). Dr. W.G. Spencer in 1923 presented a pioneering hypothesis linking melanin to neural tube development, offering early perspectives on the complex nature of melasma. Meanwhile, in 1929, the term "melanoderma" was introduced by Dr. Gupta, which significantly advanced the understanding of melanin-associated conditions.[10,11]

The first documented case of melasma in medical literature from the West appeared in the year 1934, detailing a young British woman. She came with the complaints of a well-defined, brown colored lesion on her upper lip, which worsened following sun exposure.[12]

In the 1960’s, Dr. Sorrel Resnik coined the word "melasma" and linked it to various hyperpigmentary disorders caused by the use of oral contraceptives.[13]

In 1988, Vazquez et al. established that melasma presents with similar clinico-histological traits in both men and women, despite hormonal factors playing a lesser role in men.[14]

EPIDEMIOLOGY:

INCIDENCE AND PREVALANCE:

Melasma affects individuals across all ethnicities and populations. Its global prevalence varies widely, ranging from 5–46%, depending on the group studied. Epidemiological data indicate a higher occurrence in darker skin types, particularly among Asians, Middle Eastern populations, and those of Mediterranean-African descent.[15]

An Indian study which was conducted among paddy-field workers found that melasma's prevalence in this group was approximately 41%.[16]

SEX:

A 2013 survey of more than 500 employees at a university in Brazil revealed a melasma diagnosis in 34% of women compared to only 6% of men.[17]

Multiple multicentric studies from India assessing melasma patients have indicated an incidence of 75–85% in females compared to 15–25% in males.[15,18]

AGE:

Melasma usually develops between the ages of 20 and 40. Additionally, studies have shown a correlation between the initial age of occurrence and skin phototypes, with lighter types experiencing an earlier onset compared to phototypes IV–VI ($P < 0.0001$). This indicates that melanin has a photoprotective function, delaying the onset of the lesions.[19,20]

In Indians, the melasma onset typically occurs at a later age, ranging from 35 to 45 years.[15,21]

SKIN TYPE:

Fitzpatrick skin-types categorize human skin into 'six' groups (I-VI) according to its response to ultraviolet (UV) radiation, mainly focusing on its tendency to either tan or burn.[22]

Melasma, a localized pigmentary condition, primarily affects individuals with medium to dark skin tones, particularly those with skin-types III–V. It is rarely observed at the extremes of the Fitzpatrick skin-type spectrum. It is hypothesized that people with lighter skin-types are unable to generate added pigmentation, whereas those with darker skin-types are already producing pigmentation at peak capacity. Therefore, types I and VI are more stable and resilient.[9]

A multicenter study in Brazil found that most participants presented with type III (36%) and type IV (40%) skin, with smaller proportions exhibiting type II (13%) and type V (10%).[19]

In a survey conducted on Tunisian women revealed a higher prevalence of type IV (45%) and type V (40%) skin, while 14% displayed type III.[20]

Likewise, an Indian multicenter study reported that nearly half of the participants exhibited type IV (49%), followed by type V (40%) and type III (11%).[15]

ETIOLOGY:

The etiology of melasma remains ambiguous, though it is considered to be multifactorial. Known factors that trigger melasma include UV exposure, pregnancy, genetics, oral contraceptives and hormonal replacement therapy, intestinal parasitosis,

ovarian cancers, procedures and inflammation of the skin, cosmetics, photosensitizing medications, and stressful events.[23–25]

ULTRAVIOLET RADIATION (UVR):

Ultraviolet (UV) radiation is the main trigger for melasma. Exposure to both UVA and UVB rays from sunlight can stimulate melanogenesis, resulting in the formation of epidermal pigmentation.[24,26]

This is evidenced by the fact that it most commonly develops in sun-exposed areas, and many patients have observed that sun exposure further worsens the condition. The pigmentation tends to improve in winter and intensifies during summer. Additionally, its prevalence is higher in regions near the equator.[27–29]

Visible light, as well as infrared radiation can trigger melanogenesis, but they have markedly lower potential to produce melanin as compared to UVA and UVB.[15,30,31]

HORMONES:

Female reproductive hormones (estrogen and progesterone) are known etiological risk factors for causing melasma, and the higher occurrence of the condition in women of reproductive age group further validates the theory. Disruptions in hormone levels due to hormonal therapy, pregnancy status, ovarian malignancies, and contraceptive use can stimulate melanin production. In the affected areas, an increased presence of estrogen and progesterone receptors has been identified in the dermis and epidermis, respectively.[13,32]

Studies have shown that South Asian women with melasma exhibit elevated circulating levels of prolactin, estradiol, luteinizing, and follicle-stimulating hormones

during the early menstrual cycle, indicating that serum estrogens may act as a risk factor and contribute to the persistence of the condition. In contrast, Puerto Rican women with melasma had reduced estradiol but increased LH levels compared to controls.[33–35]

Pregnancy leads to higher secretion of pituitary hormones, which may induce physiological pigmentation and contribute to melasma, particularly in the third trimester.[36]

The global prevalence among pregnant women, of melasma, varies from 5% to 46%. In up to half of women, the melasma is induced following childbirth or the use of oral contraceptives. Melasma affects upto 34% of females on combined oral contraceptives (COCs) and has also been observed in those undergoing hormone replacement therapy.[9,13,37]

Elevated gonadotropin levels and reduced testosterone concentrations were observed in fifteen Indian men with melasma compared to the control group.[38]

ENDOCRINE ALTERATIONS:

Autoimmune thyroid diseases have been proposed as a potential factor associated with melasma, but the findings of relevant studies remain inconclusive, and none have used adequate methodology to establish a reasonable hypothesis.[35,39]

Studies have indicated that there is no significant variation in the serum levels of thyroid hormones between individuals with disease and control groups.[40,41]

In Indian patients with facial melasma, 7.5% were diagnosed with hypothyroidism, while 0.9% had polycystic ovary syndrome (PCOS).[15]

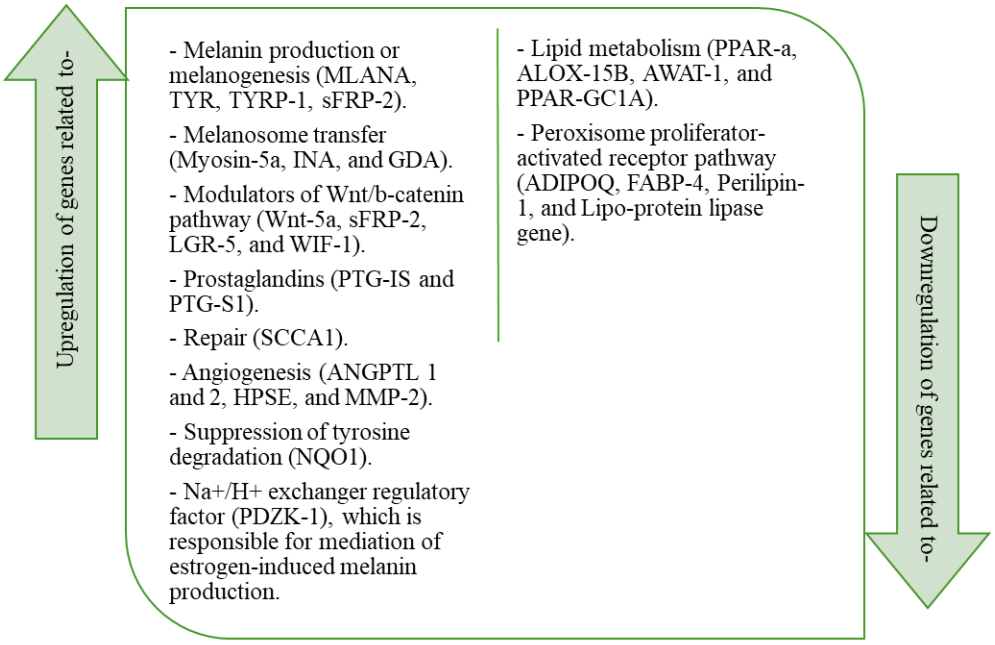
GENETIC FACTORS:

The genetic link to melasma was first identified in the 1980s in two identical twins in England, where the condition was exacerbated by sunlight exposure and there was a history of oral contraceptive use. However, their third sister (a non-twin) was unaffected, supporting the hypothesis that genetic susceptibility is significant for the disease's evolution.[42]

In a worldwide study involving over 300 melasma patients, it was revealed that almost 50% had a family history of the condition.[43]

In a Brazilian study, 56% of patients had disease history in the family, compared to 33% of Indian patients and 10% of Singaporean patients who reported a similar history. These variations suggest that the melasma onset may be affected by both hormonal and epigenetic factors, as well as environmental influences.[21,44,45]

Transcriptomic analyses comparing affected facial skin with neighboring unaffected skin have identified over 300 genes, with the key ones listed below.[46–48]



In the affected skin, the H19 gene was also found to be downregulated, and it is responsible for the transcription of noncoding RNA in mixed melanocyte-keratinocyte cultures. This stimulated greater melanin production and enhanced transfer of melanin when compared to melanocytes in monoculture.[49]

The melanocortin type-1 receptor (MC1R) is the primary controller of eumelanin production, influencing various hair and skin color phenotypes and affecting the skin's sensitivity to ultraviolet radiation.[26]

Studies on primary fibroblast cultures from facially affected skin and neighboring unaffected skin have shown increased expression of various genes such as WNT-3A, matrix metalloproteinases-1, and SOD-2. There is decreased expression of COL-4A1 and -7A1, colony-stimulating factor-2, tissue inhibitor of metalloproteinases-4, CCL-2, and OB-cadherin genes. These genes linked to inflammation, increased melanin production, and impaired skin repair may contribute to damage of upper dermis and persistent pigmentation in melasma. These genetic factors promote chronic inflammation and excessive melanogenesis, while defective repair mechanisms hinder skin recovery, leading to long-term hyperpigmentation.[50]

These findings further support the concept that melasma arises due to a multifaceted interaction of various factors, primarily affecting individuals with an inherent genetic susceptibility.

INFLAMMATORY PROCESSES AND POST INFLAMMATORY HYPERPIGMENTATION:

Certain cosmetic treatments that induce inflammation in the skin, such as peels and light/laser therapies provoke melanin production. Studies on the connection between melasma and intense pulsed light (IPL) treatments indicate that individuals with subclinical disease may experience a worsening of the condition following IPL therapy.[26,51,52]

Skin affected by melasma has been shown to exhibit increased expression of several inflammatory mediators, along with a higher density of inflammatory cells and neo angiogenesis compared to adjacent unaffected skin. These findings back the hypothesis of an enhanced inflammatory response in damaged skin.[53,54]

Patients with melasma were six times more prone to develop postinflammatory hyperpigmentation.[19,55]

DRUGS AND COSMETICS:

Hyperpigmentation of the skin can result from cosmeceuticals, photosensitizing agents, and heavy metals. Additionally, medications like chloroquine, hydroxychloroquine, minocycline, phenytoin, carbamazepine, amiodarone, and sulfonylureas may contribute to skin discoloration either by precipitating in the superficial layers of the skin or by triggering melanin production.[9,56,57]

A case of melasma has also been associated with finasteride use in a male patient undergoing treatment for androgenetic alopecia. The medication reduced

dihydrotestosterone (DHT) levels while increasing the amount of testosterone available for peripheral conversion into estrogen.[58]

In some parts of India, mustard oil is widely applied to the face and scalp. However, as a well-recognized photosensitizer, it has the potential to trigger melasma in genetically susceptible individuals.[59]

PATHOGENESIS:

Sustained exposure to UV light leads to oxidative damage, inflammation, and premature skin aging, which maintains the ongoing production of melanin in melasma. This exposure also activates the p53 protein, which further triggers the secretion of molecules like ACTH, melanocyte-stimulating hormone, β-endorphin, and laminin-332. These molecules act through paracrine signaling to further stimulate melanogenesis.[60,61]

UVB radiation primarily affects the epidermis and the basement membrane (BM) by increasing melanocyte activity, which enhances the transfer of melanosomes (pigment-containing organelles) to keratinocytes (skin cells). This process contributes to skin pigmentation. Moreover, UVB exposure degrades heparan sulfate, a key component of the BM, allowing melanogenic signals from the dermis to reach the epidermis more easily, further stimulating melanin production. Additionally, it stimulates the secretion of mediators of inflammation like PG and VEGF, which drive endothelial cell proliferation. It also activates neuropeptides such as CGRP, which trigger melanogenesis and promote dendritic formation in melanocytes.[54,62,63]

UVA radiation differs from UVB in its effects. While UVB primarily impacts the outer epidermal layer, UVA extends deeper, reaching the upper dermis. Although UVA causes less erythema, it is more potent at inducing skin darkening, mainly in

people with darker skin tones. Unlike UVB, UVA does not exert its effects through direct interaction. Instead, UVA energy is absorbed by chromophores, leading to ROS production. These triggers oxidative stress, which indirectly damages cell structures, influences melanogenesis, and accelerates skin aging.[64,65]

When the skin is exposed to UVR, it triggers the release of several signaling molecules in the epidermis, promoting melanocyte dendrite growth and increasing TYR gene expression, which enhances melanin production.[66,67]

Fibroblasts in the upper dermis release multiple growth factors and cytokines that stimulate melanocyte activity and melanin production. They also regulate the Wnt/β-catenin pathway via sFRP2, further influencing pigmentation.[68]

Mast cells respond to external stress by releasing mediators that stimulate melanin production while also causing injury to the basement membrane and papillary dermis.[69]

Estrogen and progesterone influence melasma through nuclear receptors. ER2s are more prevalent in facial skin than in the breast and abdomen, which may explain the facial predominance of melasma.[70,71]

Estradiol promotes epithelial growth and melanin production by activating key signaling pathways like ERK1-2/MAP kinases and Wnt/β-catenin, while also stimulating keratinocyte growth factor (KGF). Additionally, estradiol influences skin thickness by boosting fibroblast activity and collagen production.[72–74]

An increased ER2 expression in the affected lesional skin is noted, which is responsible for promoting melanin production by activating MC1R and enhancing the

production of MITF, tyrosinase-Related Protein 1 and 2 in melanocytes. This process is regulated by the inhibition of protein kinase A (PKA).[50,75]

The role of progesterone and its receptors is complex. Progesterone influences melasma by increasing epidermal PR expression but does not affect tyrosinase activity. While it may play a role in skin response, progesterone contradictorily inhibits melanocyte proliferation and estrogen-driven melanin production after UV exposure.[75–77]

Melasma-affected skin shows several functional changes, including higher melanin and erythema indices, as well as altered pH levels compared to the surrounding uninvolved skin. Nevertheless, there are no significant differences in the biophysical properties across the different types.[78]

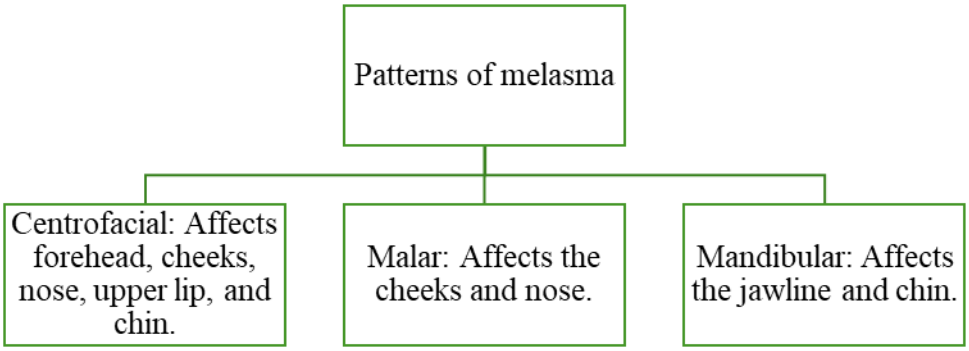
In melasma, the skin barrier is compromised, as evidenced by a thinner stratum corneum (SC) that retains more hydration compared to nearby unaffected skin. Nonetheless, trans-epidermal water loss (TEWL) and sebum levels are the same between the two. In contrast, when the skin is injured through tape stripping, melasma-affected skin exhibits increased TEWL and delayed barrier recovery.[78]

Overall, melasma-affected skin exhibits higher levels of total lipids, including phospholipids and ceramides. This increase may serve as an adaptive mechanism to preserve skin barrier integrity. However, certain essential lipids are under expressed in the presence of heightened melanocyte activity, indicating that repairing the compromised skin barrier could serve as an effective supplementary approach for managing melasma.[79]

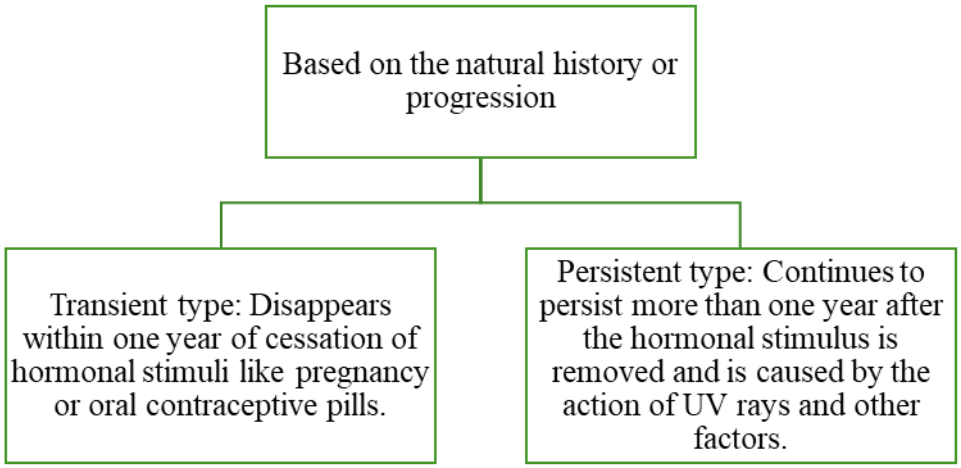
CLINICAL FEATURES:

Melasma is a prevalent cause of facial melanosis, marked by bilaterally symmetrical dark brown or grey-colored macules. It predominantly impacts the facial regions exposed to the sun. Less commonly, it can also appear on extra-facial body surfaces, like the extensor surface of forearms. These spots can have various shapes, including irregular, patchy, curved-arcuate, or poly-cyclic.[1–3]

It can be divided based on site of involvement on the face into three patterns, as follows[44,80]:



Based on the progression of the lesions, it can also be categorized as follows[81]:

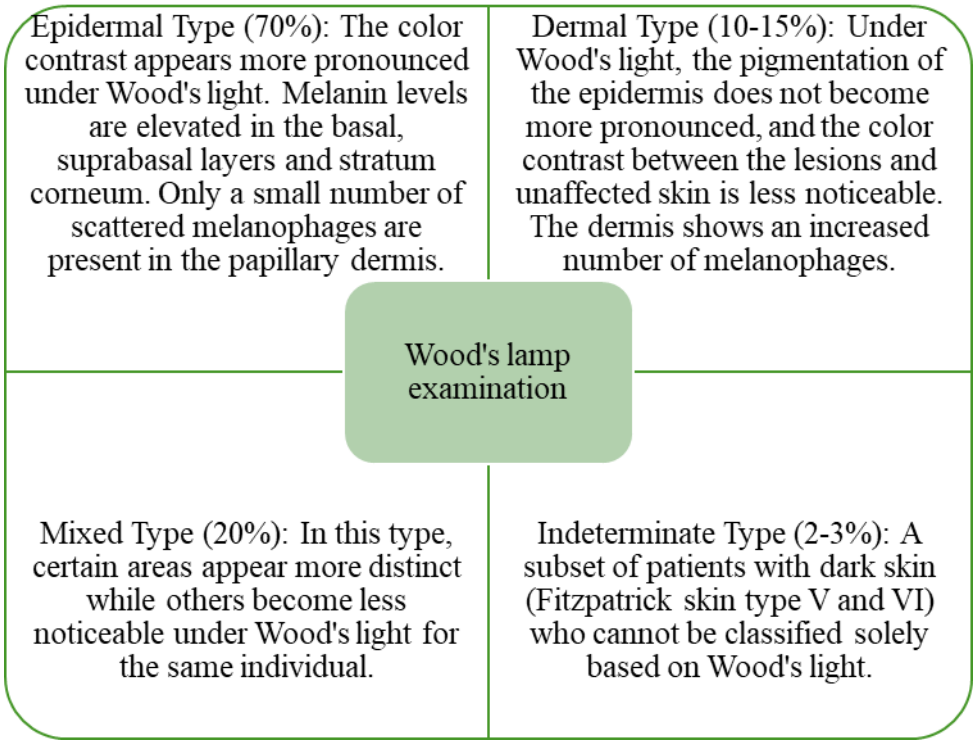


DIAGNOSIS:

WOODS LAMP EXAMINATION:

Robert W. Wood invented Wood's lamp in the 1900s. It works by emitting "long wave" ultraviolet radiation, otherwise called "black light" which is produced by mercury arc under high pressure. It is fitted with $BaSiO_3$ and 9% NiO filter, otherwise called 'Wood's filter' which filters outs all light rays apart from light belonging to wavelength between 320-400 nm.[82]

Using Wood's lamp (320-400 nm), melasma can be histologically categorized as follows[83]:



While Wood's lamp may identify epidermal type of melasma in a sub-set of patients, but all patients exhibit higher melanin amount in both the epidermis and dermis. This suggests that even when melasma appears to be epidermal under the lamp, there is likely significant dermal melanin, indicating that most cases are likely mixed melasma.[84]

DERMOSCOPY:

Dermoscopy of melasma typically shows a reticulate pseudonetwork pattern that spares the perifollicular area. Additional features, such as brown dots or globules, and annular or arcuate formations, may be present.[85,86]

The reticuloglobular pattern is linked to the epidermal type, while the dermal type often shows granular patterns. Yalamanchili et al. also reported the color varied from pale to deep brown, with diffuse patterns in 38% of cases and patchy patterns in 62%.[85,86]

The color observed in dermoscopy may correspond to the depth of pigment: uniform brown pigmentation suggests epidermal involvement, while irregular, mixed bluish-grey hues indicate pigmentation in the dermis.[85]

Various studies indicate that melasma may show an enhanced appearance of blood vessels on dermoscopy. This increased vascularity may result from growth factors that stimulate blood vessel development or from the prolonged use of topical steroid-containing creams. Evaluating vascularity is essential, as it can contribute to the skin darkening seen in melasma.[85]

REFLECTANCE CONFOCAL MICROSCOPY (RCM):

RCM offers a non-interventional, high-resolution method for assessing melasma at the cellular level, enabling detailed analysis of pigment depth and lesion heterogeneity. Unlike Wood's light examination, which has limited accuracy in determining pigment depth, RCM provides precise visualization of epidermal and dermal features, making it a valuable tool for classification and treatment planning.[87]

Epidermal Changes: RCM reveals a significant increase in hyperpigmented basal keratinocytes in all melasma lesions, forming a hyper refractile cobblestone pattern. This is confirmed by histology as epidermal hyperpigmentation. Bright dendritic cells, representing activated melanocytes, are also observed in the dermo-epidermal junction.[87]

Dermal Changes: RCM detects melanophages in the papillary dermis, with their numbers being considerably more in the affected skin. The distribution of melanophages is uneven across and within the lesions. The affected skin shows more severe solar elastosis, appearing as ragged, less refractile, lacy structures under RCM. Blood vessels appear as dark, tubular structures, with higher vascularity observed in the involved skin.[87]

Topographic Variation: Melanophages are distributed heterogeneously across melasma lesions, varying both between regions and within the same lesion. This uneven distribution highlights the variability in dermal melanin deposition in melasma.[87]

HISTOPATHOLOGY:

Histologically, melasma is defined as epidermal hyperpigmentation. The corneal layer is denser, while the stratum granulosum is atrophic, with flattened ridges and a thinner epidermis. The nuclei of the basal layer appears irregular and enlarged, with a loss of polarity and chromatin variability compared to the surrounding skin. Additionally, solar elastosis is markedly more pronounced in the affected areas.[88,89]

In melasma-affected skin, melanocytes in the basal layer are hypertrophied with enlarged dendrites. Nevertheless, the number and density of melanocytes do not show a significant increase.[89–91]

Melasma-affected skin shows elevated melanin throughout the epidermis. The melanosomes in the epidermis are larger, more developed, and more numerous compared to nearby uninvolved skin. These findings are consistent across different skin types.[90,92]

The dermis of the affected skin shows no significant difference in melanin levels. However, increased vascularity, solar elastosis, and higher mononuclear and mast cell counts are observed.[84,89,90]

Evaluation under a Wood's lamp indicates a differentiation between epidermal and dermal types, but histological analysis shows no notable difference in dermal melanin levels between them. This indicates that the clinical classification based on Wood's lamp findings does not align with histological evidence.

DIFFERENTIAL DIAGNOSIS:

The most important differentials of melasma include ephelides, solar lentigines, pigmented contact dermatitis, PIH, macular amyloidosis, ochronosis (endogenous and exogenous), acanthosis nigricans, acquired dermal macular hyperpigmentation (ADMH), periorbital hyperpigmentation, dermatosis papulosis nigra, seborrheic keratosis, actinic lichen planus, cutaneous lupus, phytophotodermatitis, pellagra, oculodermal melanocytosis , Hori's nevus, and drug-induced pigmentation. These may occasionally overlap in the patients with melasma, making it crucial to distinguish between them when planning the treatment.[93,94]

SEVERITY ASSESSMENT:

THE MELASMA AREA AND SEVERITY INDEX (MASI):

The MASI was introduced by Kimbrough-Green et al. It is a more precise technique for quantifying melasma severity and monitoring response during treatment. The score is determined by a subjective evaluation of three components: area (A) of involvement, darkness (D), and homogeneity (H).

The face is divided into four areas: the forehead (f), right malar (rm), left malar (lm), each of which accounts for 30% of the total face area, and chin (c), which is 10%.[95,96]

The area in each region which is involved is scored on a scale from 0 to 6 (where, 0 = no involvement; 1 = less than 10%; 2 = 10%-29%; 3 = 30%-49%; 4 = 50%-69%; 5 = 70%-89%; and 6 = more than 90% involvement). Darkness and homogeneity are measured on a scale from 0, meaning absent, to 4, indicating maximum severity.[95]

The final score is determined by summing the darkness and homogeneity scores, then multiplying by the respective area-wise involvement for all four regions. The total score ranges from 0 to 48.[95]

MASI = [FOREHEAD] 0.3A(D+H)
+ [RT MALAR] 0.3A(D+H)
+ [LT MALAR] 0.3A(D+H)
+ [CHIN] 0.1A(D+H)

It is a reliable instrument to evaluate the severity, demonstrating consistency over time and strong validation against other assessment methods. The severity can be effectively determined by assessing darkness and the affected area, while homogeneity evaluation can be excluded without compromising the score's reliability.[96]

MODIFIED MELASMA AREA AND SEVERITY INDEX (mMASI):

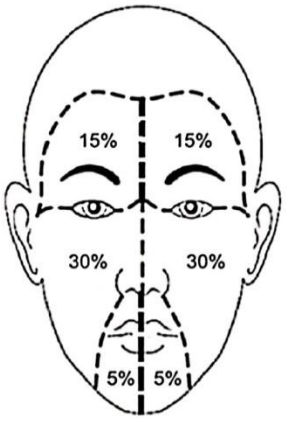
A study by Abou-Taleb et al. found that the modified MASI is highly reliable and valid compared to the standard MASI. It is more user-friendly, easier to learn, and simpler to calculate. The mMASI effectively tracks changes over time, and assessing only the affected area and pigmentation darkness is sufficient for evaluating melasma severity, making homogeneity assessment unnecessary.[97]

mMASI = [FOREHEAD] 0.3A(D)
+ [RT MALAR] 0.3A(D)
+ [LT MALAR] 0.3A(D)
+ [CHIN] 0.1A(D)

HEMI-FACE MODIFIED MELASMA AREA AND SEVERITY INDEX (HEMI-mMASI):

The modified MASI, when evaluated separately for each facial half, is called the Hemi-modified MASI (HEMI-mMASI) score. The maximum score for each hemi-face is 12.

Hemi-mMASI = [FOREHEAD] 0.15A(D)
+ [RT or LT MALAR] 0.3A(D)
+ [CHIN] 0.05A(D)



Hemi-mMASI

Frontal	15%
Malar	30%
Chin	5%

0.15(AxD) + 0.3(AxD) + 0.05(AxD)

Score range: 0-12

Area	Darkness
0=no involvement; 1=<10; 2=10–29; 3=30–49; 4=50–69; 5=70–89; and 6=90–100	0=absent; 1=slight; 2=mild; 3=marked; and 4=severe

QUALITY OF LIFE (QOL):

Melasma profoundly influences an individual's appearance, triggering emotional distress, social challenges, and a diminished quality of life. Patients often incur significant costs for treatments and procedures, many of which fall short of their expectations. Because melasma predominantly manifests on the face, its constant visibility in daily interactions has a deeply adverse effect on mental and emotional well-being. Individuals commonly experience intense feelings of embarrassment, loss of confidence, dissatisfaction, and reluctance to participate in social activities. In some cases, the condition has even been linked to reports of suicidal ideation in literature.[9,98]

In 2003, Balkrishnan et al. constructed a valid Melasma QOL Scale (MelasQoL), a targeted questionnaire aimed at assessing how melasma affects emotional health, social connections, and daily routines. The questionnaire includes ten items, with seven sourced from the Skindex-16 and three from the Skin Discoloration Questionnaire.[99]

The MelasQoL questionnaire measures the impact on patient's quality of life using a 7-point Likert scale, where 1 means "not bothered at all" and 7 means "bothered all the time. The overall rating ranges from 7 to 70, with elevated values indicating a greater negative impact on daily life and well-being.[98]

MelasQoL questionnaire[99]:

1. The appearance of your skin condition	☐
2. Frustration about your skin condition	☐
3. Embarrassment about your skin condition	☐
4. Feeling depressed about your skin condition	☐
5. The effects of your skin condition on your interactions with other people (eg, interactions with family, friends, close relationship, and so forth.)	☐
6. The effects of your skin condition on your desire to be with people	☐
7. Your skin condition making it hard to show affection	☐
8. Skin discoloration making you feel unattractive to others	☐
9. Skin discoloration making you feel less vital or productive	☐
10. Skin discoloration affecting your sense of freedom	☐

The MelasQoL scores show only a weak correlation with the clinical severity of melasma as calculated by the MASI. This suggests that patients' perceptions of their condition are influenced by factors beyond just its physical appearance. As a result, treatment choices should not be based only on clinical evaluations but should also contemplate the patient's emotional well-being and prioritize the factors most important to them.[100,101]

PROGNOSIS:

Overall, the prognosis for melasma is favorable. Its prevalence decreases with age, the severity of lesions diminishes over time, and pigmentation lessens with appropriate treatment.[9]

Persistent cases or relapses of melasma are frequent and inevitable without strict adherence to rigorous sun avoidance measures.[102]

TREATMENT:

There are several treatment options for melasma. First-line therapies typically involve topical agents aimed at reducing pigment production, along with sun protection and camouflage techniques. For more persistent cases, second-line treatments such as chemical peels may be considered, though they should be prescribed with vigilance, especially for patients with darker skin tones. For patients not responding to other treatments, laser.and.light therapies may offer potential alternatives. However, these treatments carry a high risk of worsening the already existing disease. It is critical to thoroughly know the possible risks and advantages of each treatment option to choose the most adequate plan of action for the patient.[103]

SUNSCREENS:

Sunscreens are essential for management and prevention of melasma. SPF of 30 or more that protect against UVA/UVB and VL are crucial. Ideally, for melasma, the sunscreen should provide strong UVA and VL protection, be cosmetically appealing, and include physical blockers like iron oxides. Tinted sunscreens are preferred for their dual benefits of protection and camouflage. For individuals whose skin color doesn't match the available sunscreen tints, a good alternative is to use non-tinted sunscreens in combination with camouflage makeup containing iron oxides. Consistent and prolonged photoprotection is vital to prevent relapses, reduce the severity of melasma, and enhance treatment outcomes, as UVA/UVB protection alone is inadequate.[104–106]

HYDROQUINONE (HQ) AND TRIPLE COMBINATION (TC):

HQ is an aromatic compound with free-radical scavenging properties that inhibit melanin production. It works by blocking the enzyme tyrosinase, which is essential for melanin synthesis. HQ also interferes with nucleic acid synthesis and disrupts melanosome synthesis, leading to melanocyte damage and cell death. These effects reduce melanin production, resulting in skin-lightening over time.[107,108]

Consistent use of sunscreen along with 4% HQ results in substantial improvement in melasma severity, with the most significant reduction occurring over a 16-week period.[109,110]

The combination of hydroquinone with tretinoin and corticosteroids, as proposed by Kligman and Willis, improves the pigment reducing effects, rendering it a more effective treatment.[111]

The triple combination (TC) treatment, containing 0.05% tretinoin and 0.01% fluocinolone acetonide in addition to 4% HQ, is usually regarded as the benchmark therapy for melasma. It is highly effective, providing rapid and significant improvement when used alongside sunscreen.[112,113]

The triple combination (TC) may cause adverse effects such as contact dermatitis, leukoderma, erythema, telangiectasias, and exogenous ochronosis.[114]

NIACINAMIDE:

It is a bioactive metabolite of vitamin B3 (niacin) that plays a crucial role in cellular metabolism. It works like a precursor for essential coenzymes like NADH and NADPH, which are vital for energy production and possess strong antioxidant

properties. These coenzymes help protect cells from oxidative stress and support various biological functions.[115]

It is believed to reduce melanin by inhibiting melanosome transfer. Additionally, it is known for its ability to decrease inflammation and mitigate sun-induced degenerative changes.[116]

In melasma treatment, using 4% niacinamide has demonstrated a substantial decrease in the hemi-MASI (hMASI) score following two months of therapy.[117]

CYSTEAMINE:

It is derived from L-cysteine degradation and has antioxidant and skin-lightening properties. It works by inhibiting peroxidase and tyrosinase enzymes, binding to iron and copper, and increasing glutathione levels in the cells, all of which contribute to its depigmenting effects.[118]

An RCT involving 50 females with melasma compared 5% cysteamine to a modified triple combination (TC) containing HQ, retinoic acid, and betamethasone over 16 weeks. There was 51% improvement in MASI for cysteamine and 42% for the modified TC. Additionally, two placebo-controlled studies tested 5% cysteamine cream applied nightly for 16 weeks, achieving MASI reductions of 50% and 59%, respectively. These findings highlight cysteamine's effectiveness in melasma treatment.[118,119]

ASCORBIC ACID:

L-ascorbic acid is often prescribed for melasma at concentrations ranging from 5% to 20%. It acts as a powerful antioxidant that reduces melanin production by binding to copper and inhibiting the enzyme tyrosinase.[120]

In an RCT involving 16 melasma patients, 4% HQ was evaluated against 5% L-ascorbic acid over 16 weeks, with more than 60% of patients using 5% L-ascorbic acid reporting good to excellent outcome by the third month, while those using 4% HQ experienced noticeable improvements as early as four weeks. However, statistical significance was not observed between the two treatments in terms of colorimetry measurements.[121]

KOJIC ACID:

KA, derived from fungi such as Acetobacter species, suppresses tyrosinase function, leading to decreased eumelanin synthesis and resulting in skin brightening. In addition to its depigmenting effects, it also possesses antiproliferative, anti-inflammatory, UV-protective, and antibacterial properties. These benefits make it useful for treating conditions like melasma and providing additional skin protection.[122]

In an RCT with 80 patients, treatment with 1% KA in combination with hydroquinone and betamethasone valerate over 3 months showed up to 71% decrease in MASI score.[122]

A study involving 60 patients compared treatments with 4% HQ cream alone and a combination cream containing HQ, 0.75% KA, and ascorbic acid over 3 months. Both treatments led to reductions in MASI scores. However, the HQ cream group showed significantly greater efficacy than the combination group, highlighting HQ's stronger effectiveness in treating melasma, although the combination cream still offered some benefit.[123]

GLYCOLIC ACID:

Glycolic acid, an AHA, works based on its concentration and pH level, weakening the bond between corneocytes and resulting in epidermal thinning with prolonged use. It enhances the absorption of other ingredients and helps distribute melanin more evenly. These properties make it effective in treating hyperpigmentation.[124]

GA is often used alongside compounds such as KA or HQ. Primary adverse events include irritation, with possible temporary redness and a burning sensation occurring shortly after application.[125]

AZELAIC ACID (AZA):

Azelaic acid (9-carbonodicarboxylic acid) functions as a non-phenolic compound obtained from Pityrosporum ovale, a yeast found on the skin. It was initially introduced in the 1980s as part of a treatment plan for melanoma, showing positive results when combined with surgery.[126,127]

AZA targets overactive melanocytes, which are more permeable than normal cells, allowing AZA to penetrate them more effectively. It disrupts cellular processes like mitochondrial respiration and endoplasmic reticulum function, inhibiting melanocyte proliferation. AZA also blocks melanin production by suppressing the enzyme tyrosinase.[126]

Azelaic acid is considered safe for topical use during pregnancy, falling under Category B. It is approved for individuals over the age of 12, indicating that its benefits outweigh any potential risks for this age group. In terms of side effects, it is mostly well-tolerated, with most users experiencing only minimal, transient

symptoms like stinging, burning, or itching at the application site. These effects typically resolve with continued use or proper application. Notably, there have been no reports of severe systemic side effects, and azelaic acid does not seem to increase sensitivity to sunlight or the likelihood of sunburn.[7,128]

TRANEXAMIC ACID (TXA):

TXA serves as an artificial derivative of lysine. It primarily prevents excessive bleeding by blocking plasminogen from transforming into plasmin. In melasma treatment, it works by inhibiting tyrosinase, decreasing mast cell activity, and suppressing the production of fibroblastic growth factors.[129]

Various formulations and routes of administration of TXA have been used, including oral tablets, topical applications, intradermal injections, microneedling, and iontophoresis, all of which have shown promising effectiveness in melasma treatment.[130]

Although oral TXA is considered more effective than the topical route, it is important to take a thorough medical history for thromboembolism, cardiovascular events, and menstrual issues before starting treatment with oral TXA. Additionally, coagulation profiles in all patients and complete baseline investigations for high-risk patients should be performed.[130,131]

TXA is not recommended in patients with renal impairment, tumors, a personal or family history of thromboembolic or cardiovascular conditions, as well as during pregnancy or when the patient is taking anticoagulants.[132]

Patients taking oral tranexamic acid have reported significant side effects, including back pain, musculoskeletal discomfort, oligomenorrhea, hypomenorrhea,

urticaria, abdominal cramps, gastritis, nausea, heartburn, and headaches. These adverse effects were more severe compared to those associated with topical administration. [132,133]

When TXA is prescribed through the topical route the side effects were minimal and were mostly short lasting. This included pruritus, dryness, skin irritation, erythema, burning, and hyperpigmentation.[134,135]

MICRONEEDLING (MN):

Microneedling is a versatile and powerful therapy for various dermatological concerns, providing a minimally invasive approach that utilizes thin, short needles to induce controlled micro-injuries in the tissue. This procedure, often performed using stamping devices, needle rollers, or electric pens.[136,137]

The effectiveness of microneedling is supported by several mechanisms:

1. Enhanced delivery of topicals through micro-punctures created by the microneedles.
2. Skin wounding, that triggers the healing process, promoting fibroblast proliferation, leading to neocollagenesis, neoelastogenesis, and thickening of the epidermis.
3. Facilitated transcutaneous removal of melanin.[6]

The increased effectiveness of topical treatments with microneedling has been demonstrated in studies, regardless of needle length. The stratum corneum is quite thick (about 0.01–0.02 mm) and resistant, acting as a key barrier against the absorption of topical agents. Microneedles with sufficient depth enhance the permeability of this layer, enabling better absorption of topical therapies.[6]

It was observed that most researchers favored the use of 1.5 mm microneedles for treating melasma, with significant symptom improvement noted within 8–12 weeks. This is likely because 1.5 mm microneedles penetrate the skin to an optimal depth, effectively activating the dermal repair process while minimizing excessive skin damage, which is essential for achieving the best therapeutic outcomes.[137]

Various studies have assessed the therapeutic value of microneedling combined with TXA compared to other treatments for melasma. Three studies evaluating MN with topical TXA to microinjections of intradermal TXA found that microinjections were more effective. Microneedling, however, resulted in greater patient satisfaction and better tolerance, as it caused less skin damage, minimized discomfort, and shortened the duration of treatment sessions.[138–140]

Studies comparing MN combined with TXA to MN alone showed that the combination group experienced significantly better results. This suggests that TXA enhances the effectiveness of microneedling, particularly for treating pigmentation issues like melasma.[141–143]

Additionally, various studies comparing MN with TXA to MN with ascorbic acid revealed that either combination was similarly effective in reducing melasma severity. However, the patients in the TXA group reported considerably higher improvement scores.[144,145]

Two trials comparing microneedling with 4% TXA to 4% HQ found no statistical difference in efficacy for melasma treatment. Although patients in HQ were more satisfied, this benefit was minimal and non-significant.[146,147]

Lastly, two studies comparing microneedling, fractional carbon dioxide, and Q-switched Nd:YAG laser for delivering TXA concluded that all three methods were equally effective and had a similar side effect profile in treating facial melasma.[148,149]

The predominant adverse effects associated with microneedling were erythema, slight discomfort, localized irritation at the treated area, PIH, and transient sensations of burning and itching.[145]

Azelaic acid has only been used in one study by Kusumawardani et al. with microneedling as Liposomal serum with combination of 4-n-butylresorcinol, AZA, and retinol. The modified MASI improved in all three patients with scores of 33.3, 41.7, 85% respectively. Safety profile was not evaluated.[150]

MATERIALS AND METHODS

SOURCE OF DATA: The study was conducted in the Department of Dermatology, Venereology, and Leprosy.

STUDY DESIGN: Open label, non-randomized, split-face interventional study.

STUDY DURATION: The study was conducted between 1st April 2023 to 31st March 2024.

ETHICAL CLEARANCE: Approval from the institute's Ethical Committee was secured before the study began.

CTRI REGISTRATION: The study was subsequently registered with the Clinical Trials Registry - India, and a CTRI number (CTRI/2023/11/059632) was obtained.

SAMPLE SIZE: The sample size was determined based on the following formula:

$$n = (Z_{1-\alpha/2} + Z_{1-\beta})^2 (SD_1^2 + SD_2^2) / (\bar{x}_1 - \bar{x}_2)^2$$

where n is the sample size required, $Z_{1-\alpha/2}$ value is 1.96 for 95% confidence interval and $Z_{1-\beta}$ value is 2.36 at 99% confidence interval.

The sample size came out to be 34. Considering 20% attrition, the sample size required was 41.

SAMPLING TECHNIQUE: Purposive sampling was done.

INCLUSION CRITERIA:

- Patients aged between 18 and 60 years with bilaterally symmetrical melasma.
- Individuals with Fitzpatrick skin phototypes: III-V.
- Patients who have not used any topical or oral treatments for melasma, except for topical sunscreen, in the past month.

EXCLUSION CRITERIA:

- Patients using oral contraceptive pills or undergoing hormone replacement therapy.
- Patients on anticonvulsants, antidepressants, thyroid medications, or anticoagulants.
- Pregnant or lactating women.
- Patients with any known systemic illness or endocrine disorders.
- Patients with bleeding disorder history.
- Patients who are ELISA positive for HIV or HBsAg positive.
- Patients with a drug allergy to Tranexamic acid or Azelaic acid.
- Patients with a keloidal tendency.
- Patients with any active facial infection.

STUDY PROTOCOL AND DATA COLLECTION PROCEDURE:

PRE-PROCEDURE:

A comprehensive patient history was taken, followed by a thorough clinical examination before initiating treatment.

The patient was then informed about the treatment to be given and possible side effects that might occur.

Informed written consent was taken from each patient.

Data was collected and recorded in a case proforma.

Hemi-mMASI score was calculated for each side separately at baseline (T0), and digital photographs were taken.

Melasma Quality of Life (MelasQoL) score was also calculated at baseline (T0).

PROCEDURE:

The face of the patient was cleaned before the procedure using mild baby soap and water.

Then topical anaesthetic cream (eutectic mixture of lidocaine and prilocaine) was applied under occlusion for 45-60 mins before procedure, to anaesthetize the area.

Microneedling was done on both sides of the face over the melasma lesions, using Dr. Pen Ultima A6, with needle depth being fixed to 1.5 mm.

The microneedling was carried out in four different directions (horizontally, vertically, and diagonally right and left) about four to five times. The end point was the appearance of erythema with pinpoint bleeding.

Immediately after microneedling, Azelaic acid 10% gel was applied to the right side of the face, and Tranexamic acid 5% gel was applied to the left side of the face. One hour later, a SPF 50+ sunscreen was applied to the entire face.

FOLLOW-UP:

A total of three microneedling sessions were conducted at one-month intervals (T0, T1, T2, where T0 denotes the baseline, T1 denotes one month after the baseline, and T2 denotes two months after the baseline)..

Patients were asked to apply topical SPF 50+ sunscreen over their whole face daily, twice during the day. Azelaic acid 10% gel was applied on the right side of the face and Tranexamic acid 5% gel on the left side of the face daily, once at night, over a total period of 12 weeks.

Hemi-mMASI score for each side was again calculated every month for 3 months (T1, T2, T3).

Melasma quality of life (MelasQoL) score was again calculated at the end of 3 months (T3).

The safety of the treatment was assessed in terms of the immediate and delayed side effects associated with the treatment.

DATA PROCESSING AND STATISTICAL ANALYSIS:

- Data was analyzed using Microsoft Excel and statistical software Epi Info™.
- Categorical variables were represented by frequencies and percentages.
- Continuous variables were represented by Mean ± Standard Deviation (SD).
- The Chi-Square test was used to check the association between categorical variables.
- Normality of variable was checked by Shapiro Wilk test and QQ plot. If the data was normal, then parametric test was used. Otherwise, a non-parametric test was used.
- Two sample t-test/Mann Whitney U test were used to compare means/distributions of variables between the groups (Right and Left sides of face).
- Paired t test/Wilcoxon test was used to compare means/distributions of variables over timepoints.
- P-value less than or equal to 0.05 indicates statistical significance.

RESULTS

In the current study a total of 41 patients were recruited, out of which 7 were lost to follow-up, and the remaining 34 patients completed the 12-week-long study. The mean age of the patients who participated in the study was 37.92 years, with a standard deviation of 7.83. The maximum age being 52 years and the minimum age being 23 years. The average age of the onset of melasma was 32.99 years. The mean duration of melasma was 4.94 years.

A. DEMOGRAPHIC DATA

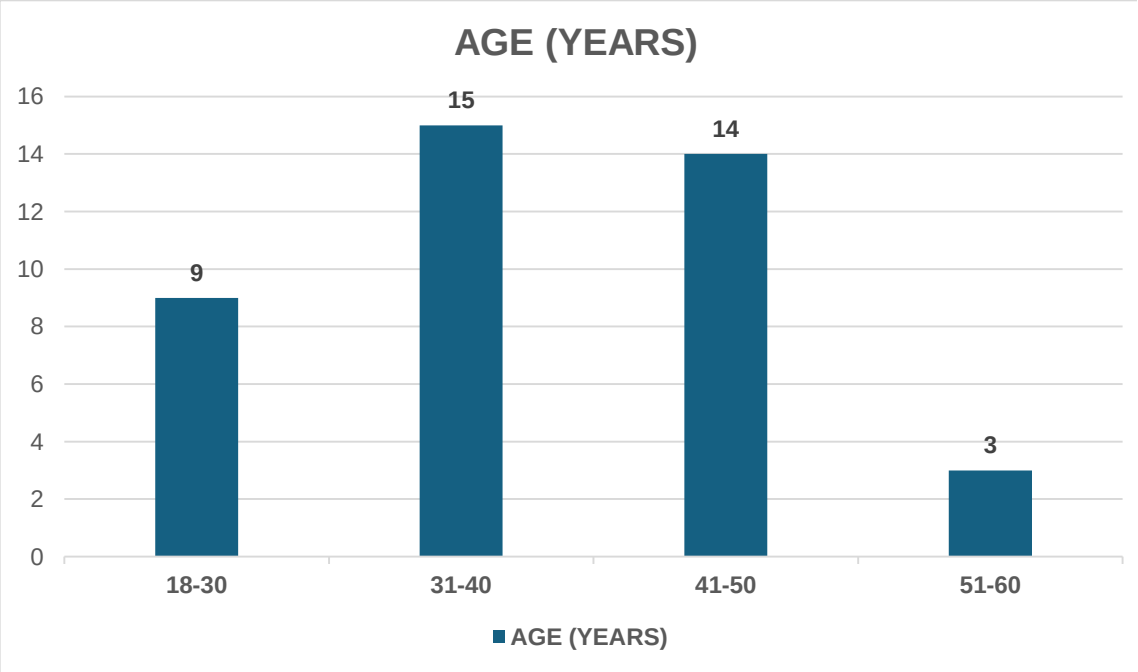
1. Age Distribution:

Out of the total of 41 patients, 9 (22%) patients were less than or equal to 30 years of age, 15 (36.5 %) patients were between 31 to 40 years of age, 14 (34.1%) patients were between 41 to 50 years and only 3 (7.3%) patients were aged above 50 years.

Table 1: Age Distribution.

Age groups (Years)	Number of patients	Percentage (%)
18-30	9	22
31-40	15	36.6
41-50	14	34.1
51-60	3	7.3
Total	41	100

Figure 1: Age Distribution.



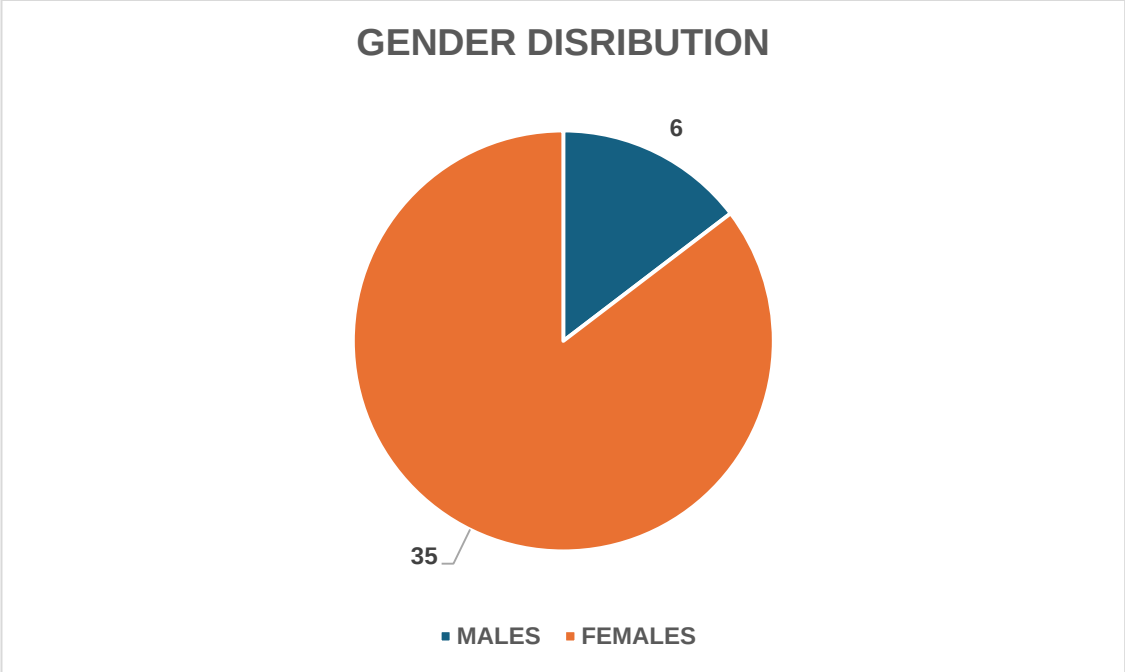
2. Gender Distribution:

Out of a total of 41 patients, the majority, i.e. 35 (85.4%) of the patients were females and only 6 (14.6%) of the patients were males, showing a clear female preponderance.

Table 2: Gender Distribution

Gender	Number of patients	Percentage (%)
Males	6	14.6
Females	35	85.4
Total	41	100

Figure 2: Gender Distribution.



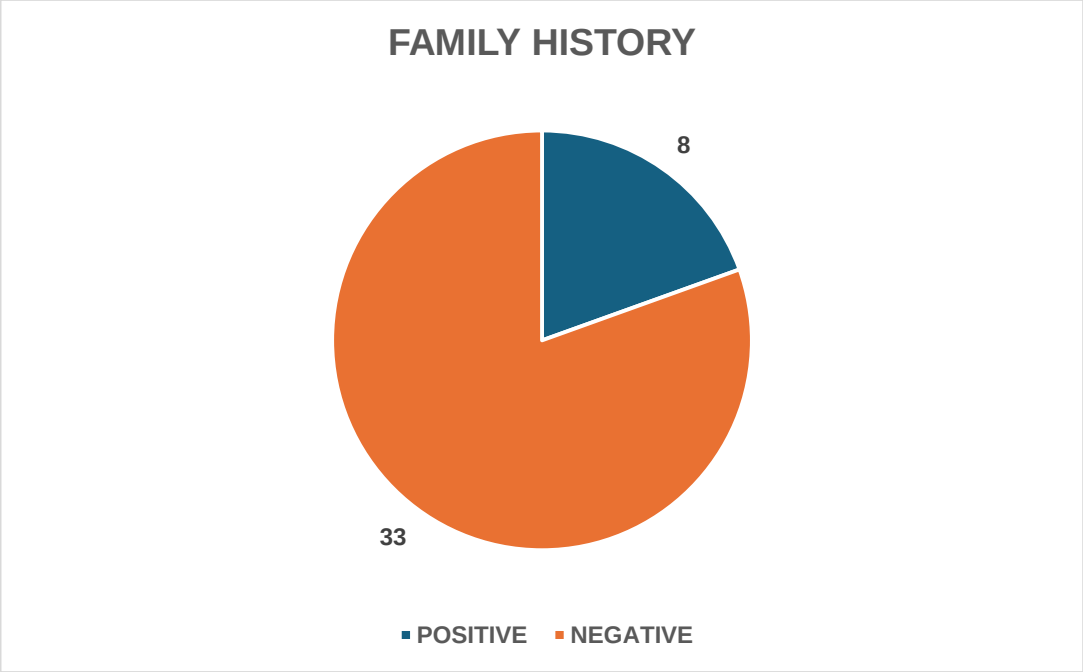
3. Distribution on the basis of family history:

A total of 8 (19.5%) patients gave a positive family history, whereas the rest of the 33 (80.5%) patients had no history of melasma in the family.

Table 3: Distribution on the basis of family history.

Family History	Number of patients	Percentage (%)
Positive.	8	19.5
Negative.	33	80.5
Total.	41	100

Figure 3: Distribution on the basis of family history.



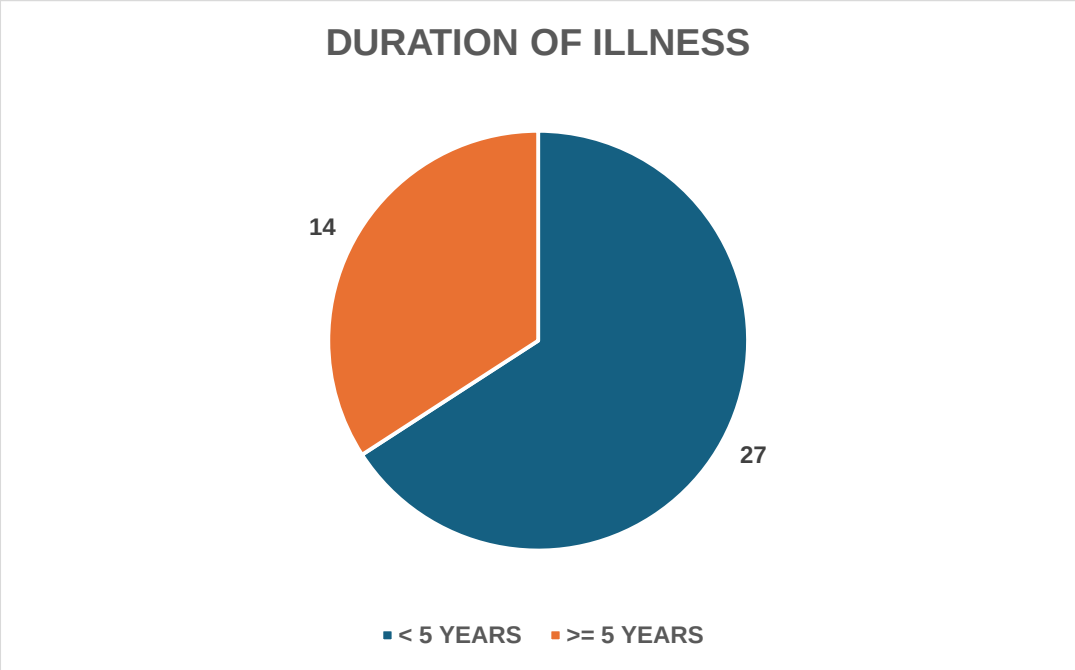
4. Distribution on the basis of duration of illness:

Around 27 (65.9%) of the patients have a total duration of melasma for less than 5 years, whereas 14 (34.1%) patients have been suffering from melasma for more than or equal to 5 years.

Table 4: Distribution on the basis of duration of illness.

Duration of illness	Number of patients	Percentage (%)
< 5 yrs	27	65.9
>= 5 yrs	14	34.1
Total.	41	100

Figure 4: Distribution on the basis of duration of illness.



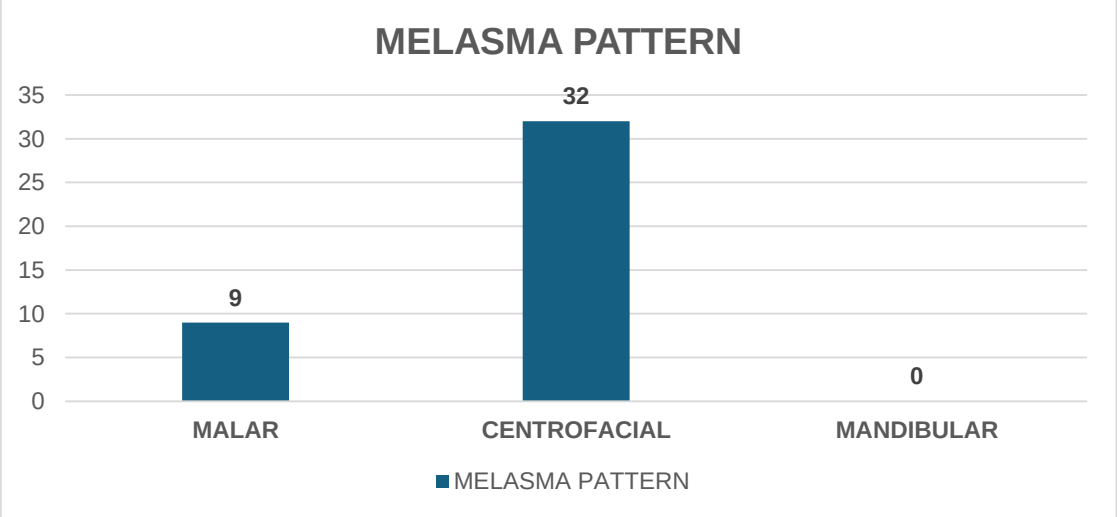
5. Distribution on the basis of pattern of melasma:

The most common pattern of melasma seen in the study was that of centrofacial type seen in 32 (78%) patients, followed by malar type seen in 9 (22%) patients, and there were no patients suffering from mandibular type of melasma.

Table 5: Distribution on the basis of pattern of melasma.

Pattern of melasma	Number of patients	Percentage (%)
Malar	9	22
Centrofacial	32	78
Mandibular	0	0
Total	41	100

Figure 5: Distribution on the basis of pattern of melasma.



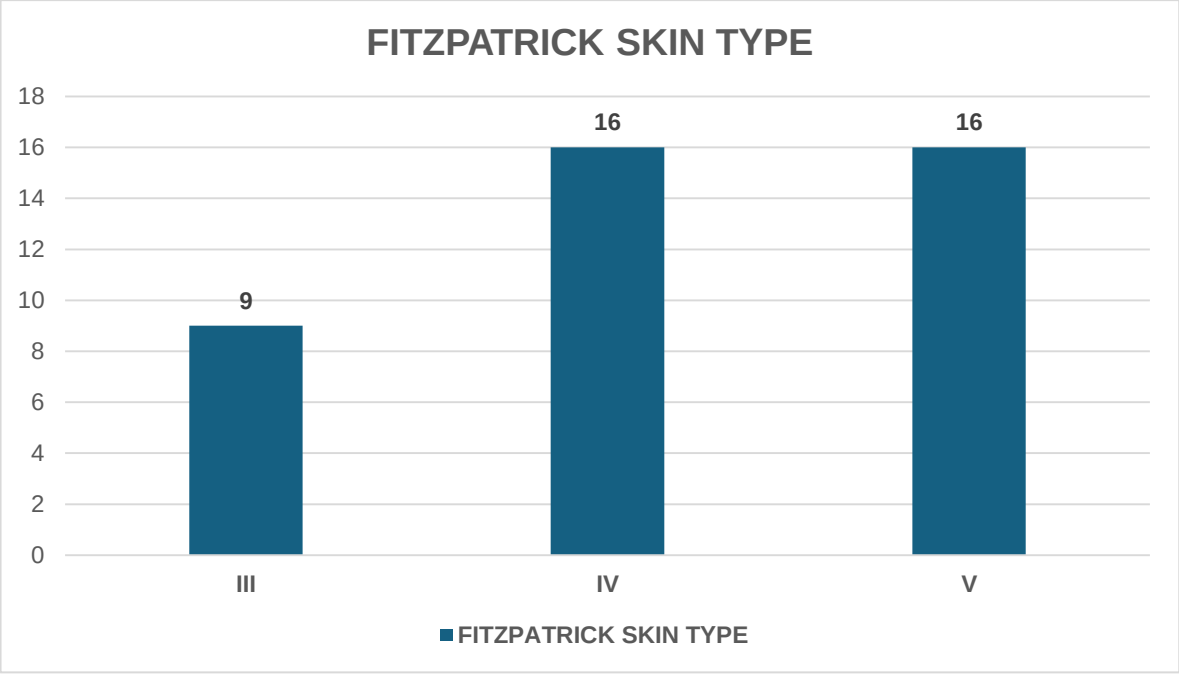
6. Distribution on the basis of Fitzpatrick skin type:

9 (22%) patients in the study had Fitzpatrick skin type III, while 16 (39%) had type IV and another 16 (39%) had type V.

Table 6: Distribution on the basis of Fitzpatrick skin type.

Fitzpatrick skin type	Number of patients	Percentage (%)
III	9	22
IV	16	39
V	16	39
Total	41	100

Figure 6: Distribution on the basis of Fitzpatrick skin type.



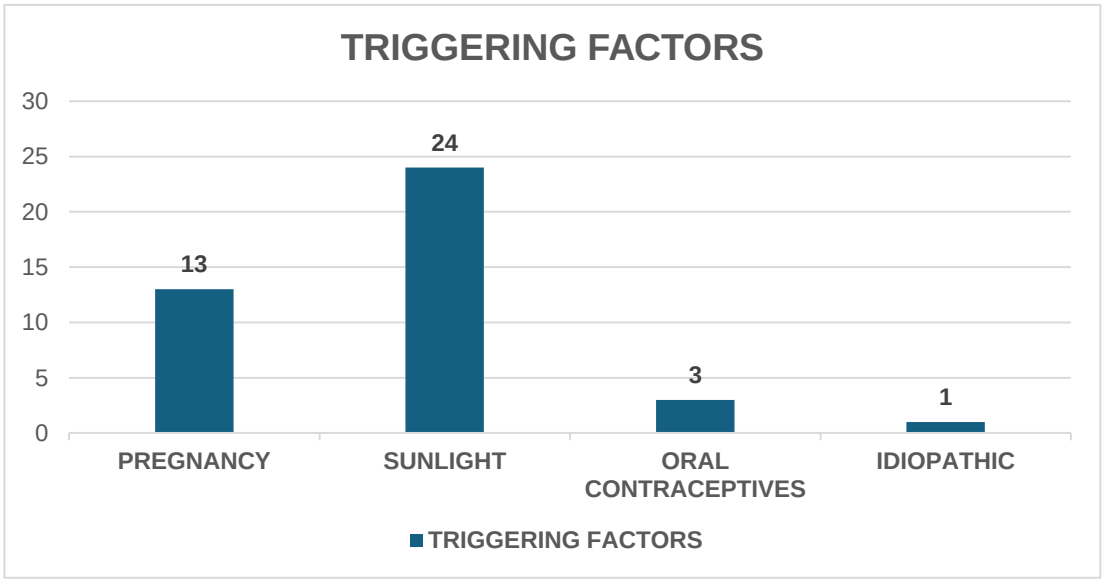
7. Distribution on the basis of triggering factors:

The history of onset during or after the pregnancy was given by 13 (31.8%) of the patients, while 24 (58.5%) of the patients had onset or worsening after prolonged exposure to sunlight, 3 (7.3%) patients had onset after they started taking oral contraceptives, and in 1 (2.4%) patient it was idiopathic i.e. no specific triggering factor was found.

Table 7: Distribution on the basis of triggering factors.

Triggering factors	Number of patients	Percentage (%)
Pregnancy	13	31.8
Sunlight	24	58.5
Oral contraceptives	3	7.3
Idiopathic	1	2.4
Total	41	100

Figure 7: Distribution on the basis of triggering factors



8. Distribution on the basis of duration of sun exposure:

16 (39%) patients had sun exposure for up to one hour, 13 (31.7%) for up to two hours, 5 (12.2%) for up to three hours, 3(7.3%) for up to four hours, while 2(4.9%) patients were exposed for five hours and another 2(4.9%) for six hours.

Table 8: Distribution on the basis of duration of sun exposure.

Duration of sun exposure	Number of patients	Percentage (%)
1 Hour	16	39
2 Hours	13	31.7
3 Hours	5	12.2
4 Hours	3	7.3
5 Hours	2	4.9
6 Hours	2	4.9

B. CATEGORICAL DATA ANALYSIS

1. Distribution of triggering factors across Fitzpatrick skin types.(III-V):

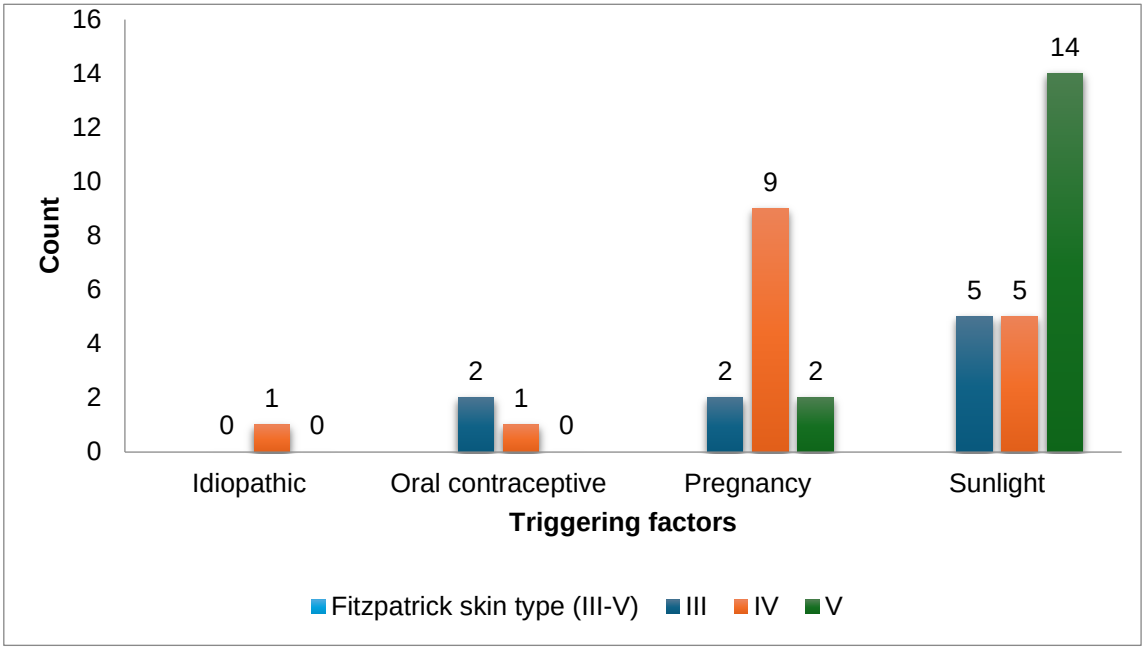
The distribution of triggering factors among Fitzpatrick types III, IV, and V was analyzed, revealing significant differences in the prevalence of these factors (p = 0.020). Idiopathic causes were rare, with only one case reported in skin type IV. The use of oral contraceptives as a trigger was observed in two cases for type III and one in type IV, while no cases were noted for skin type V. Pregnancy was a more common factor, particularly in skin type IV, with nine cases, compared to two cases each in types III and V. Sunlight exposure was the most frequently reported trigger, particularly among individuals with Fitzpatrick skin type V (14 cases), followed by equal distribution in types III and IV (five cases each). The significant p-value indicates a statistically meaningful difference in triggering factor distribution across skin types, suggesting that sunlight exposure might be a predominant factor, particularly in darker skin tones.

Table 9: Distribution of triggering factors across Fitzpatrick skin types (III-V).

		Fitzpatrick type (III-V)			P value
		III	IV.	V	
Triggering factors	Idiopathic	0	1	0	0.020*
	Oral contraceptive	2	1	0	
	Pregnancy	2	9	2	
	Sunlight	5	5	14	

*Significant
Chi Square Test

Figure 8: Distribution of triggering factors across Fitzpatrick skin types (III-V).



2. Distribution of Fitzpatrick skin types (III-V) according to duration of sun exposure:

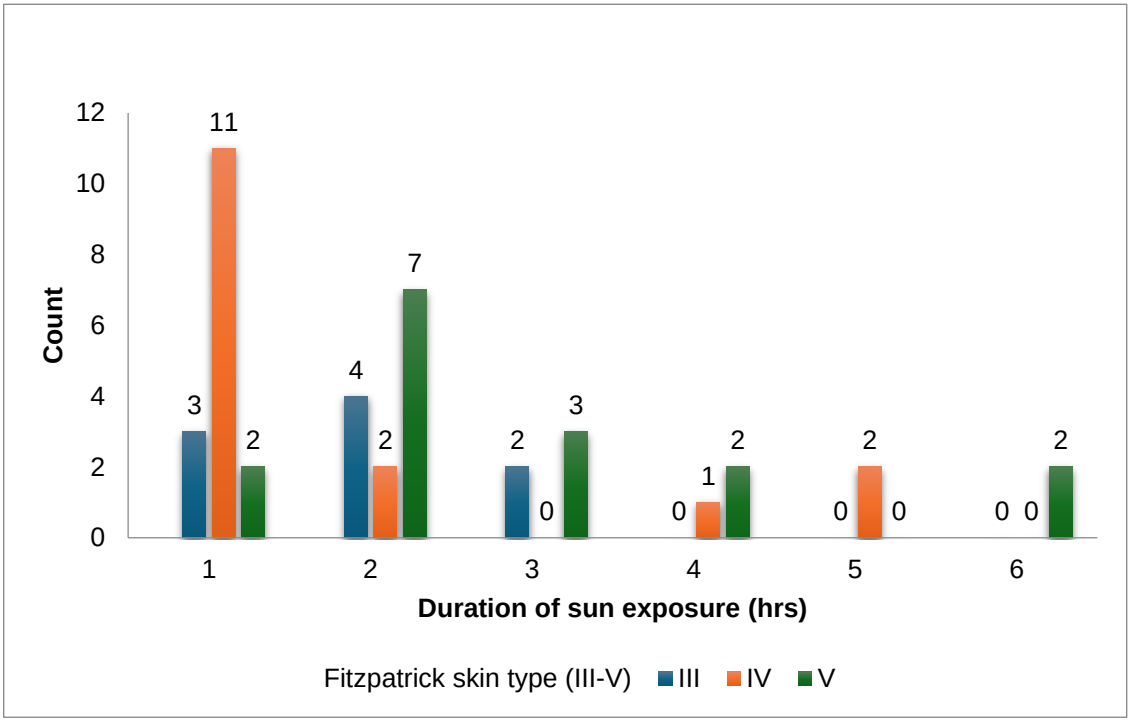
The analysis examines the relation among Fitzpatrick types (III-V) and sun exposure duration, with a Chi-Square Test yielding a statistically significant P-value (P = 0.026), indicating a non-random distribution of sun exposure across skin types. Skin Type IV has the highest proportion of individuals with only 1 hour of sun exposure (11 individuals), while Skin Type V shows a higher proportion of individuals exposed for 2 hours (7 individuals). Skin Type III is more evenly distributed across shorter durations (1–3 hours) but lacks participants with prolonged exposure beyond 3 hours. Longer sun exposure (4+ hours) is relatively uncommon, with only a few individuals reporting extended periods outdoors. The significant P-value suggests that skin type influences sun exposure behaviors, with lighter-skinned individuals (III and IV) limiting their exposure due to a higher sensitivity to UV radiation, whereas darker-skinned individuals (V) tend to have longer exposure times.

Table 10: Distribution of Fitzpatrick types (III-V) according to duration of sun-exposure.

		Fitzpatrick type (III-V)			P value
		III	IV	V	
Duration of sun exposure (hrs)	1 hour	3	11	2	0.026*
	2 hours	4	2	7	
	3 hours	2	0	3	
	4 hours	0	1	2	
	5 hours	0	2	0	
	6 hours	0	0	2	

*Significant
Chi Square Test

Figure 9: Distribution of Fitzpatrick skin types (III-V) according to duration of sun exposure.



3. Distribution of triggering factors according to duration of sun exposure:

This analysis examines the relationship between triggering factors (Idiopathic, Oral Contraceptive Use, Pregnancy, and Sunlight) and sun exposure duration, with a Chi-Square Test yielding a statistically significant P-value (P = 0.001), indicating a strong association among these factors and the duration of sun exposure. The data shows that sunlight is the predominant triggering factor among individuals with prolonged sun exposure, as all participants exposed for 3 or more hours had sunlight as the primary trigger. In contrast, Idiopathic, Oral Contraceptive, and Pregnancy-related triggers were associated only with shorter sun exposure (1–2 hours). The significant P-value suggests that sun exposure duration is not randomly distributed among different triggering factors, with sunlight being the key factor in prolonged exposure cases. This implies that individuals whose condition is triggered by sunlight are more likely to have extended sun exposure compared to those with other triggers, reinforcing the effect of UV exposure in disease onset or aggravation.

Table 11: Distribution of triggering factors according to duration of sun exposure.

		Triggering factors				P value
		Idiopathic	Oral contraceptive	Pregnancy	Sunlight	
Duration of sun exposure (hrs)	1 hour	1	2	13	0	0.001*
	2 hours	0	1	0	12	
	3 hours	0	0	0	5	
	4 hours	0	0	0	3	
	5 hours	0	0	0	2	
	6 hours	0	0	0	2	

*Significant

Chi Square Test

4. Distribution of triggering factors on the basis of family history:

This analysis examines the association between family history (positive or negative) and triggering factors (Idiopathic, Oral Contraceptive Use, Pregnancy, and Sunlight), with a Chi-Square Test yielding a P-value of 0.071, indicating that there is no significant association among family history and triggering factors.

The data shows that all cases of idiopathic, oral contraceptive, and pregnancy-related triggers occurred in patients with a negative history of similar complaints in the family, while sunlight was the most common trigger in both groups, affecting 8 patients with a positive family history and 16 with a negative family history. Although sunlight appears more frequently as a trigger in both groups, the lack of statistical significance suggests that family history does not play a decisive role in determining which triggering factor is more likely to be present in an individual.

Table 12: Distribution of triggering factors on the basis of family history.

		Family history		P value
		Positive	Negative	
Triggering factors	Idiopathic	0	1	0.071
	Oral contraceptive	0	3	
	Pregnancy	0	13	
	Sunlight	8	16	

Chi Square Test

5. Distribution of sex (Male vs. Female) based on family history:

A Chi-square Test used to analyze the relationship among sex and family history yielded a P-value = 1, depicting that there is no association. The data shows that females make up the majority of cases in both family history categories (7 with positive history, 28 with negative history), while males are much fewer (1 with positive history, 5 with negative history). However, since the proportions are consistent across groups, family history does not appear to influence whether a person is male or female in this dataset.

Table 13: Distribution of sex (Male vs. Female) based on family history.

		Family history		P value
		Positive	Negative	
SEX	M	1	5	1
	F	7	28	

Chi Square Test

6. Distribution of sex (Males vs. Female) based on duration of sun exposure:

An independent t-test performed to evaluate the variation in the mean duration of sun exposure between males and females. The results showed that males had a mean average sun exposure of 3.3 ± 1.37 hours, while females had an average exposure of 2.03 ± 1.32 hours. The test yielded a p-value = 0.036, highlighting a statistically significant difference.

Table 14: Distribution of sex (Males vs. Female) based on mean duration of sun exposure.

		Mean duration of sun exposure (Hrs)	P value
SEX	M	3.3 ± 1.37	0.036*
	F	2.03 ± 1.32	

*Significant
Independent T Test

7. Distribution by mean age of onset and family history:

Comparing the mean age of onset (± standard deviation) of individuals with positive and negative family history, resulted in a P-value of 0.830, indicating no statistically significant difference. The mean age of onset for individuals having a positive family history is 32.5 years (± 6.28), while for those with a negative family history, it is 33.1 years (± 9.28). The small difference in mean ages of onset suggests that the distribution is similar regardless of family history status.

Table 15: Distribution by mean age of onset and family history.

		Age of onset(Yrs) [Mean ± SD]	P value
Family history	Positive	32.5 ± 6.28	0.830
	Negative	33.1 ± 9.28	

Independent T Test

8. Distribution by mean age of onset by sex:

The Independent T-Test result (P = 0.059) suggests a borderline significant difference for the mean age of onset of melasma between males and females. The mean age of onset for males is 28.1 years (± 5.43), while for females, it is 33.8 years (± 8.97), showing that females in the study have higher age of onset than males.

Table 16: Distribution by mean age of onset by sex.

		AGE (Yrs) [Mean ± SD]	P value
SEX	M	28.1 ± 5.43	0.059
	F	33.8 ± 8.97	

Independent T Test

9. Distribution of mean age of onset by Fitzpatrick skin type:

The one-way ANOVA test was conducted to compare the mean age of onset of melasma among individuals with Fitzpatrick types III, IV, and V. The mean age of onset was 36.89 ± 8.2 years for type III, 30.06 ± 8.42 years for type IV, and 33.71 ± 8.43 years for type V. The results showed a P-value = 0.169, indicating that there is no significant difference in the mean age of onset among the three groups. This suggests that Fitzpatrick skin type does not have a strong effect on the age of melasma onset in this study population.

Table 17: Distribution of mean age of onset by Fitzpatrick skin type.

		AGE (Yrs) [Mean ± SD]	P value
Fitzpatrick skin type	III	36.89 ± 8.2	0.169
	IV	30.06 ± 8.42	
	V	33.71 ± 8.43	

One-way ANOVA test

10. Relationship between sun exposure duration (hours) and duration of illness:

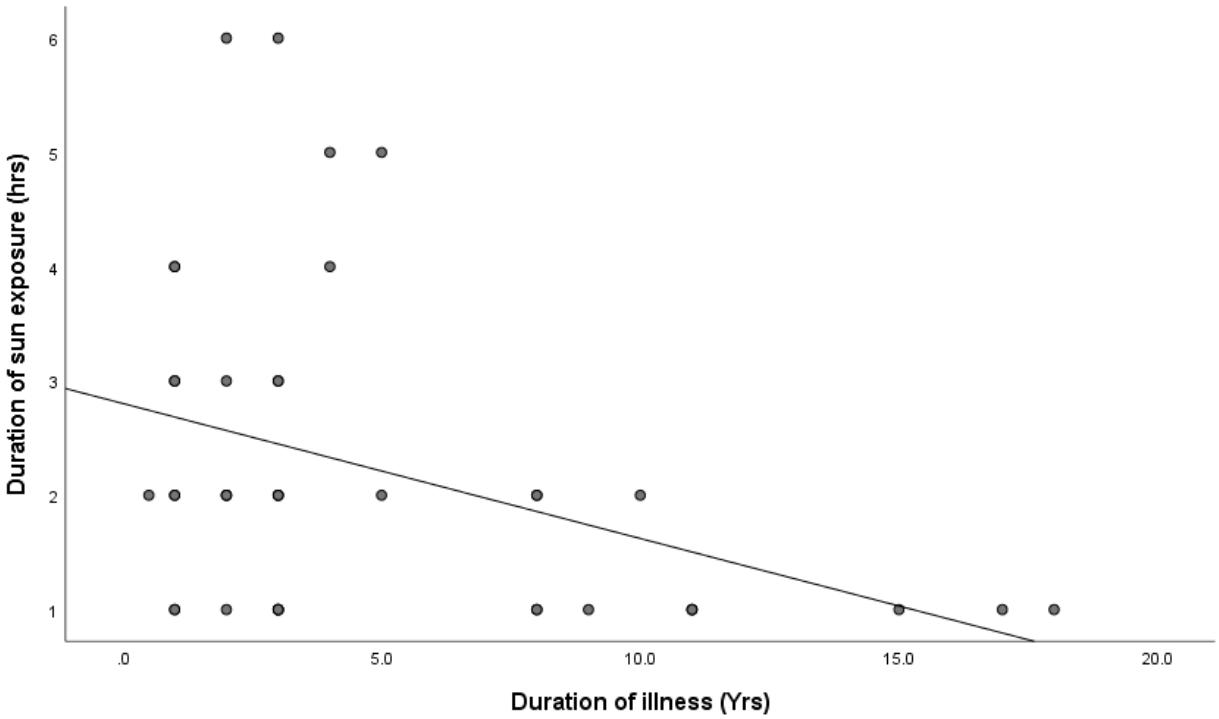
On examining the relationship between duration of illness (in years) and duration of sun exposure (in hours) using Spearman's correlation, resulted in a correlation coefficient of -0.383 and a statistically significant P-value = 0.013. The negative correlation (-0.383) suggests an inverse relationship between the two variables—individuals with higher sun exposure tend to have developed the illness more recently, while those with lower sun exposure have had the illness for a longer duration. The significant P-value (0.013) indicates that this relationship is unlikely to be due to chance. This finding implies that higher sun exposure may be associated with a faster onset of illness, meaning the disease may develop sooner in individuals exposed to more sunlight.

Table 18: Relationship between sun exposure duration (hours) and duration of illness.

		Duration of illness (Yrs)
Duration of sun exposure (hrs)	Correlation Coefficient	-0.383
	P value	0.013*

*Significant
Spearman Correlation

Figure 10: Relationship between sun exposure duration (hours) and duration of illness.



11. Comparison of illness duration by melasma pattern:

The Mann-Whitney U.Test was applied to analyze duration of illness (in years) between centrofacial and malar melasma patterns. The median illness duration for centrofacial melasma was 3 years (IQR: 2 to 8 years), while for malar melasma, it was 2 years (IQR: 1 to 4 years).

The P-value = 0.092 reflects that the difference is not statistically significant, proving that melasma pattern type does not significantly affect illness duration.

Although the centrofacial pattern appears to have a longer illness duration than the malar pattern, this result does not confirm a meaningful difference, implying that other factors may influence the disease's course.

Table 19: Comparison of illness duration by melasma pattern.

		Duration of illness (Yrs) [Median(IQR)]	P value
Melasma pattern	Centrofacial	3(2,8)	0.092
	Malar	2(1,4)	

Mann Whitney U Test

12. Comparison of illness duration by family history:

The Mann-Whitney U test applied to analyze the duration of illness (in years) based on family history of melasma. The median illness duration for individuals having positive family history was 4.5 years (IQR: 3 to 6.5 years), while for those with a negative family history, it was 3 years (IQR: 1 to 8 years). The P-value = 0.248 indicates that the difference is not significant, suggesting that having a family history of melasma does not significantly impact the duration of illness.

Table 20: Comparison of illness duration by family history.

		Duration of illness (Yrs) [Median(IQR)]	P value
Family history	Positive	4.5(3,6.5)	0.248
	Negative	3(1,8)	

Mann Whitney U Test

13. Comparison of illness duration by Fitzpatrick skin type.

The Kruskal-Wallis test was applied to analyze the duration of illness (in years) across different Fitzpatrick skin types (III-V). The median illness duration for individuals with skin type III was 3 years (IQR: 2 to 3 years), for skin type IV it was 4.5 years (IQR: 3 to 11 years), and for skin type V it was 2 years (IQR: 1 to 4 years). The P-value = 0.010 depicts that the difference in illness duration across skin types is statistically significant.

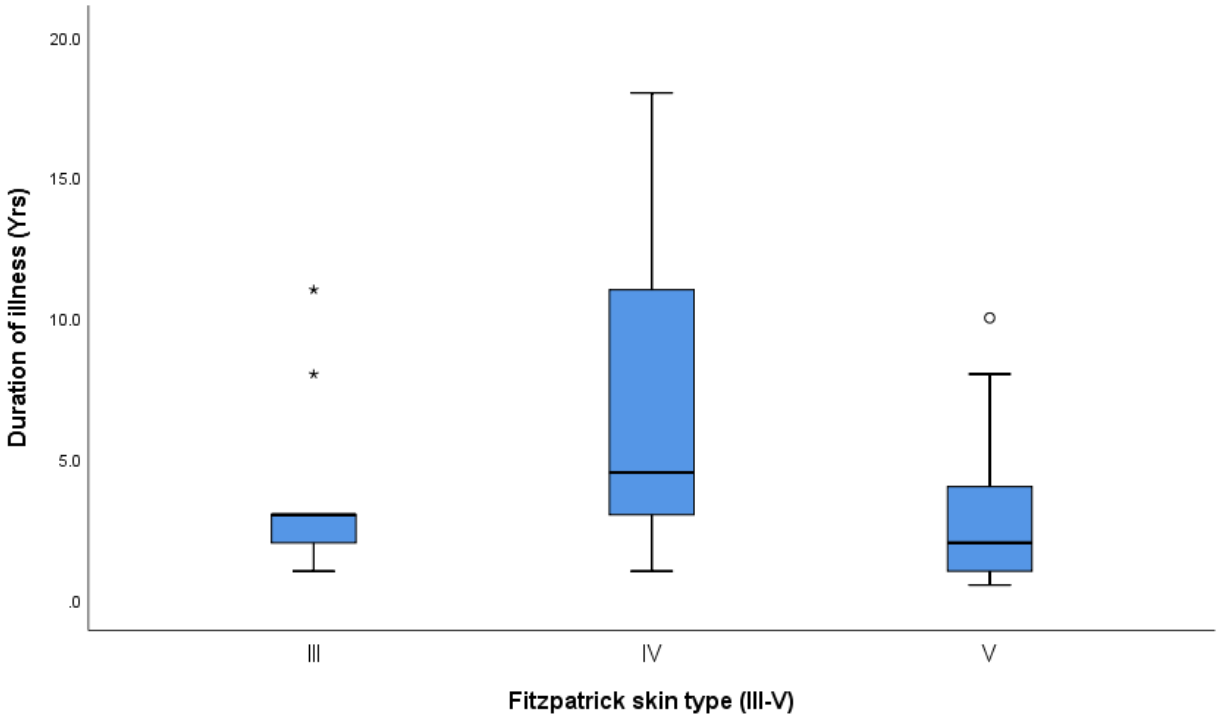
The pairwise comparison further reveals that individuals with skin type IV had a significantly longer illness duration compared to those with skin type V (IV > V, P < 0.05).

Table 21: Comparison of illness duration by Fitzpatrick skin type.

		Duration of illness (Yrs) [Median(IQR)]	P value	Pairwise Comparison
Fitzpatrick skin type (III-V)	III	3(2,3)	0.010*	IV > V *
	IV	4.5(3,11)		
	V	2(1,4)		

*Significant
Kruskal Wallis Test

Figure 11: Comparison of illness duration by Fitzpatrick skin type.



14. Distribution of melasma patterns by sex (Males vs Females):

The results of Chi-Square Test revealed a significant association among sex and melasma pattern, indicating that melasma distribution differs between males and females. Among males (n = 6), malar melasma was more common (4 cases) than centrofacial melasma (2 cases), whereas among females (n = 35), centrofacial melasma was predominant (30 cases) compared to malar melasma (5 cases). With a P-value of 0.015, this suggests that centrofacial melasma is significantly more frequent in females, while malar melasma is relatively more common in males. The findings imply a potential gender-related influence on melasma patterns.

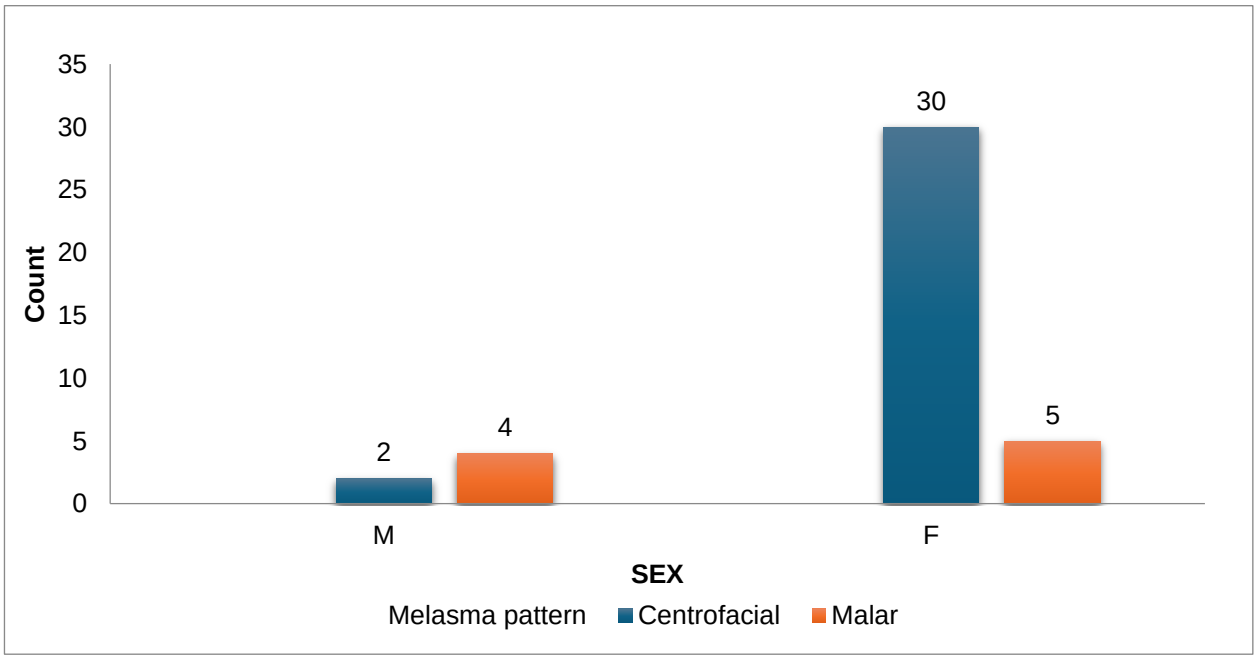
Table 22: Distribution of melasma patterns by sex (Males vs Females).

		Melasma pattern		P value
		Centrofacial	Malar	
SEX	M	2	4	0.015*
	F	30	5	

*Significant

Chi Square Test

Figure 12: Distribution of melasma patterns by sex (Males vs Females).



15. Distribution of melasma patterns by family history:

The Chi-Square test result (P-value = 1) indicates that no significant association is present between family history and the melasma pattern (centrofacial vs. malar). This means that having a positive or negative family history does not appear to influence the type of melasma a person develops. Since P-value of 1 suggests no difference between groups, family history is unlikely to be a determining factor in the distribution of melasma patterns in this dataset.

Table 23: Distribution of melasma patterns by family history.

		Melasma pattern		P value
		Centrofacial	Malar	
Family history	Positive	6	2	1
	Negative	26	7	

Chi Square Test

16. Distribution of melasma patterns by triggering factors:

The analysis resulted in a P-value = 0.214, indicating that association among triggering factors and melasma patterns (centrofacial vs. malar) is not significant. This suggests that the distribution of triggering factors—idiopathic, oral contraceptive use, pregnancy, and sunlight exposure—does not differ significantly between the two melasma patterns.

Table 24: Distribution of melasma patterns by family history.

		Melasma pattern		P value
		Centrofacial	Malar	
Triggering factors	Idiopathic	1	0	0.214
	Oral contraceptive	3	0	
	Pregnancy	12	1	
	Sunlight	16	8	

Chi Square Test

17. Distribution of melasma pattern by Fitzpatrick skin type:

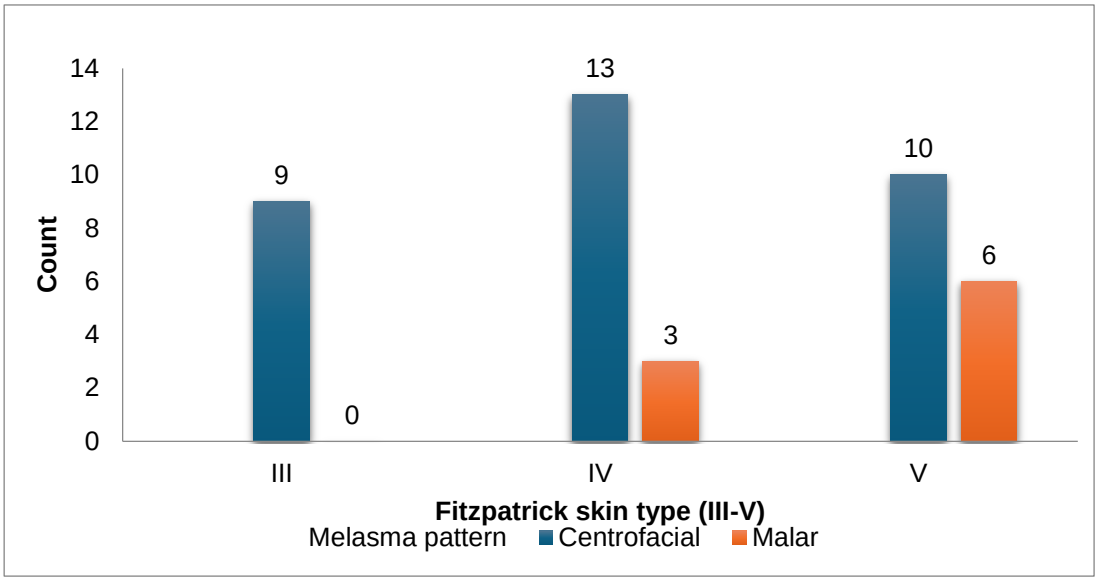
The analysis yielded a P-value = 0.087, depicting that no statistically significant association is present among Fitzpatrick skin type (III-V) and melasma pattern (centrofacial vs. malar) at the conventional significance level (typically $P < 0.05$). However, the relatively low P-value suggests a possible trend where malar melasma appears more frequently in patients with darker skin types (Fitzpatrick type V) compared to lighter skin types. While centrofacial melasma is seen across all types, it is more common in Fitzpatrick types III and IV.

Table 25: Distribution of melasma pattern by Fitzpatrick skin type.

		Melasma pattern		P value
		Centrofacial	Malar	
Fitzpatrick skin type (III-V)	III	9	0	0.087
	IV	13	3	
	V	10	6	

Chi Square Test

Figure 13: Distribution of melasma pattern by Fitzpatrick skin type.



18. Distribution of melasma pattern by duration of sun exposure:

The analysis resulted in a P-value = 0.42, depicting that there is no significant correlation among duration of sun exposure and melasma pattern (centrofacial vs. malar). This suggests that the duration of sun exposure does not significantly influence whether a person develops centrofacial or malar melasma.

Table 26: Distribution of melasma pattern by duration of sun exposure.

		Melasma pattern		P value
		Centrofacial	Malar	
Duration of sun exposure (hrs)	1	15	1	0.42
	2	9	4	
	3	4	1	
	4	2	1	
	5	1	1	
	6	1	1	

Chi Square Test

C. ANALYSIS OF TREATMENT RESPONSE

1. Comparison of mean hemi-mMASI scores between each side on each visit:

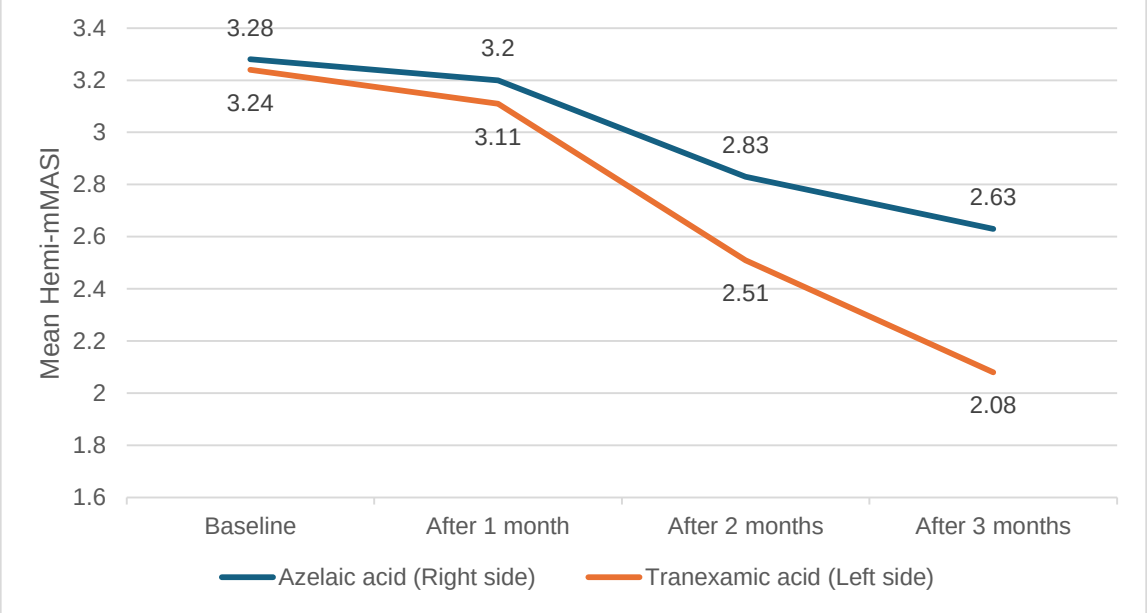
The mean hemi-mMASI was assessed for each side at baseline and each monthly visit. The mean difference was then calculated. The mean difference at baseline was 0.035 (p-value=0.921), after 1 month it was 0.093 (p-value=0.818), after 2 months it was 0.322 (p-value=0.371) and after end of 3rd month it was 0.547 (p-value=0.129). This indicated that there was gradual improvement on either sides of the face, but a notable degree of improvement was seen on the tranexamic acid side with each subsequent visit.

Table 27: Comparison of mean Hemi-mMASI scores between each side at each visit.

	Group	Mean ± SD	Mean Difference	P Value
hemi-mMASI at baseline	Azelaic acid	3.28 ± 1.6	0.035	0.921
	Tranexamic acid	3.24 ± 1.63		
hemi-mMASI after one month	Azelaic acid	3.2 ± 1.8	0.093	0.818
	Tranexamic acid	3.11 ± 1.72		
hemi-mMASI after two months	Azelaic acid	2.83 ± 1.53	0.322	0.371
	Tranexamic acid	2.51 ± 1.41		
hemi-mMASI after three months	Azelaic acid	2.63 ± 1.54	0.547	0.129
	Tranexamic acid	2.08 ± 1.39		

Independent T Test

Figure 14: Comparison of mean Hemi-mMASI scores between each side at each visit



2. Comparison of mean hemi-mMASI change from (T0) to end of 3 months (T3):

An independent t-test was done to compare hemi-mMASI scores at baseline and after three months for both the azelaic acid and tranexamic acid groups. For the azelaic acid group, the mean hemi-mMASI score decreased from 3.28 ± 1.6 at baseline to 2.63 ± 1.54 after three months, but the p-value was 0.079, indicating that the reduction was not significant. In contrast, the tranexamic acid group showed a significant reduction in the mean hemi-mMASI score from 3.24 ± 1.63 at baseline to 2.08 ± 1.39 after three months, with a p-value = 0.0016, demonstrating a significant improvement. These outcomes suggest that while both treatments led to a reduction in hemi-mMASI scores over time, tranexamic acid showed a significant effect, whereas azelaic acid did not.

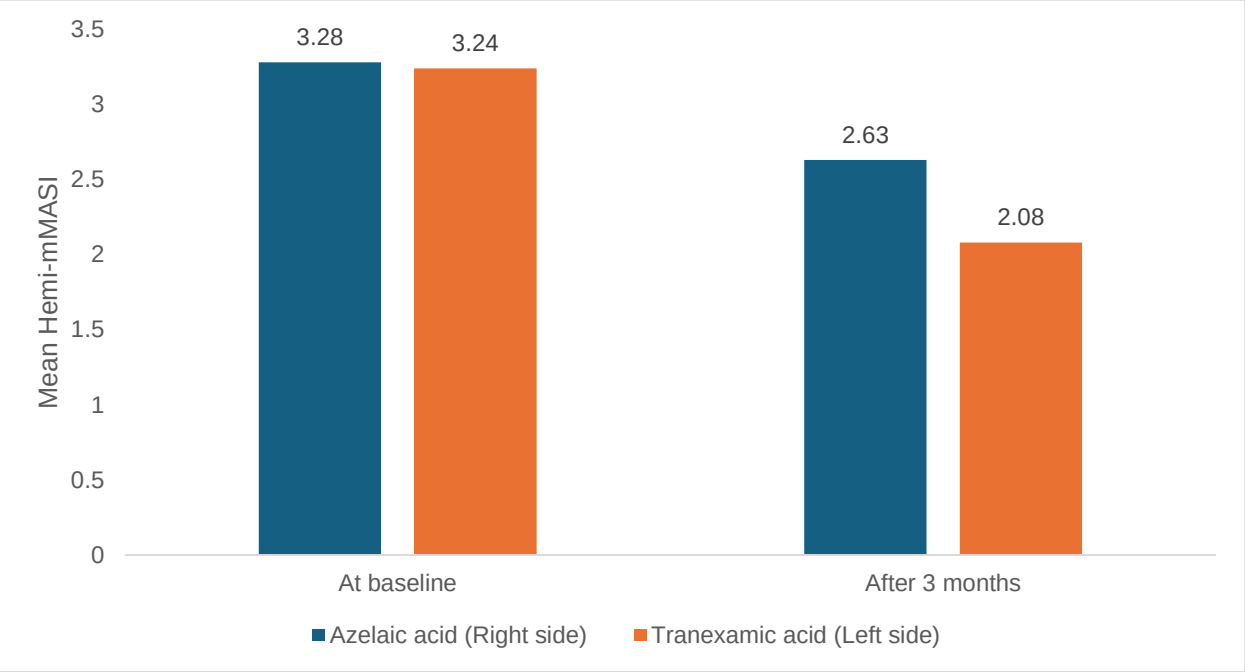
Table 28: Comparison of mean Hemi-mMASI change from (T0) to end of 3 months (T3).

	Group	Mean ± SD	P Value
hemi-mMASI at baseline	Azelaic acid	3.28 ± 1.6	0.079
hemi-mMASI after three months	Azelaic acid	2.63 ± 1.54	
hemi-mMASI at baseline	Tranexamic acid	3.24 ± 1.63	0.0016*
hemi-mMASI after three months	Tranexamic acid	2.08 ± 1.39	

Independent t-test

*Significant

Figure 15: Comparison of mean Hemi-mMASI change from (T0) to end of 3 months (T3).



3. Hemi-mMASI Score Change (Median [IQR]) from baseline (T0) to end of 3 months (T3):

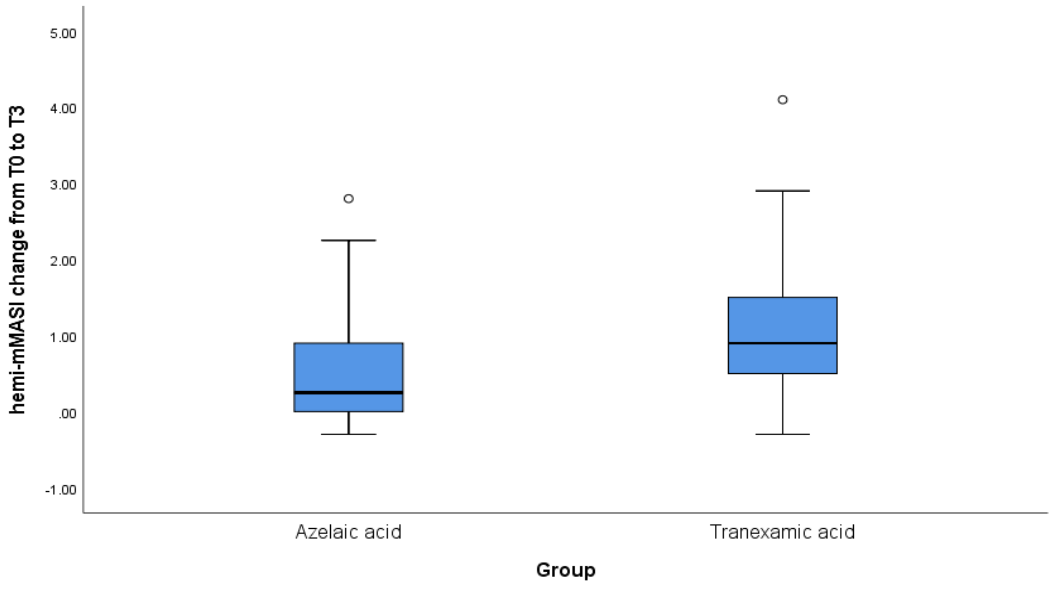
Tranexamic acid appears to be more effective in lowering hemi-mMASI scores than Azelaic acid, as indicated by a higher median reduction (0.9 vs. 0.25). The P-value = 0.009 supports that the change is statistically significant, reinforcing the treatment's effectiveness. The Interquartile Range (IQR) shows more variability in response for Azelaic acid, whereas Tranexamic acid provided a more consistent improvement.

Table 29: Comparison of hemi-mMASI score changes from T0 to T3.

Group	Hemi-mMASI change from T0 to T3 [Median(IQR)]	P Value
Azelaic acid	0.25(0,0.9)	0.009*
Tranexamic acid	0.9(0.5,1.5)	

*Significant
Mann Whitney U Test

Figure 16: Box plot comparing hemi-mMASI score changes from T0 to T3.



4. Comparison of hemi-mMASI score changes from baseline (T0) to follow-up (T3) in different melasma patterns:

The Mann-Whitney U test used to contrast the change in hemi-mMASI scores between azelaic acid and tranexamic acid for different melasma patterns. For centrofacial melasma, the median score change was 0.45 (0, 0.9) for azelaic acid and 1.05 (0.6, 1.8) for tranexamic acid, with a P-value = 0.014, indicating a significant improvement favoring tranexamic acid. In contrast, for malar melasma, the median change was 0 (0, 0.3) for azelaic acid and 0.45 (0, 0.75) for tranexamic acid, but the P-value = 0.234 suggests that this difference was not significant. These results indicate that tranexamic acid is significantly more effective than azelaic acid for centrofacial melasma, while both treatments show similar effects for malar melasma.

Table 30: Comparison of hemi-mMASI score changes from baseline (T0) to follow-up (T3) in different melasma patterns.

Melasma pattern	Group	hemi-mMASI change from T0 to T3 [Median(IQR)]	P Value
Centrofacial	Azelaic acid	0.45(0,0.9)	0.014*
	Tranexamic acid	1.05(0.6,1.8)	
Malar	Azelaic acid	0(0,0.3)	0.234
	Tranexamic acid	0.45(0,0.75)	

*Significant

Mann Whitney U Test

5. Comparison of Hemi-mMASI score changes based on duration of illness:

The analysis was done to compare hemi-mMASI score changes between AZA and TXA groups in patients with different durations of melasma. In patients with illness duration <5 years, the median score change was 0.15 (0, 0.8) for Azelaic acid and 0.9 (0.45, 1.5) for Tranexamic acid, with a P-value = 0.049, indicating a significant greater improvement with Tranexamic acid. However, in patients with melasma lasting >5 years, the median score change was 0.3 (0, 0.825) for Azelaic acid and 1 (0.55, 1.35) for Tranexamic acid, but the P-value = 0.088 suggests that this difference was not significant. These findings indicate that Tranexamic acid is significantly more effective than Azelaic acid for melasma cases of shorter duration (<5 years), but for longer-duration cases (>5 years), the response is not significantly different between the two treatments.

Table 31: Comparison of Hemi-mMASI score changes based on duration of illness.

Duration of illness (Yrs)	Group	Hemi-mMASI change from T0 to T3 [Median(IQR)]	P Value
<5	Azelaic acid	0.15(0,0.8)	0.049*
	Tranexamic acid	0.9(0.45,1.5)	
>5	Azelaic acid	0.3(0,0.825)	0.088
	Tranexamic acid	1(0.55,1.35)	

*Significant
Mann Whitney U Test

6. Comparison of Hemi-mMASI score changes based on family history of melasma:

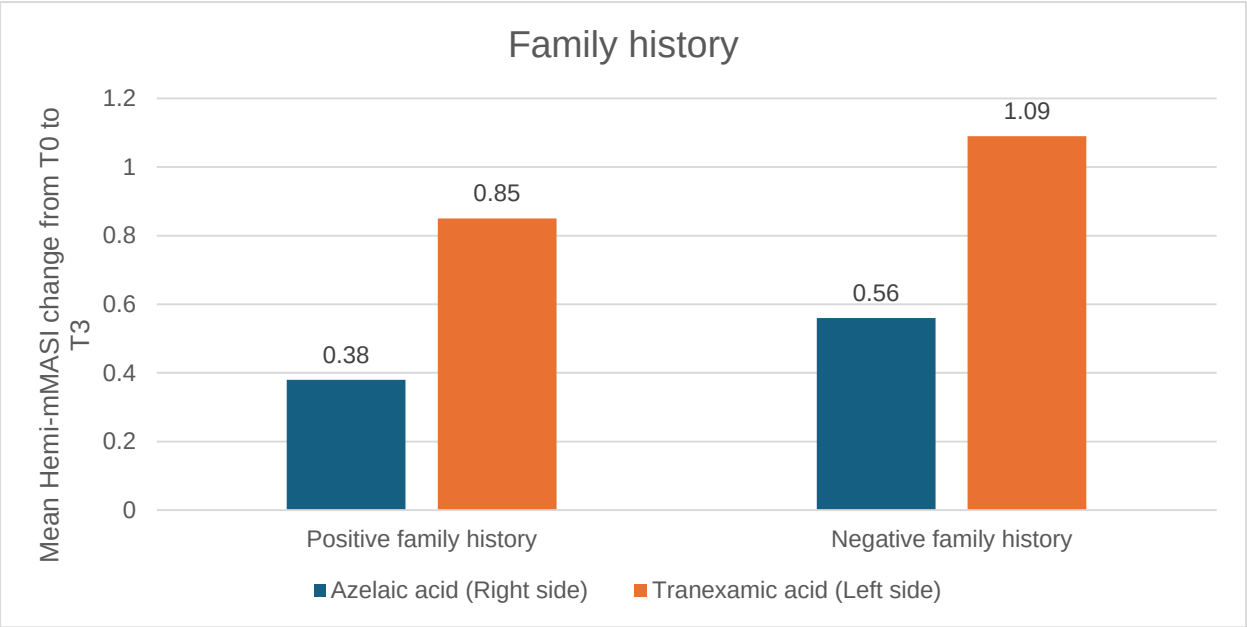
The analysis was done to compare the mean hemi-mMASI score changes between AZA and TXA groups, considering patients with and without a family history of melasma. In patients with history, the mean score change was 0.38 ± 0.43 for azelaic acid and 0.85 ± 0.55 for tranexamic acid, with a mean difference of -0.475 and a P-value = 0.075, depicting that the difference was not significant. However, in patients with no family history, the mean score change was 0.56 ± 0.75 for azelaic acid and 1.09 ± 1.04 for tranexamic acid, with a mean difference of -0.536 and a P-value = 0.038, which was significant. This suggests that tranexamic acid showed greater improvement than azelaic acid in both groups, but the difference was only statistically significant in patients without a family history of melasma. These findings imply that family history may influence treatment response, with Tranexamic acid demonstrating a stronger and significant effect in patients without a genetic predisposition.

Table 32: Comparison of Hemi-mMASI score changes based on family history of melasma.

Family history	Group	hemi-mMASI change from T0 to T3 [Mean ± SD]	Mean Difference	P Value
Positive	Azelaic acid	0.38 ± 0.43	-0.475	0.075
	Tranexamic acid	0.85 ± 0.55		
Negative	Azelaic acid	0.56 ± 0.75	-0.536	0.038*
	Tranexamic acid	1.09 ± 1.04		

*Significant
Independent T Test

Figure 17: Bar plot comparing Hemi-mMASI score changes based on family history.



7. Comparison of mean Hemi-mMASI scores on the right side (azelaic acid side) across Fitzpatrick skin types (III–V):

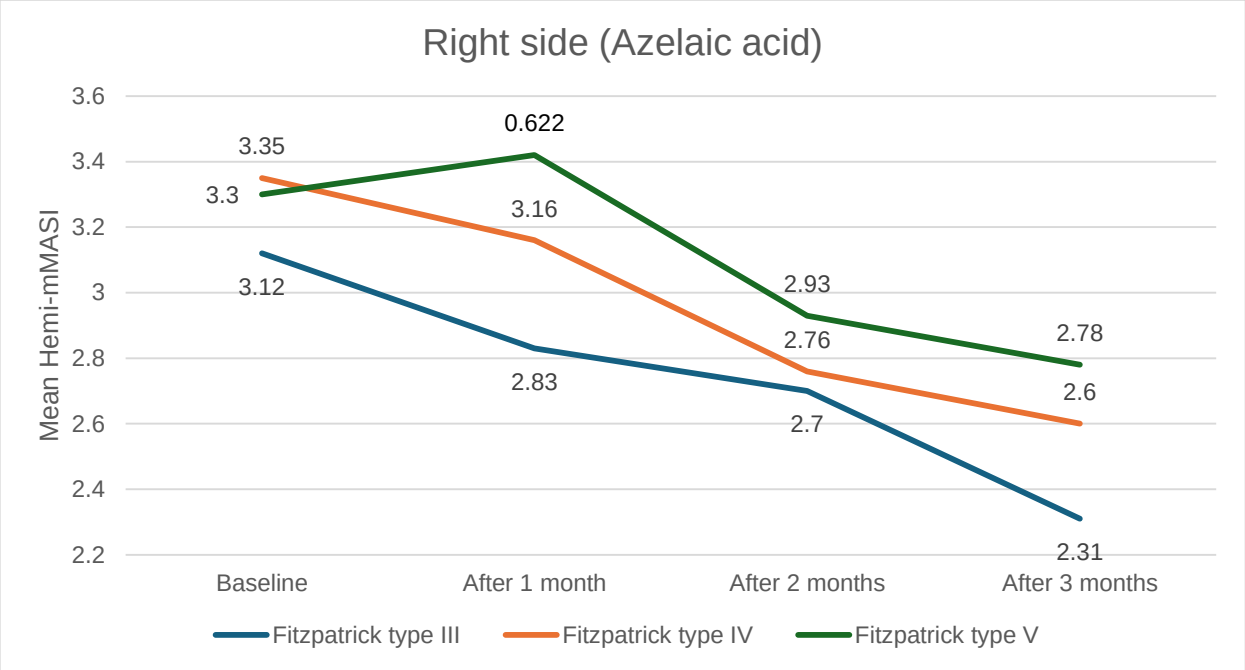
The One-Way ANOVA test was done. At baseline, the scores were similar across all three skin types ($p = 0.941$), indicating no significant difference in initial melasma severity. After one-month, slight variations were observed, but the difference remained statistically insignificant ($p = 0.759$). By two months, the scores continued to decrease, yet no significant difference was noted ($p = 0.938$). After three months, the greatest reduction was observed, particularly in Fitzpatrick Type III, but the p-value (0.805) indicated that this difference was not significant. Overall, the results suggest that azelaic acid had a comparable effect across all Fitzpatrick skin types.

Table 33: Comparison of mean Hemi-mMASI scores on the right side (azelaic acid side) across Fitzpatrick skin types (III–V).

	Fitzpatrick skin type (III-V)	Mean ± SD	P Value
hemi-mMASI at baseline	III	3.12 ± 1.65	0.941
	IV	3.35 ± 1.53	
	V	3.3 ± 1.74	
hemi-mMASI after one month	III	2.83 ± 1.72	0.759
	IV	3.16 ± 1.57	
	V	3.42 ± 2.09	
hemi-mMASI after two months	III	2.7 ± 1.52	0.938
	IV	2.76 ± 1.58	
	V	2.93 ± 1.6	
hemi-mMASI after three months	III	2.31 ± 1.4	0.805
	IV	2.6 ± 1.51	
	V	2.78 ± 1.68	

One Way ANOVA Test

Figure 18: Comparison of mean Hemi-mMASI scores on the right side (azelaic acid side) across Fitzpatrick skin types (III–V).



8. Comparison of mean Hemi-mMASI scores on the left side (tranexamic acid side) across Fitzpatrick skin types (III–V):

The One-Way ANOVA test was done. At baseline, the mean hemi-mMASI scores were 3.02 ± 1.6 for Fitzpatrick type III, 3.46 ± 1.6 for type IV, and 3.16 ± 1.76 for type V, with a p-value = 0.794, indicating no significant difference. After one month of treatment, there was a slight reduction in scores for types III (2.67 ± 1.64) and V (3.12 ± 1.87), whereas type IV remained relatively stable at 3.34 ± 1.65. The p-value (0.689) suggests that the differences between the groups were not significant.

At the two-month mark, the mean scores continued to decline, reaching 2.32 ± 1.35 for type III, 2.5 ± 1.49 for type IV, and 2.59 ± 1.47 for type V, with a p-value of 0.922, indicating a similar response across skin types.

By the third month, further improvement was observed, with the mean scores dropping to 1.61 ± 1.3 for type III, 2.22 ± 1.58 for type IV, and 2.19 ± 1.34 for type V. However, the p-value (0.622) suggests that the differences among the groups were still not statistically significant.

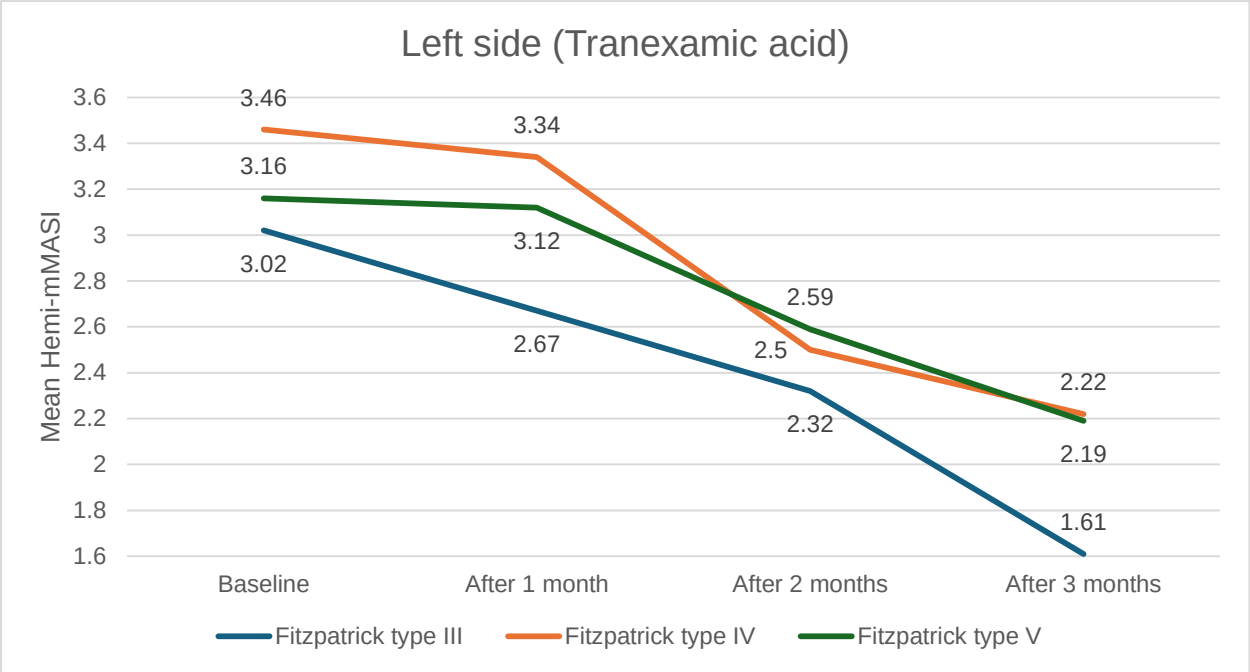
Overall, the results indicate that while all Fitzpatrick skin types showed a gradual reduction in hemi-mMASI scores over time with tranexamic acid, the response was comparable across the groups, with no difference in treatment efficacy based on type of the skin.

Table 34: Comparison of mean Hemi-mMASI scores on the Left side (Tranexamic acid Side) across Fitzpatrick skin types (III–V).

	Fitzpatrick type (III-V)	Mean ± SD	P Value
hemi-mMASI at baseline	III	3.02 ± 1.6	0.794
	IV	3.46 ± 1.6	
	V	3.16 ± 1.76	
hemi-mMASI after one month	III	2.67 ± 1.64	0.689
	IV	3.34 ± 1.65	
	V	3.12 ± 1.87	
hemi-mMASI after two months	III	2.32 ± 1.35	0.922
	IV	2.5 ± 1.49	
	V	2.59 ± 1.47	
hemi-mMASI after three months	III	1.61 ± 1.3	0.622
	IV	2.22 ± 1.58	
	V	2.19 ± 1.34	

One Way ANOVA Test

Figure 19: Comparison of mean Hemi-mMASI scores on the Left side (Tranexamic acid Side) across Fitzpatrick skin types (III–V).



D. SAFETY ANALYSIS:

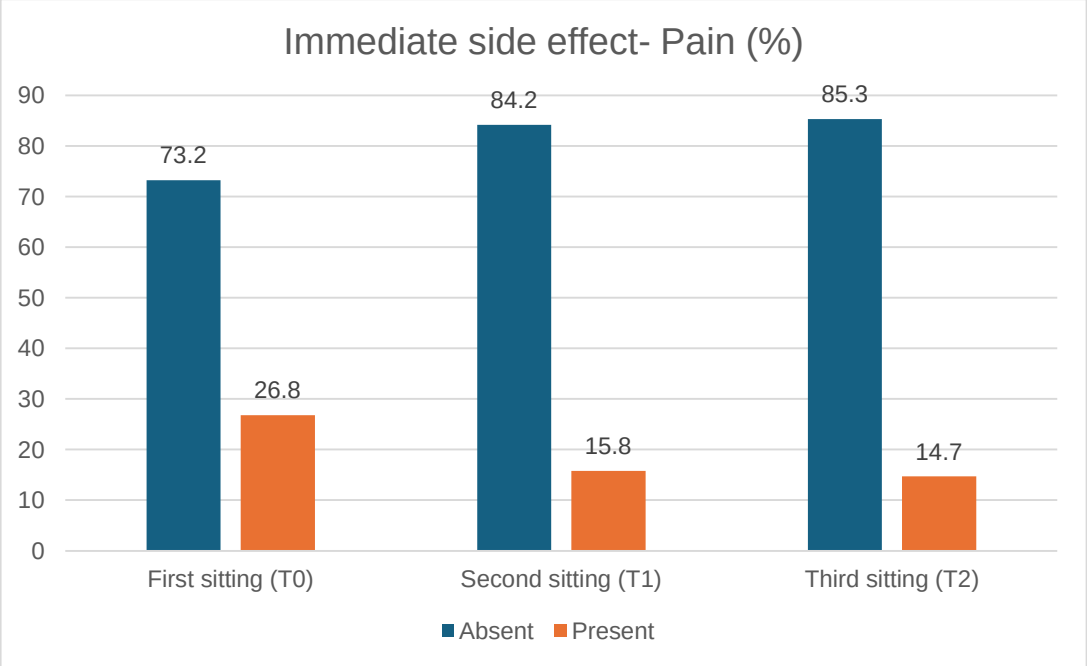
1. Immediate side effects (Pain):

The data on immediate side effects indicate that pain was most commonly reported during the first sitting (T0), where 26.8% (11 out of 41) experienced pain, while 73.2% (30 out of 41) did not. By the second sitting (T1), the proportion of pain-free participants increased to 84.2% (32 out of 38), with only 15.8% (6 out of 38) reporting pain. Similarly, at the third sitting (T2), 85.3% (29 out of 34) remained pain-free, while 14.7% (5 out of 34) still experienced pain. This trend suggests that pain was most prominent during the initial session but decreased over time, possibly indicating adaptation or increased tolerance to the treatment.

Table 35: Presence or absence of the immediate side effect—pain across treatment sittings.

	ABSENT	PRESENT
First sitting (T0)	30/41 (73.2%)	11/41 (26.8%)
Second sitting (T1)	32/38 (84.2%)	6/38 (15.8%)
Third sitting (T2)	29/34 (85.3%)	5/34 (14.7%)

Figure 20: Presence or absence of the immediate side effect—pain across treatment sittings.



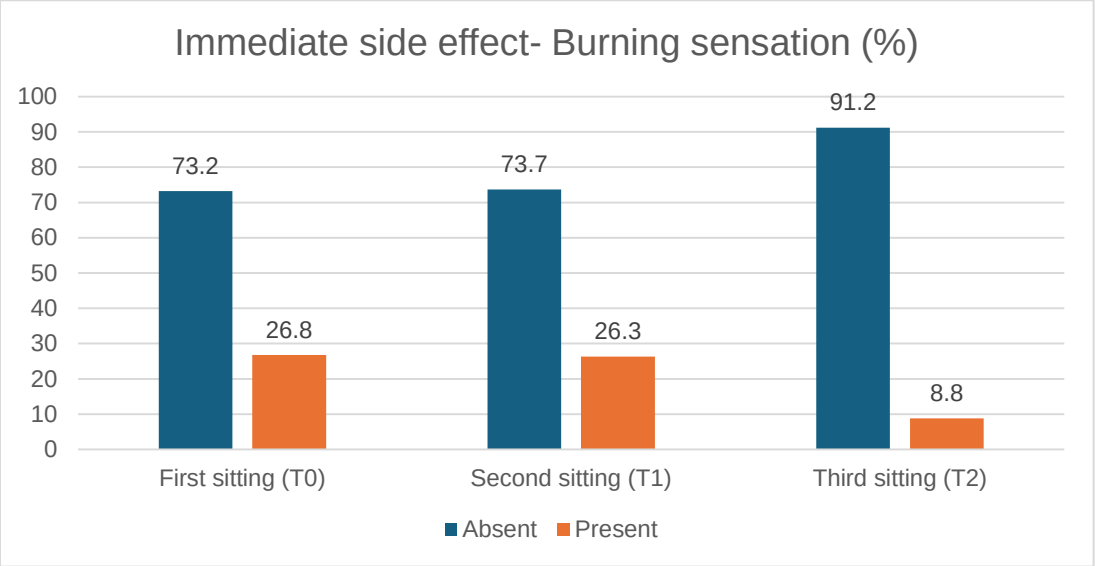
2. Immediate side effects (Burning sensation):

The data on the immediate side effect of burning sensation across treatment sittings show a varying trend. During the first sitting (T0), 73.2% (30 out of 41) of participants did not experience a burning sensation, while 26.8% (11 out of 41) reported its presence. In the second sitting (T1), the proportion of participants without a burning sensation remained similar at 73.7% (28 out of 38), with 26.3% (10 out of 38) still experiencing it. However, by the third sitting (T2), the number of participants reporting a burning sensation significantly decreased to only 8.8% (3 out of 34), while 91.2% (31 out of 34) did not experience this side effect. This trend suggests that the burning sensation was more common in the early sittings but reduced significantly over time, again possibly indicating increased tolerance or adaptation to the treatment.

Table 36: Presence or absence of the immediate side effect—burning across treatment sittings.

	ABSENT	PRESENT
First sitting (T0)	30/41 (73.2%)	11/41 (26.8%)
Second sitting (T1)	28/38 (73.7%)	10/38 (26.3%)
Third sitting (T2)	31/34 (91.2%)	3/34 (8.8%)

Figure 21: Presence or absence of the immediate side effect—burning across treatment sittings.



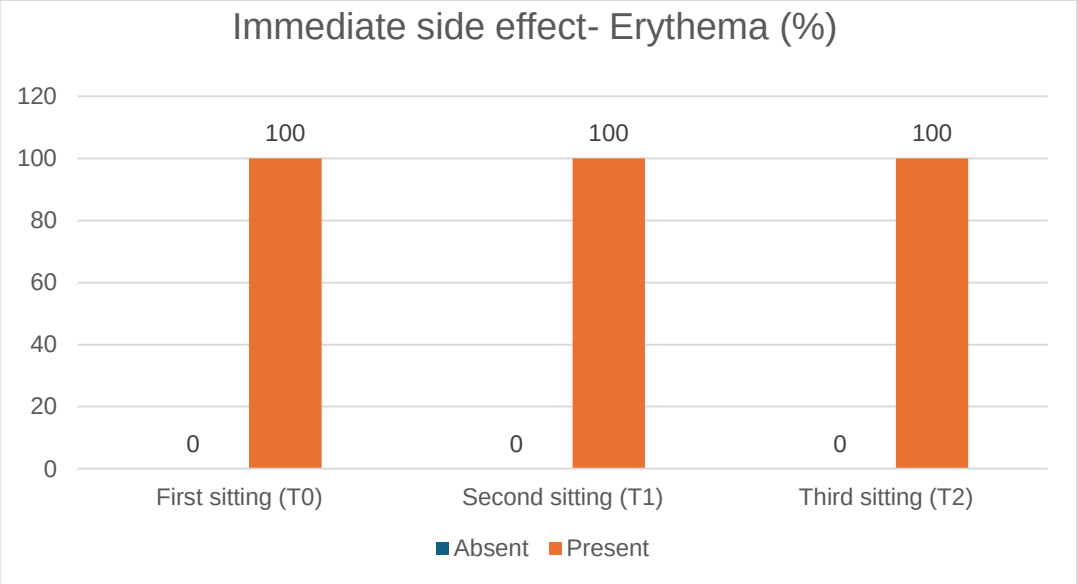
3. Immediate side effects (Erythema):

The data show that erythema was universally present across all treatment sittings. In the first (T0), second (T1), and third (T2) sittings, 100% of participants experienced erythema immediately after the procedure, with no cases of absence. However, in individuals with higher Fitzpatrick skin types, erythema was slightly more difficult to assess, but it was still present. This consistency suggests that erythema is an expected and unavoidable reaction to the treatment.

Table 37: Presence or absence of the immediate side effect—erythema across treatment sittings.

	ABSENT	PRESENT
First sitting (T0)	0/41 (0%)	41/41 (100%)
Second sitting (T1)	0/38 (0%)	38/38 (100%)
Third sitting (T2)	0/34 (0%)	34/34 (100%)

Figure 22: Presence or absence of the immediate side effect—erythema across treatment sittings.



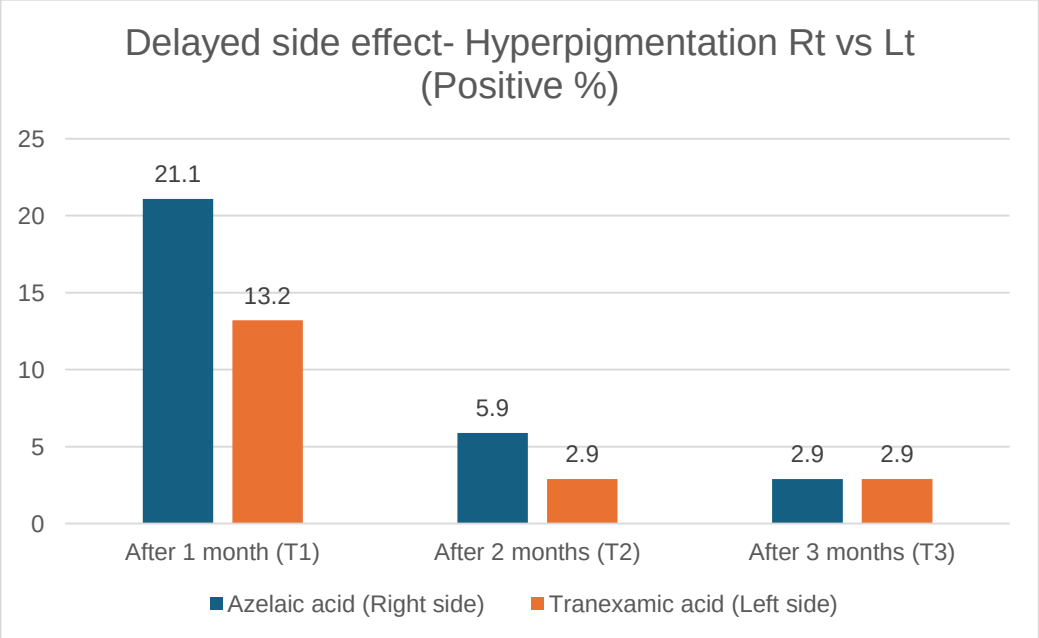
4. Delayed side effects (Hyperpigmentation) Right vs Left:

The comparison of hyperpigmentation as a delayed side effect between azelaic acid (right side) and tranexamic acid (left side) reveals a higher initial incidence on the azelaic acid side. At one month (T1), 21.1% of patients reported hyperpigmentation with azelaic acid, compared to 13.2% with tranexamic acid, and the difference among the two groups was not significant (P value=0.54). By two months (T2), the occurrence dropped significantly to 5.9% on the AZA side and 2.9% on the TXA side. At three months (T3), both groups showed a 2.9% prevalence, indicating a gradual resolution over time.

Table 38: Comparison (Rt vs Lt) of presence of the delayed side effect—hyperpigmentation.

	Azelaic acid (Rt side)	Tranexamic acid (Lt side)	P-value
After 1 month (T1)	8/38 (21.1%)	5/38 (13.2%)	0.542
After 2 months (T2)	2/34 (5.9%)	1/34 (2.9%)	1
After 3 months (T3)	1/34 (2.9%)	1/34 (2.9%)	1

Figure 23: Comparison (Rt vs Lt) of presence of the delayed side effect—hyperpigmentation.



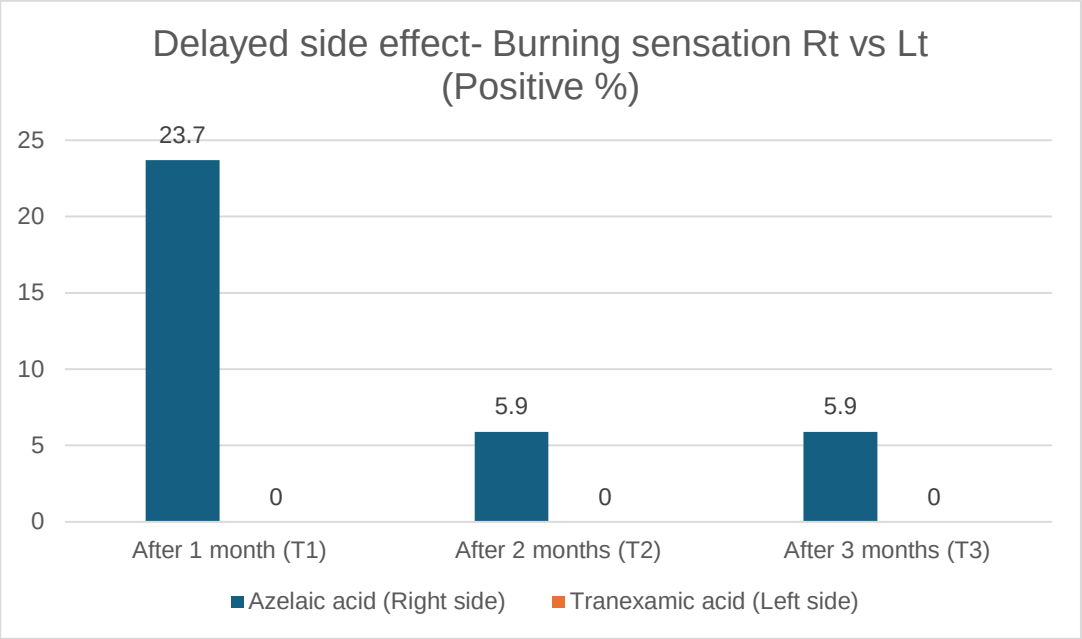
5. Delayed side effects (Burning sensation) Right vs Left:

The comparison of burning sensation as a delayed side effect between Azelaic acid (right side) and Tranexamic acid (left side) reveals a notable difference. At one month (T1), 23.7% of participants reported burning sensation with Azelaic acid, while none experienced it on the Tranexamic acid side (P value = 0.0001) . By two months (T2), the incidence dropped to 5.9%, and this remained unchanged by the end of three months (T3). In contrast, the Tranexamic acid side consistently showed no cases throughout the study. It is also noteworthy that two patients persistently reported burning sensation throughout the three-month period on the Azelaic acid side.

Table 39: Comparison (Rt vs Lt) of presence of the delayed side effect—burning sensation.

	Azelaic acid (Rt side)	Tranexamic acid (Lt side)	P-value
After 1 month (T1)	9/38 (23.7%)	0/38 (0%)	0.0001*
After 2 months (T2)	2/34 (5.9%)	0/34 (0%)	0.493
After 3 months (T3)	2/34 (5.9%)	0/34 (0%)	0.493

Figure 24: Comparison (Rt vs Lt) of presence of the delayed side effect—burning sensation.



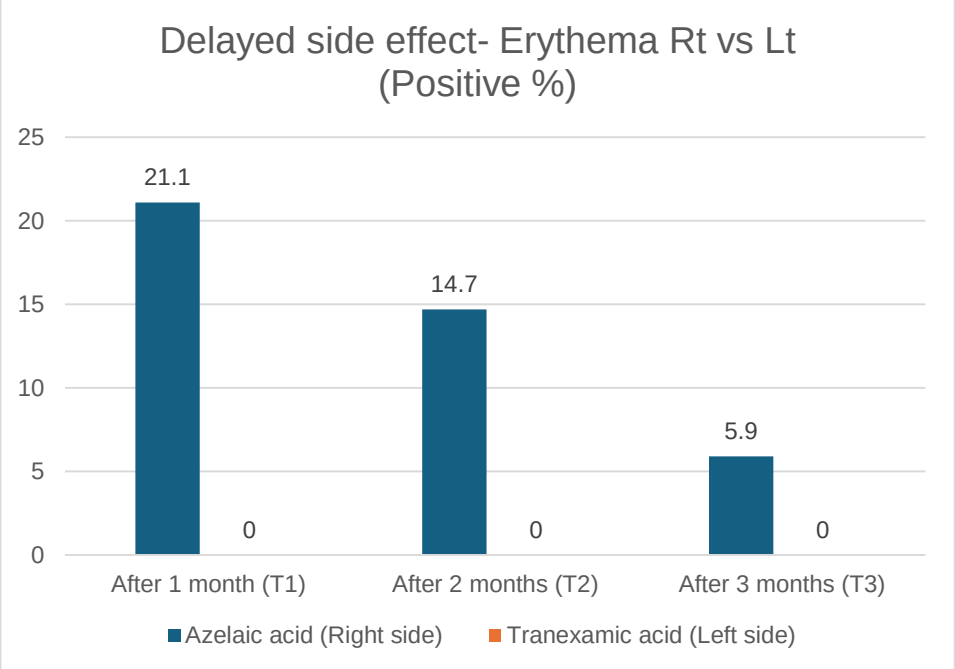
6. Delayed side effects (Erythema) Right vs Left:

The comparison of erythema as a delayed side effect between Azelaic acid (right side) and Tranexamic acid (left side) shows a clear contrast. At one month (T1), 21.1% of participants experienced erythema on the Azelaic acid side, while none reported it on the Tranexamic acid side (P value=0.005, significant). By two months (T2), the occurrence dropped to 14.7%, and by three months (T3), it further declined to 5.9%. Also to note that two patients persistently reported erythema throughout the three-month period on the Azelaic acid side.

Table 40: Comparison (Rt vs Lt) of presence of the delayed side effect—erythema.

	Azelaic acid (Rt side)	Tranexamic acid (Lt side)	P-value
After 1 month (T1)	8/38 (21.1%)	0/38 (0%)	0.005*
After 2 months (T2)	5/34 (14.7%)	0/34 (0%)	0.053
After 3 months (T3)	2/34 (5.9%)	0/34 (0%)	0.493

Figure 25: Comparison (Rt vs Lt) of presence of the delayed side effect—erythema.



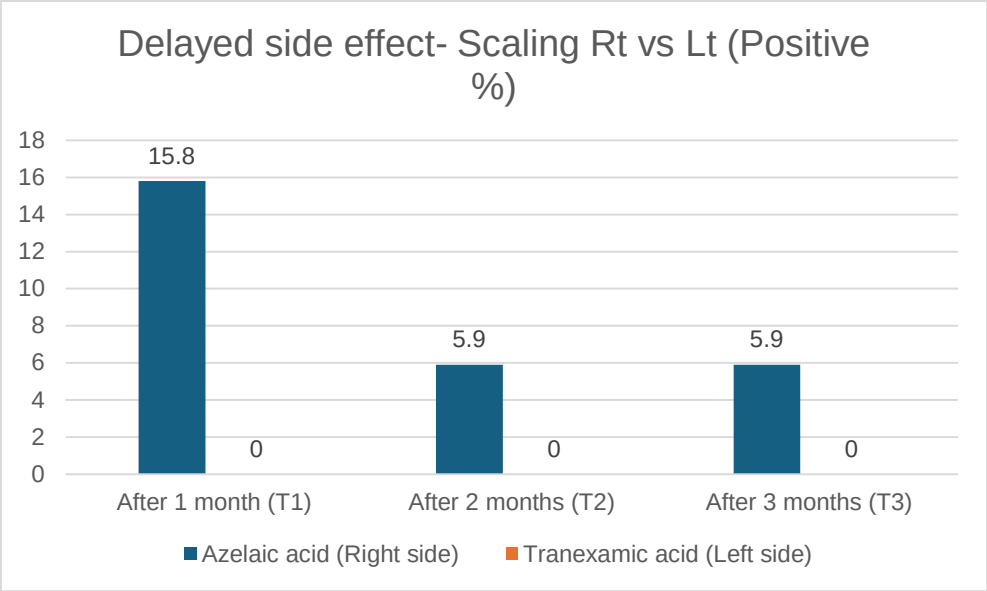
7. Delayed side effects (Scaling) Right vs Left:

The comparison of scaling as a delayed side effect between Azelaic acid (right side) and Tranexamic acid (left side) highlights a notable difference in their effects. At one month (T1), 15.8% of patients experienced scaling on the Azelaic acid side, whereas none reported it on the Tranexamic acid side (P value=0.33, significant). By two months (T2), the occurrence decreased to 5.9%, and it remained at the same level at the end of three months (T3). Throughout the study, no cases of scaling were observed on the Tranexamic acid side. Also to note that two patients persistently reported scaling throughout the three-month period on the Azelaic acid side.

Table 41: Comparison (Rt vs Lt) of presence of the delayed side effect—scaling.

	Azelaic acid (Rt side)	Tranexamic acid (Lt side)	P-value
After 1 month (T1)	6/38 (15.8%)	0/38 (0%)	0.025*
After 2 months (T2)	2/34 (5.9%)	0/34 (0%)	0.493
After 3 months (T3)	2/34 (5.9%)	0/34 (0%)	0.493

Figure 26: Comparison (Rt vs Lt) of presence of the delayed side effect—scaling.



E. MELASMA QUALITY OF LIFE ANALYSIS:

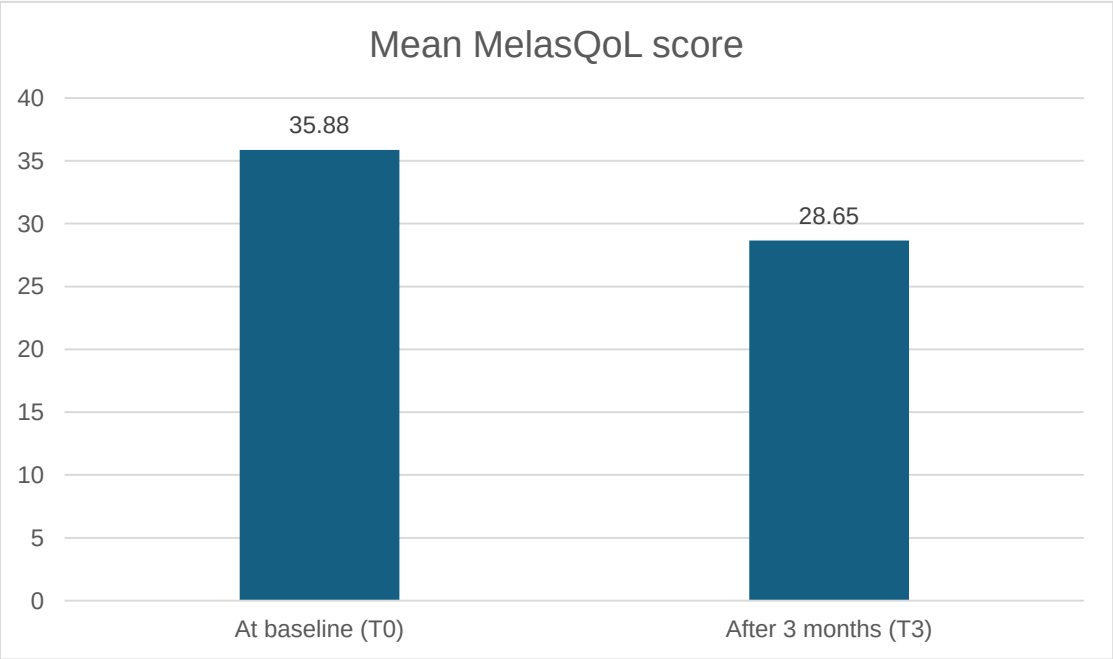
The paired t-test was done to contrast the MelasQoL scores at baseline (T0) and after three months (T3) for the 34 patients who completed the study. The mean score significantly reduced from 35.89 ± 10.46 at baseline to 28.65 ± 7.47 at the end of three months. The p-value < 0.001 depicts a significant reduction in MelasQoL scores, suggesting an enhancement in the patients' quality of life.

Table 42: Mean MelasQol score change from baseline (T0) to after 3 months (T3).

N=34	MelasQoL score (Mean ± SD)	P-value
At baseline (T0)	35.89 ± 10.46	<0.001*
After 3 months (T3)	28.65 ± 7.47	

*Significant
Paired t-test

Figure 27: Mean MelasQol score change from baseline (T0) to after 3 months (T3).



DISCUSSION

An open-label, non-randomized, split-face interventional study was conducted at a tertiary hospital in southern India to compare the efficacy and safety profile of microneedling with azelaic acid 10% gel versus microneedling with tranexamic acid 5% gel in the treatment of melasma. The quality of life of the patients enrolled in the study was also assessed using the MelasQoL questionnaire.

Age and sex:

In our study, a total of 41 patients were recruited, of whom 35 (85.4%) were female and 6 (14.6%) were male, with a female to male ratio of 5.8. Similarly, a study conducted by Sarkar et al.[15] involving 1001 Indian patients reported a comparable ratio of 5.63. However, this was higher than the previously reported female-to-male ratio of 4:1 in other Indian studies.[18,21]

The study population had a mean age of 37.92 ± 7.83 years, which aligns with the findings of KrupaShankar et al.[18], who reported a mean age of 37.2 ± 9.3 years. The mean age of melasma onset in our study was approximately 33 years, which is slightly higher than that observed by Achar et al.[21] but slightly lower than the global average of 34 years.[43]

In our study, the association between the mean age of onset of melasma and sex showed a borderline significant difference (P = 0.059), suggesting a potential trend toward an earlier onset in males compared to females. This may be explained by the findings of Sivayathorn et al.[151], who observed that individuals with higher outdoor sun exposure tend to develop melasma earlier. Consistent with this, the males in our study had a significantly longer duration of sun exposure than females (P = 0.036), which could contribute to their earlier onset of melasma.

Fitzpatrick skin type and triggering factors:

In our study, 9 patients (22%) had Fitzpatrick skin type III, while 16 (39%) had type IV, and 16 (39%) had type V. In contrast, a multicentric study conducted in India by Sarkar et al.(15) reported that approximately 11% had type III, 48% had type IV, 40% had type V, and less than 1% had type VI. Similarly, in a study by Guinot et al.(20) on Tunisian patients found that 14% had type III, 46% had type IV, and 40% had type V. Another study by Hexsel et al.(19) from Brazil reported that approximately 36% had type III, 40% had type IV, and 10% had type V. These differences may be attributed to the regional variations.

In our study, the majority of patients (58.5%) experienced the onset or worsening of melasma following prolonged sun exposure, while 31.8% reported its onset during or after pregnancy. Similarly, in the study by Hexsel et al.(19), sun exposure (44.4%) and pregnancy (24.2%) were the most commonly observed triggering factors.

A history of melasma onset after starting oral contraceptives was reported by 7.3% of patients in our study, similar to the study by Sarkar et al.(15), where only a small proportion (5.4%) had a history of oral contraceptive use. However, in the study by Guinot et al. oral contraceptives were identified as a triggering factor in 26.4% of the patients. This difference may be influenced by sociocultural factors prevalent in India.(15)

Our study found a significant association ($P = 0.020$) between Fitzpatrick skin type (III–V) and triggering factors for melasma. Sunlight was the most common trigger, particularly in skin type V, while pregnancy was more frequent in skin type IV. These findings align with previous studies by KrupaShankar et al.[18], Hexsel et

al[19], and Guinot et al.[20], which also identified prolonged sun exposure and pregnancy as predominant triggering or aggravating factors.

In our study, the association between Fitzpatrick skin type (III–V) and sun exposure duration was also statistically significant (P = 0.026). A higher proportion of patients with Fitzpatrick type V reported sun exposure of two hours or more. This pattern may be attributed to the greater melanin content in darker skin, which provides natural protection against UV damage[152]. As a result, individuals with darker skin (type V) may tolerate prolonged sun exposure before developing sun-related conditions compared to those with lighter skin types (III and IV).

The association between the mean age of onset of melasma among individuals with Fitzpatrick skin types III, IV, and V indicates no statistically significant difference among the three groups (P = 0.169), suggesting that Fitzpatrick skin type does not strongly influence the age of melasma onset in this study population. However, these findings differ from those of previous studies by Sarkar et al.[15] and Hexsel et al.[19], which found that individuals with Fitzpatrick types II and III experienced an earlier onset of melasma compared to those with type V.

In our study, a statistically significant association (P = 0.013) was found between the total duration of illness and the duration of sun exposure. Individuals with longer sun exposure reported a shorter duration of illness at the time of presentation, whereas those with less sun exposure experienced a longer duration of illness. Similarly, Guinot et al.[20] found that in individuals with lower lifetime sun exposure, melasma took a longer cumulative time to develop. In contrast, for those with a history of significant lifetime sun exposure, sunlight acted as an aggravating factor and was associated with faster onset.

Family history:

A total of 8 patients (19.5%) had a positive family history of melasma, while the remaining 33 patients (80.5%) had no similar complaints in their family. This is consistent with the study by Sarkar et al.(15), which reported that 20% of patients had a positive family history, while 80% did not. Studies by Achar et al.(21) and KrupaShankar et al.(18) reported a positive family history in 33.3% and 31.1% of the patients with melasma, respectively. The variation in family history may be due to genetic factors, sample size differences, environmental influences, recall bias, and hormonal or lifestyle factors.

Our study found a moderately significant association (P = 0.071) between family history (positive or negative) and triggering factors for melasma. Oral contraceptive and pregnancy-related triggers were observed only in individuals with a negative family history. While sunlight was a common trigger in both groups (8 with a positive and 16 with a negative family history), the results suggest a possible influence of family history, though not strong enough to be statistically conclusive and requiring further studies with a larger sample size.

The association between the mean age of onset and family history was not significant (P = 0.830) in our study, suggesting that genetic predisposition does not influence the age at which melasma develops. This means that the onset occurred at a similar age in individuals with and without a family history. These findings align with those of KrupaShankar et al.[18] but differ from those reported by Hexsel et al.[19]

Melasma pattern:

In our study, two predominant patterns of melasma were observed. The centrofacial pattern was the most common, affecting 78% of patients, while the malar

pattern was seen in 22%. No cases of the mandibular type were recorded. This distribution closely aligns with the findings of Guinot et al.(20), who reported 75% centrofacial, 24% malar, and 1% mandibular cases. However, other Indian studies have reported a higher prevalence of the malar type, with Achar et al.(21) reporting 43.3%, KrupaShankar et al.(18) reporting 39%, and Sarkar et al.(15) reporting 42.8%, respectively. This variation may be due to differences in study populations, genetic factors, and environmental influences.

A significant association (P = 0.015) was found between sex (male vs. female) and melasma patterns, indicating that melasma distribution differs between the two sexes. Among males, malar melasma (66.7%) was more common than centrofacial melasma, whereas among females, centrofacial melasma was predominant. Similarly, another study by Sarkar et al.[153] reported that 61% of men had malar melasma in their study. These findings suggest a potential gender-related influence on melasma patterns.

In the present study, melasma patterns were also analyzed in relation to family history, Fitzpatrick skin type, triggering factors, duration of sun exposure, and duration of illness, but no significant associations were found.

Efficacy:

In our study, when comparing the mean hemi-mMASI scores between the microneedling with azelaic acid and microneedling with tranexamic acid sides, both showed gradual improvement; however, the tranexamic acid side demonstrated a greater degree of improvement with each subsequent visit (P_{T0} = 0.921, P_{T1} = 0.818, P_{T2} = 0.371, P_{T3} = 0.129).

The comparison of hemi-mMASI scores at baseline and after three months showed differing outcomes for the azelaic acid (AZA) and tranexamic acid (TXA) groups. In the AZA group, the mean hemi-mMASI score decreased from 3.28 ± 1.6 to 2.63 ± 1.54, but this reduction was not statistically significant (P = 0.079).

Although various studies[154–159] have demonstrated comparable or superior efficacy of topical 20% AZA over 2–4% HQ in reducing melasma severity, it has never been compared head-to-head with TXA.

Azelaic acid has been used in only one case series of three patients with microneedling along with liposomal serum combination containing 4-n-butylresorcinol, AZA, and retinol. An improvement in modified MASI score was reported in all three patients, with reductions of 33.3%, 41.7%, and 85%, respectively.[150]

In contrast, when comparing hemi-mMASI scores at baseline and after three months, the TXA group showed a significant decline from 3.24 ± 1.63 to 2.08 ± 1.39 (P = 0.0016).

Similar results were observed in the tranexamic acid (TXA) with microneedling group for the treatment of melasma in the study by Saleh et al.[141], where the MASI score significantly decreased from 14.9 ± 4.2 to 6.1 ± 4.9 (P = 0.001). Likewise, Kaur et al.[142] reported similar findings, with a significant reduction in the mMASI score from 4.313 ± 1.7781 to 1.471 ± 1.1242 (P < 0.05).

Banihashemi et al.[160] evaluated the efficacy of 5% liposomal TXA compared with 4% HQ cream. Although the liposomal TXA group showed a greater reduction in the MASI score than the HQ group, the difference was not significant. In the liposomal TXA group, the MASI score was reduced, from 14.72 ± 2.2 to 7.69 ± 2.4,

which was consistent with the findings of Shamsi et al.[161] (12.89 ± 0.94 to 6.84 ± 0.78 in the TXA plus microneedling group. Thus, doing microneedling before TXA application can enhance its penetration, achieving an efficacy comparable to the liposomal form.

The association between hemi-mMASI score changes with melasma pattern in our study revealed that microneedling with TXA was significantly more efficacious than microneedling with AZA for centrofacial melasma (P = 0.014), while both treatments had similar effects in malar melasma.

Regarding illness duration, the tranexamic acid group in our study showed significantly greater improvement in hemi-mMASI score changes in patients with melasma lasting less than 5 years (P = 0.049). However, for cases persisting more than 5 years, the difference among the treatments was not significant (P = 0.088), suggesting that microneedling with tranexamic acid is more effective in early stage melasma, whereas both treatments perform similarly in chronic cases.

The impact of family history on treatment response was also evaluated. While microneedling with tranexamic acid showed greater improvement than microneedling with azelaic acid in both groups, the difference was significant only in patients without a family history of melasma (P = 0.038). In contrast, those with a positive family history showed no significant difference between treatments (P = 0.075). These findings suggest that genetic predisposition may influence treatment response, with microneedling with tranexamic acid demonstrating a stronger effect in patients without a hereditary risk.

Both azelaic acid and tranexamic acid led to a gradual reduction in hemi-mMASI scores across all Fitzpatrick skin types, but neither treatment showed a

significant difference in efficacy between skin types. Notably, a slight increase in scores at one month for Fitzpatrick type V on the azelaic acid side suggests a potential early variation in response. However, overall, both treatments demonstrated comparable effectiveness across skin types.

Safety Profile:

In the study by Sivayathorn et al. (155), 36.5% of patients in the azelaic acid arm experienced mild itching and a burning sensation. The safety profile was not assessed in the study by Kusumawardani et al.[150].

Common side effects of microneedling with tranexamic acid were evaluated in a meta-analysis by Feng et al.[145]. The reported side effects included erythema (56.36%), mild pain (41.27%), irritation at the microneedling site (19.64%), PIH (6.67%), and transient burning and itching (7%).

In our study, immediate side effects were assessed immediately after each microneedling session for both sides. It was observed that they were the same on both treatment sides and were primarily attributed to microneedling.

In our study, pain was most prominent during the first session (T0), affecting 26.8% of participants, but decreased to 15.8% at T1 and 14.7% at T2, suggesting increased tolerance over time. Burning sensation was reported by 26.8% at T0, remained stable at 26.3% at T1, but dropped to 8.8% at T2, indicating a decline with continued treatment. Erythema was universally present in all sessions (T0, T1, and T2) in 100% of participants, though it was slightly harder to assess in those with higher Fitzpatrick skin types. Overall, pain and burning sensation decreased over time, while erythema remained a consistent post-procedural side effect.

Similarly, in the study by Saleh et al.[141], no significant side effects were reported for microneedling with tranexamic acid or microneedling alone, except for slight transient erythema and a burning sensation, which some patients experienced a few hours after the sessions and were attributed to microneedling as the cause.

The delayed side effects of hyperpigmentation, burning sensation, erythema, and scaling were compared between azelaic acid (right side) and tranexamic acid (left side), revealing a consistently higher incidence with azelaic acid.

Hyperpigmentation was initially more on the azelaic acid side, with 21.1% affected at one month (T1) compared to 13.2% on the tranexamic acid side, though this variation was not significant (P = 0.54). By two months (T2), the incidence dropped to 5.9% and 2.9%, respectively, and by three months (T3), both sides showed a prevalence of 2.9%, indicating gradual resolution over time.

Hyperpigmentation was also noted in the study by Kaur et al.[142], but it affected only 5% of the patients in the test group. This difference can be explained by the fact that their study primarily included participants from Northern India who had a much lower Fitzpatrick skin type compared to the participants in our study.

A notable difference was observed in burning sensation, erythema, and scaling, which were significantly more frequent with azelaic acid than with tranexamic acid. At T1, burning sensation (23.7%, P = 0.0001), erythema (21.1%, P = 0.005), and scaling (15.8%, P = 0.33) were reported only on the azelaic acid side, with no cases on the tranexamic acid side. In the azelaic acid group, by T2, both burning sensation and scaling dropped to 5.9% and remained unchanged at T3, while erythema decreased to 14.7% at T2 and further to 5.9% at T3. No complaints of erythema, burning sensation, or scaling were observed in the tranexamic acid group.

Notably, two patients experienced persistent burning, two had erythema, and two had scaling throughout the three-month period with azelaic acid. No such cases were observed with tranexamic acid.

The MelasQoL scores at baseline (T0) and after three months (T3) were compared for the 34 patients who completed the study. A statistically significant reduction in the mean score was observed from 35.89 ± 10.46 at T0 to 28.65 ± 7.47 at T3 ($p < 0.001$), indicating an overall improvement in melasma-related quality of life.

CONCLUSION

This study demonstrates that microneedling with tranexamic acid is more effective than microneedling with azelaic acid in reducing melasma severity, particularly in centrofacial melasma and early-stage cases. The presence of a positive family history appeared to reduce the difference in treatment response, suggesting a potential genetic influence on treatment efficacy. While both treatments were effective across Fitzpatrick skin types, a transient increase in hemi-mMASI scores was observed in Fitzpatrick type V patients treated with AZA, indicating possible variations in response based on skin type.

The safety profile of both treatments was generally favorable, though delayed adverse effects were significantly more frequent with AZA. TXA-treated patients reported fewer side effects and no persistent cases of burning, erythema, or scaling, reinforcing its superior tolerability. Additionally, the observed improvement in MelasQoL scores suggests a positive effect on patients' life quality, further supporting the clinical utility of microneedling with TXA for melasma management.

STRENGTHS

Direct head-to-head comparison: This study provides a direct comparison of two commonly used agents (AZA and TXA) in microneedling-based melasma treatment, offering important understanding of their relative efficacy and safety.

Objective assessment: The hemi-mMASI scores and MelasQoL scale allowed for a structured and quantifiable evaluation of treatment outcomes.

Evaluation of multiple factors: The study considered the impact of melasma pattern, duration, family history, and Fitzpatrick skin type on treatment response, providing a comprehensive analysis.

Longitudinal follow-up: Patients were followed for three months, enabling the assessment of both short-term and delayed treatment effects.

LIMITATIONS

Small sample size: The study included a relatively small number of patients, which may limit the generalizability of findings to larger populations.

Short follow-up duration: Although three months provided useful insights into efficacy and safety, a longer follow-up would be beneficial to assess the long-term sustainability of treatment benefits.

Potential bias in self-reported outcomes: Subjective symptoms such as pain and burning sensation rely on patient-reported outcomes, which may introduce variability.

SUMMARY

This study aimed to compare the efficacy and safety of microneedling with azelaic acid (AZA) and microneedling with tranexamic acid (TXA) in the treatment of melasma. Among the patients studied, the majority (58.5%) reported the onset or worsening of melasma following prolonged sun exposure, while 31.8% developed melasma during or after pregnancy. A small percentage (7.3%) reported onset after oral contraceptive use, which was comparable to previous studies, though variations in findings across studies suggest sociocultural influences on melasma triggers.

Both treatment modalities demonstrated a gradual reduction in hemi-mMASI scores, indicating improvement in severity over time. However, the TXA-treated side consistently showed a greater degree of improvement at each follow-up visit. When analyzing treatment efficacy by melasma pattern, TXA was found to be significantly more effective than AZA in treating centrofacial melasma, whereas both treatments exhibited similar effects in malar melasma. The duration of melasma also influenced treatment response, with TXA demonstrating significantly greater improvement in patients with melasma of less than five years in duration. In cases persisting for more than five years, the difference in treatment efficacy was not statistically significant, suggesting that TXA may be more beneficial in early-stage melasma, while both treatments perform similarly in chronic cases.

The study also evaluated the impact of family history on treatment response. While TXA showed greater improvement than AZA in both groups, the difference was only statistically significant in patients without a family history of melasma. This suggests that genetic predisposition may influence treatment response, with TXA demonstrating a stronger effect in patients without a hereditary risk.

In terms of safety, immediate side effects such as pain and erythema were present in both treatment groups and were primarily attributed to microneedling rather than the topical agents themselves. Pain was most intense during the first session but decreased over subsequent treatments, suggesting increased tolerance over time. However, delayed side effects, including burning sensation, erythema, and scaling, were significantly more frequent with AZA than with TXA. While hyperpigmentation was initially more common on the AZA-treated side, it gradually resolved over time in both groups. Notably, some patients experienced persistent burning, erythema, and scaling throughout the three-month study period with AZA, whereas no such cases were reported with TXA.

Quality of life assessment using the MelasQoL scale revealed a significant reduction in mean scores from baseline to three months, reflecting overall improvement in melasma-related quality of life. These findings highlight the potential of microneedling-assisted tranexamic acid therapy as an effective and well-tolerated treatment option for melasma.

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ANNEXURE – I - INFORMED CONSENT FORM

Title of the study: An open label non-randomized split face interventional study to compare the efficacy and safety of microneedling with azelaic acid 10% gel versus microneedling with tranexamic acid 5% gel in the treatment of melasma.

Name of Student/Principal Investigator: DR

Name of Guide/Co Investigators: DR

Introduction:

Melasma is an acquired pigmentary disorder characterized by symmetrically distributed, hyperpigmented patches and macules with well-defined margins, affecting the forehead, temples, malar and mandibular regions of the cheeks, nose, and chin.

Explanation of Procedure:

You will be taken for the study if you fall under the inclusion criteria. Prior to the procedure, a detailed history and examination will be conducted. Baseline melasma scores and facial photographs will be recorded.

After anesthetizing the face with a topical agent, microneedling with Dr. Pen Ultima A6 will be carried out on both sides of the face, followed by the application of Azelaic acid 10% gel on one side and Tranexamic acid 5% gel on the other side.

Thereafter, two more sessions of microneedling will be performed, each one a month apart.

You are advised to apply sunscreen over the full face twice a day and Azelaic acid gel on the right side and Tranexamic acid gel on the left side of the face at night once

daily for three months. Melasma scores and photographs will be recorded monthly and at the end of the study period (three months).

Withdrawal from Participation in the Study:

Participation in this study is voluntary. You are free to decide whether to participate or to continue participation once enrolled. If you decide to withdraw, you may do so at any time. However, please inform the principal investigator of your decision.

Possible Benefits from Participating in the Study:

Participation in this study may provide you with benefits. The data gathered will help the population at large.

Possible Risks from Participating in the Study:

You may experience mild erythema and irritation after the procedure.

Privacy and Confidentiality:

The information collected from you will be coded to prevent anyone from identifying 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 the aggregated data will be published for scientific purposes and/or presented to scientific groups. However, your identity will never be revealed.

Questions: In case of any questions regarding this study, you are free to 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: " **An Open label Non-Randomized Split Face Interventional Study to Compare the Efficacy and Safety of Microneedling with Azelaic Acid 10% gel versus Microneedling with Tranexamic Acid 5% gel in the treatment of Melasma**". 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 they were 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 Data		
Name:		
Phone Number:		
OP/IP Number:		
Age (yrs):		
Sex:		
Family history (First degree relative)	Positive Negative	
Duration of illness	< 5 yrs ≥ 5 yrs	
Melasma pattern	Centro facial Malar Mandibular	
Skin photo type- Fitzpatrick skin type (III-V)	III IV V	
Triggering factors	Sun exposure Oral contraception Pregnancy Idiopathic	
Duration of sun exposure (hrs):		

Severity of melasma (Hemi-mMASI score)		
	Right side (Azelaic acid)	Left side (Tranexamic acid)
T0 (At Baseline)		
T1 (After 1 month)		
T2 (After 2 months)		
T3 (After 3 months)		

IMMEDIATE SIDE EFFECTS				
			Right side (Azelaic acid)	Left side (Tranexamic acid)
T0 (At first sitting)	Pain	Present Absent		
	Burning	Present Absent		
	Erythema	Present Absent		
T2 (At second sitting)	Pain	Present Absent		
	Burning	Present Absent		
	Erythema	Present Absent		
T3 (At third sitting)	Pain	Present Absent		
	Burning	Present Absent		
	Erythema	Present Absent		

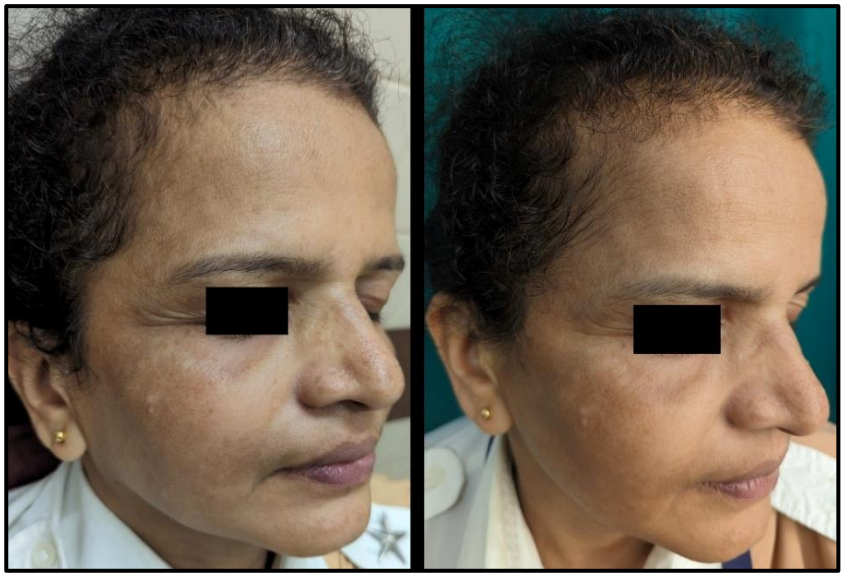
DELAYED SIDE EFFECTS				
			Right side (Azelaic acid)	Left side (Tranexamic acid)
T1 (After 1 month)	Hyperpigmentation	Present Absent		
	Burning	Present Absent		
	Erythema	Present Absent		
	Scaling	Present Absent		
T2 (After 2 months)	Hyperpigmentation	Present Absent		
	Burning	Present Absent		
	Erythema	Present Absent		
	Scaling	Present Absent		
T3 (After 3 months)	Hyperpigmentation	Present Absent		
	Burning	Present Absent		
	Erythema	Present Absent		
	Scaling	Present Absent		

	MelasQoL Scale	**Score (1-7)**
1.	The appearance of your skin condition	
2.	Frustration about your skin condition	
3.	Embarrassment about your skin condition	
4.	Feeling depressed about your skin condition	
5.	The effects of your skin condition on your interactions with other people (eg, interactions with family, friends, close relationship, and so forth.)	
6.	The effects of your skin condition on your desire to be with people	
7.	Your skin condition making it hard to show affection	
8.	Skin discoloration making you feel unattractive to others	
9.	Skin discoloration making you feel less vital or productive	
10.	Skin discoloration affecting your sense of freedom	
	TOTAL (10-70):	

	MelasQoL Score (10-70)
T0 (At baseline)	
T3 (At end of 3 months)	

ANNEXURE III- PHOTOGRAPHS

Case #1a: Left side (Azelaic acid) Baseline vs After 3 months.

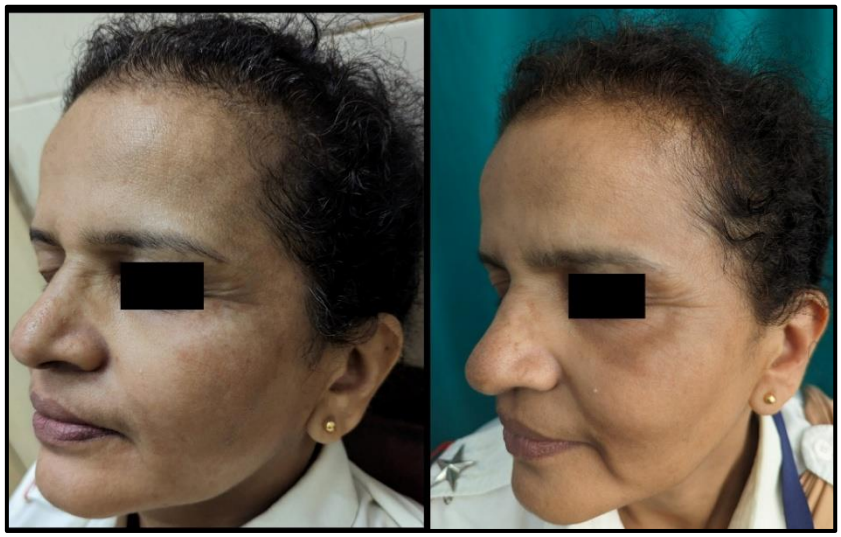


Right Hemi-mMASI (Baseline)=4.85

Right Hemi-mMASI (After 3 Months)=3.2

Hemi-mMASI Difference = 1.65

Case #1b: Right side (Tranexamic acid) Baseline vs After 3 months.

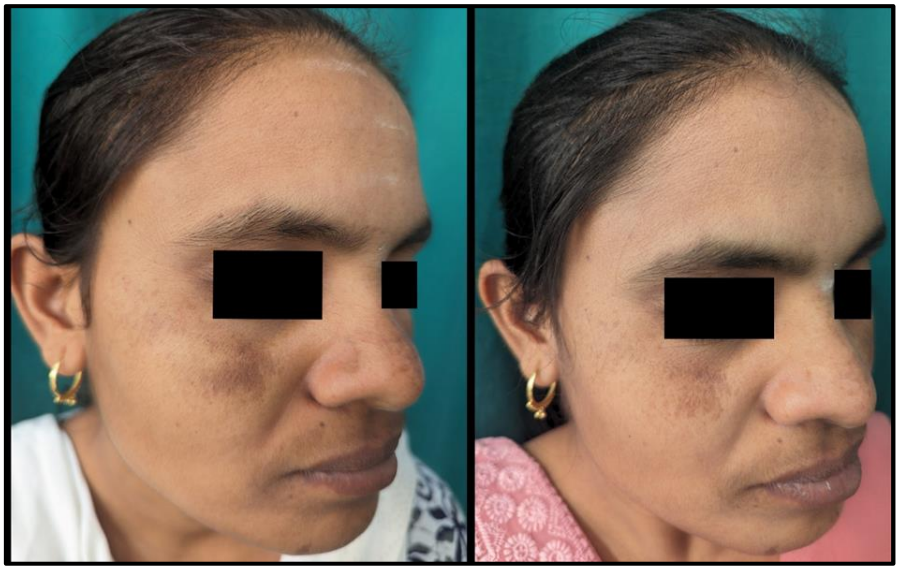


Left Hemi-mMASI (Baseline)=3.8

Left Hemi-mMASI (After 3 months)=1.8

Hemi-mMASI Difference = 2.0

Case #2a: Left side (Azelaic acid) Baseline vs After 3 months.

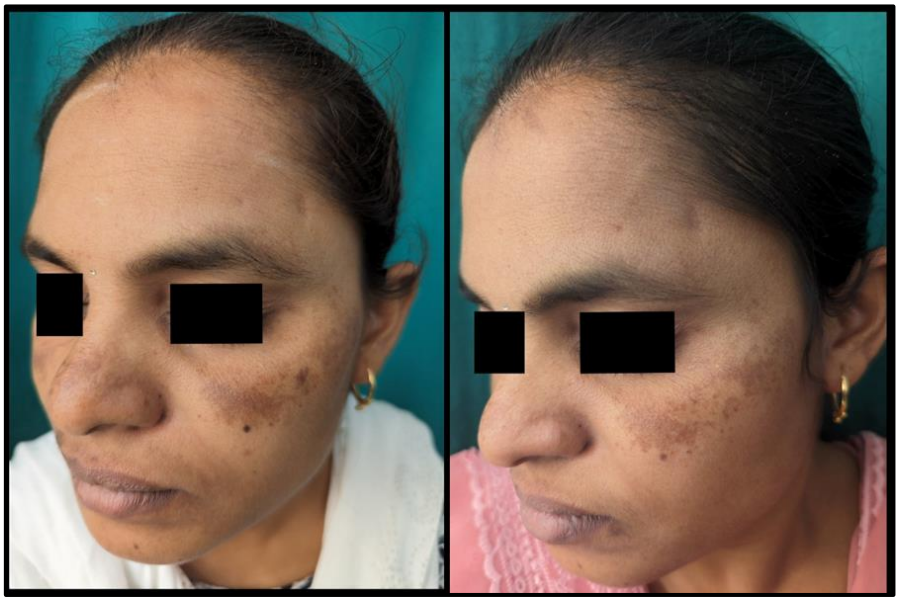


Right Hemi-mMASI (Baseline)=4.15

Right Hemi-mMASI (After 3 Months)=3.25

Hemi-mMASI Difference = 0.9

Case #2b: Right side (Tranexamic acid) Baseline vs After 3 months.

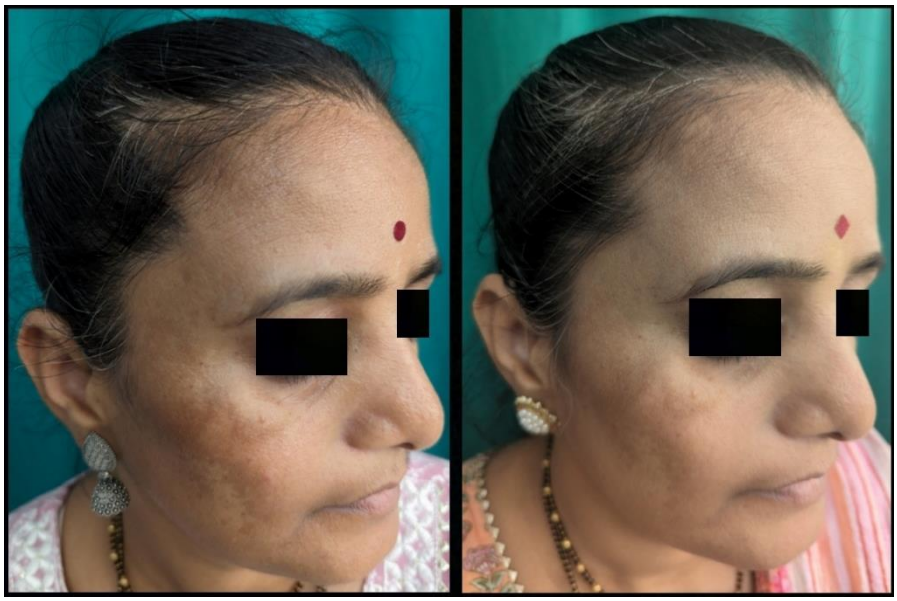


Left Hemi-mMASI (Baseline)=4.15

Left Hemi-mMASI (After 3 months)=2.35

Hemi-mMASI Difference = 1.8

Case #3a: Left side (Azelaic acid) Baseline vs After 3 months.

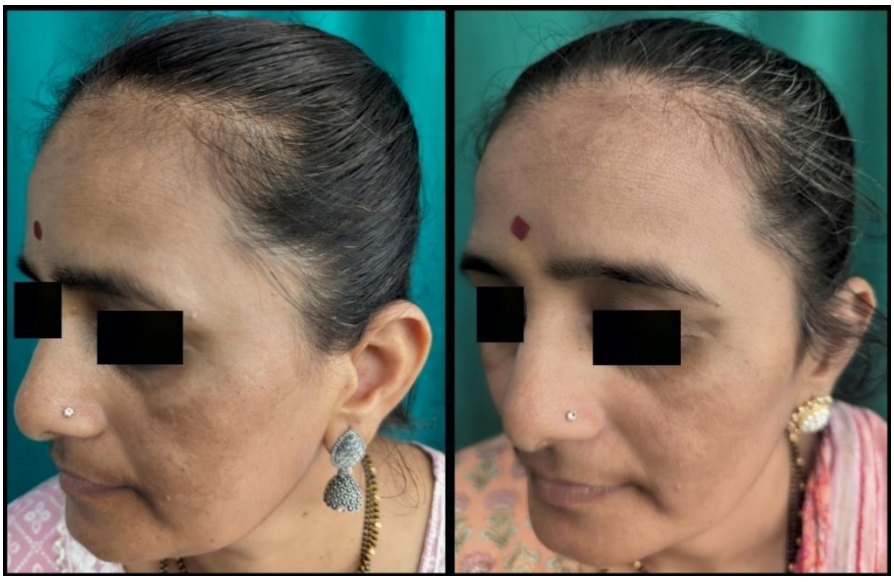


Right Hemi-mMASI (Baseline)=3.6

Right Hemi-mMASI (After 3 Months)=3.6

Hemi-mMASI Difference = 0

Case #3b: Right side (Tranexamic acid) Baseline vs After 3 months.



Left Hemi-mMASI (Baseline)=3.6

Left Hemi-mMASI (After 3 months)=2.4

Hemi-mMASI Difference = 1.2

ANNEXURE IV- KEY TO MASTER CHART

T0	-	At Baseline.
T1	-	After 1 month.
T2	-	After 2 months.
T3	-	After 3 months.
L	-	Left face.
R	-	Right face.
I	-	Immediate side effects.
D	-	Delayed side effects.
mMASI	-	Modified melasma severity index.
MelasQoL	-	Melasma quality of life score.
N/A	-	Not applicable.

Sr No.	OP no.	AGE (Yrs)	SEX	Family history	Duration of illness (Yrs)	Melasma pattern	Type of melasma on dermoscopy	Fitzpatrick skin type (III-V)	Triggering factors	Duration of sun exposure (hrs)	R_mMASI_T0	R_mMASI_T1	R_mMASI_T2	R_mMASI_T3	L_mMASI_T0	L_mMASI_T1	L_mMASI_T2	L_mMASI_T3	I_PAIN_T0	I_PAIN_T1	I_PAIN_T2	I_BURNING_T0	I_BURNING_T1	I_BURNING_T2	I_ERYTHEMA_T0	I_ERYTHEMA_T1	I_ERYTHEMA_T2	D_R_HYPERPIGMENTATION_T1	D_R_HYPERPIGMENTATION_T2	D_R_HYPERPIGMENTATION_T3	D_R_BURNING_T1	D_R_BURNING_T2	D_R_BURNING_T3	D_R_ERYTHEMA_T1	D_R_ERYTHEMA_T2	D_R_ERYTHEMA_T3	D_R_SCALING_T1	D_R_SCALING_T2	D_R_SCALING_T3	D_L_HYPERPIGMENTATION_T1	D_L_HYPERPIGMENTATION_T2	D_L_HYPERPIGMENTATION_T3	D_L_BURNING_T1	D_L_BURNING_T2	D_L_BURNING_T3	D_L_ERYTHEMA_T1	D_L_ERYTHEMA_T2	D_L_ERYTHEMA_T3	D_L_SCALING_T1	D_L_SCALING_T2	D_L_SCALING_T3	MelasQoL_T0	MelasQoL_T3
1	7022658	33	F	NEGATIVE	11	Centrofacial	Mixed	IV	Pregnancy	1	5.25	5.25	5.25	4.35	5.4	5.4	3.75	3.75	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	41	35
2	7054354	26	M	POSITIVE	2	Malar	Mixed	V	Sunlight	6	1.8	2.4	1.8	1.8	1.2	1.8	1.2	1.2	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	36	31
3	7024241	28	M	NEGATIVE	1	Centrofacial	Mixed	V	Sunlight	4	5.25	5.1	3	3	4.05	3.9	2.85	1.95	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	51	38
4	7028439	50	F	NEGATIVE	1	Centrofacial	Mixed	V	Sunlight	3	3.9	1.65	1.45	1.1	5.4	2.85	1.3	1.3	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	43	31
5	4951416	42	M	NEGATIVE	3	Centrofacial	Mixed	V	Sunlight	3	5.5	7.3	5.5	5.5	5.5	7.3	5.5	5.5	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	53	37
6	7088608	52	F	NEGATIVE	2	Centrofacial	Mixed	IV	Idiopathic	1	4.15	3.25	3.25	3.25	4.15	3.25	3.25	2.35	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	PRESENT	PRESENT	ABSENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	27	22
7	2804081	47	F	NEGATIVE	11	Centrofacial	Mixed	III	Pregnancy	1	4.85	4.7	4.7	3.2	4.7	4.7	3.8	1.8	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	49	20
8	7122995	45	F	NEGATIVE	2	Malar	Mixed	V	Sunlight	2	0.6	1.2	1.2	0.6	0.6	0.6	1.2	0.3	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	29	25
9	3683747	24	F	NEGATIVE	3	Centrofacial	Mixed	III	Sunlight	3	2.15	2.3	2.15	1.25	1.55	1.55	1.55	0.65	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	55	32
10	6734407	43	F	POSITIVE	10	Malar	Mixed	V	Sunlight	2	3.6	3.6	3.6	3.6	3.6	3.6	3.6	2.4	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	31	30
11	7216118	34	F	NEGATIVE	3	Centrofacial	Mixed	IV	Sunlight	2	3.75	3.75	3.75	3.75	4.65	4.65	3.75	3.6	PRESENT	ABSENT	ABSENT	PRESENT	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	46	38
12	7258963	40	F	NEGATIVE	15	Centrofacial	Mixed	IV	Pregnancy	1	5.8	5.8	5.8	5.8	5.8	5.8	5.8	5.8	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	39	37
13	3258947	30	M	NEGATIVE	0.5	Malar	Mixed	V	Sunlight	2	0.6	0.6	0.9	0.9	0.6	0.6	0.9	0.9	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	25	25
14	3174922	43	F	NEGATIVE	3	Centrofacial	Mixed	IV	al contracep	1	2.15	2.15	2	2	2.15	2.15	1.4	1.4	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	34	28
15	5595672	40	F	POSITIVE	8	Centrofacial	Mixed	III	Sunlight	2	0.8	0.45	0.75	0.6	1.1	1.05	1.05	0.6	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	30	21
16	3343419	43	F	NEGATIVE	2	Centrofacial	Mixed	V	Sunlight	2	6.35	7.85	6.35	6.35	6.35	6.95	5.45	3.95	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	42	35
17	3852589	42	F	NEGATIVE	1	Malar	Mixed	V	Sunlight	4	1.2	1.8	1.2	0.6	1.2	1.8	0.6	0.3	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	29	26
18	7288915	45	F	POSITIVE	3	Centrofacial	Mixed	III	Sunlight	2	5.85	5.85	4.65	4.65	5.4	5.4	4.2	4.2	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	55	47
19	7322508	34	F	POSITIVE	4	Centrofacial	Mixed	IV	Sunlight	4	2	2	2	1.3	3.2	3.2	2.3	1.4	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	32	27
20	7151893	30	F	NEGATIVE	1	Centrofacial	Mixed	V	Pregnancy	1	2.1	2.1	2.1	2.1	1.8	1.8	1.8	1.8	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	20	18
21	6149875	36	F	NEGATIVE	9	Centrofacial	Mixed	IV	Pregnancy	1	5.25	5.25	N/A	N/A	5.7	5.7	N/A	N/A	PRESENT	PRESENT	N/A	PRESENT	PRESENT	N/A	PRESENT	PRESENT	N/A	ABSENT	N/A	N/A	PRESENT	N/A	N/A	PRESENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	36	N/A
22	6249725	35	F	NEGATIVE	8	Malar	Mixed	IV	Pregnancy	1	2.4	2.4	N/A	N/A	2.4	2.4	N/A	N/A	PRESENT	ABSENT	N/A	ABSENT	ABSENT	N/A	PRESENT	PRESENT	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	40	N/A
23	6484106	51	F	NEGATIVE	3	Centrofacial	Mixed	III	Sunlight	2	4.6	N/A	N/A	N/A	4.6	N/A	N/A	N/A	ABSENT	N/A	N/A	PRESENT	N/A	N/A	PRESENT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	57	N/A
24	7022569	38	F	NEGATIVE	18	Centrofacial	Mixed	IV	Pregnancy	1	4.8	N/A	N/A	N/A	4.8	N/A	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	PRESENT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	28	N/A
25	4956025	27	F	NEGATIVE	3	Centrofacial	Mixed	IV	Pregnancy	1	3.45	N/A	N/A	N/A	3	N/A	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	PRESENT	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	25	N/A
26	7032596	32	F	NEGATIVE	8	Centrofacial	Mixed	V	Pregnancy	1	3.15	2.25	2.25	2.1	3.6	2.7	2.4	2.1	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	24	20
27	7854921	48	F	NEGATIVE	1	Centrofacial	Mixed	V	Sunlight	2	4.1	4.55	3.5	3.5	3.8	3.8	3.1	3.1	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	28	24
28	4426971	37	F	NEGATIVE	3	Centrofacial	Mixed	IV	Pregnancy	1	5.1	5.1	N/A	N/A	5.1	5.1	N/A	N/A	ABSENT	ABSENT	N/A	ABSENT	ABSENT	N/A	PRESENT	PRESENT	N/A	ABSENT	N/A	N/A	PRESENT	N/A	N/A	PRESENT	N/A	N/A	PRESENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	28	N/A
29	7589356	46	F	POSITIVE	5	Centrofacial	Mixed	IV	Sunlight	5	1.5	1.8	1.5	1.5	1.5	1.8	1.5	0.9	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	23	16
30	7865423	35	F	NEGATIVE	2	Centrofacial	Mixed	III	al contracep	2	2.5	2.2	1.6	1.45	3.1	2.2	1.45	0.75	PRESENT	PRESENT	PRESENT	ABSENT	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	29	19
31	6845102	32	F	NEGATIVE	11	Centrofacial	Mixed	IV	Pregnancy	1	2.8	2.2	2.2	2.05	2.2	2.05	1.15	1.15	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	33	25
32	6632015	44	F	NEGATIVE	4	Malar	Mixed	IV	Sunlight	5	1.8	1.8	1.2	1.2	1.8	1.8	1.2	1.2	PRESENT	ABSENT	PRESENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	27	24
33	4498562	29	F	POSITIVE	5	Centrofacial	Mixed	V	Sunlight	2	2.9	2.6	2.6	2.6	2.9	2.6	2.6	2	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	28	26
34	7901254	23	M	NEGATIVE	1	Malar	Mixed	V	Sunlight	3	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	29	27
35	6589825	51	F	NEGATIVE	2	Centrofacial	Mixed	III	Sunlight	3	1.95	1.8	N/A	N/A	1.35	1.2	N/A	N/A	PRESENT	ABSENT	N/A	PRESENT	ABSENT	N/A	PRESENT	PRESENT	N/A	ABSENT	N/A	N/A	PRESENT	N/A	N/A	PRESENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	ABSENT	N/A	N/A	35	N/A
36	6659817	41	F	NEGATIVE	8	Centrofacial	Mixed	V	Sunlight	2	4.2	4.2	3.9	3.9	4.2	3.9	3.8	3.2	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	33	29
37	4692315	41	F	NEGATIVE	3	Centrofacial	Mixed	III	al contracep	1	2.1	2.1	1.95	1.95	2.1	1.95	1.05	1.05	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	40	25
38	7956842	39	F	NEGATIVE	17	Centrofacial	Mixed	IV	Pregnancy	1	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	34	32
39	7569840	28	M	NEGATIVE	1	Malar	Mixed	IV	Sunlight	2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.6	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	28	28
40	7560301	37	F	POSITIVE	3	Centrofacial	Mixed	V	Sunlight	6	4.8	4.8	4.8	4.2	3	3	2.4	2.4	PRESENT	PRESENT	ABSENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	36	29
41	7742396	34	F	NEGATIVE	1	Centrofacial	Mixed	III	Pregnancy	1	3.25	3.25	3.1	3.1	3.3	3.3	3.15	2.25	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	PRESENT	PRESENT	PRESENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	ABSENT	61	47