OPEN COMPARATIVE STUDY OF MELAGENINA PLUS LOTION AND MELAGENINA PLUS LOTION ALONG WITH UVA TO EVALUATE THE RATE OF REPIGMENTATION IN VITILIGO PATIENTS

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Certificate

Certified that this dissertation entitled **"OPEN COMPARATIVE STUDY OF MELAGENINA PLUS LOTION AND MELAGENINA PLUS LOTION ALONG WITH UVA TO EVALUATE THE RATE OF REPIGMENTATION IN VITILIGO PATIENTS"** is a bonafide work done by **Dr. K.JAGANNATHAN**, Post graduate student of the Department of Dermatology and Leprology and Institute of Venereology, Madras Medical College, Chennai- 3, during the academic year 2004 – 2007. This work has not previously formed the basis for the award of any degree or diploma.

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Declaration

I, **Dr. K.JAGANNATHAN,** solemnly declare that dissertation titled, "**OPEN COMPARATIVE STUDY OF MELAGENINA PLUS LOTION AND MELAGENINA PLUS LOTION ALONG WITH UVA TO EVALUATE THE RATE OF REPIGMENTATION IN VITILIGO PATIENTS"** is a bonafide work done by me at Madras Medical College during 2004-2007 under the guidance and supervision of **Prof. Dr. B. PARVEEN, M.D.,D.D.,** Professor and Head, Department of Dermatology, Madras Medical College, Chennai-600 003.

The dissertation is submitted to The Tamilnadu, Dr. M.G.R. Medical University, towards partial fulfillment of requirement for the award of **M.D. Degree in Dermatology, Venereology and Leprology (BRANCH – XII A).**

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CONTENTS

INTRODUCTION

Vitiligo is a pigmentary disorder of universal distribution and unknown etiology with various precipitating factors. Vitiligo is asymptomatic and benign in terms of human life, of uncertain prognosis and fatal from the aesthetic point of view. It modifies the conduct of the patients, and quite frequently affects the family dynamics.

Throughout history this has lead to sustained research on its etiology and to the search for a medication that will effectively induce repigmentation without undesirable side effects.

At present the various therapeutic options include topical and systemic photochemotherapy with psoralens, topical and systemic steroids and immunomodulators. Off late topical tacrolimus and narrow band UVB therapy is gaining importance. Apart from medical therapies various surgical and cosmetic procedures are being carried out in patients with stable vitiligo.

Since far back in time, numerous drugs for topical and systemic therapy have been used for vitiligo, many only with a placebo effect, which in their times were considered a panacea for the treatment of vitiligo. Time itself has served to discard them, be it for their inefficacy or for the higher incidence of side effects.

Hence there is a need to find an alternative therapy that will offer the patient an option for improvement or cure, avoiding these side effects.

Melagenina is one such biological product, manufactured in Placental Histotherapy Center, Havana, Cuba since 1973. The center claims the repigmentation rate of 80-84%, 2 years after starting treatment with Melagenina, without local and systemic side effects. It was also claimed that addition of calcium chloride to Melagenina enhances its potency and it was marketed under the name Melagenina plus lotion. This product was available in India since 2003. This product was tested in various other countries with encouraging results. The aim of our study is to find the efficacy of Melagenina plus lotion in patients with localized stable vitiligo, when used alone and in combination with UVA in our Indian population.

REVIEW OF LITERATURE

VITILIGO:

Vitiligo is a common, specific, usually progressive, melanocytopenic, often heritable, acquired disorder characterized by well circumscribed milky white cutaneous macules and patches, affecting skin and mucous membranes.

Historical Aspects:

The term Vitiligo was first used by the Roman physician Celsus in his Latin medical classic De Medicina in 30 AD.

According to Bateman 'the white and glistening appearance, bearing some resemblance to the pale pink flesh of calves (vituli) seems to have given rise to the generic term vitiligo. El Mofty suggests that vitiligo is derived from the Latin word 'Vitelius' which means 'calf' referring to the characteristic white patches of the disease resembling the white patches of a spotted calf.[1] Other authors however believe that the term is derived from the Latin word 'vitium' meaning a fault or blemish.[2]

It is interesting to note that the Rigveda (6000 BC or earlier) named leukoderma as 'kilas', meaning a white spotted deer.The earliest reference to the disease is in 2200 BC in the ancient literature of Iran, 'Tarkh-e-Tibbl-e-Iran'. The oldest information concerning vitiligo comes from the 'Ebers Papyrus' writings about Pluraonic medicine in 1550 BC.[3]

In 1400 BC leukoderma has been mentioned as a variety of leprosy (Swetha Kustha) and several herbal remedies have been mentioned in the

Ancient Indian sacred book, 'Atharva Veda'.[4] These remedies highlighted the value of Vasuchika which was much later identified with the plant Psoralia corylifolia, oil from the seeds (bouchi seeds) contain active furocoumarin.[5] A similar drug, 'pu-ku-c' for treating leukoderma has been mentioned in the ancient Chinese literature.

Much later in the 13th century, Ibn Eb Bitar in Egypt mentioned the cure of leukoderma by an Egyptian herb known as Ammi Majus, from the fruit extract of which important furocoumarins were eventually identified in the 20th century.[6]

In Amarkosha the term svitra has been used synonymously with 'padosphota' meaning 'flower of legs', 'twakpuspi', meaning 'flower of skin', and 'sishmati', which means 'spreading whiteness'.In Vinayak Pitak (624-544 BC) the sacred book of Buddhism, there is mention of a disease associated with white spots and persons so suffering were not eligible for ordainment. A disease suggestive of vitiligo finds mention in the writings of Greek historian Herodotus (484-425 BC) .[7] In ancient Arabic books, 'white skin' was expressed as 'baras'. The word baras is mentioned in the Koran Ch 3 v 48 and Ch 5 v 109.

The white spots were also described in the Old Testament under the Hebra word 'Zora at', and this word was translated as 'lepra' in the Greek and English translations of the Bible.[7]

Stigma attached to Vitiligo:

The Stigma associated with vitiligo dates back to ancient times and continues to date in some culture. In ancient times Vitiligo was considered as a God's punishment for sins.

…....The priest shall put him in isolation for seven days … (Leviticus 1405 BC)

……Man and women suffering from this disease (white spot) are not eligible for ordainment (Vinayak Pitak 624-544 BC)

…….He must have sinned against the sun' and often the sufferers were shunned by their society…..Must be forced to leave the country (Herodotus 484-425 BC)

…….A person who had stolen clothes in his former existence may suffer from 'svitra' (Manusmruti 200BC)

Pandemics of leprosy in the middle ages as well as abundant myths particularly in underdeveloped countries, cast the 'unclean spell of leprosy; on those with vitiligo.[8] In South India, vitiligo is still called 'Ven Kushtam' translated as 'white leprosy'. As the social stigma makes patient conceal their white spots, even today in the Indian Villages, the disease is often called 'Charak' meaning 'the secret disease'.

EPIDEMIOLOGY

Vitiligo is relatively common disorder affecting all races across the globe.[9] Both sexes are equally affected.[10] The female prevalence in some studies probably attributed to greater concern about a cosmetic defect.[11]

The Incidence ranges from 0.14 to 8.8% across the globe.[12] The highest incidence has been recorded in India and Mexico. The Incidence is roughly estimated to be between 3-4% in India[13]. In India highest Incidence has been reported from the states of Gujarat and Rajasthan[14]. Vitiligo appears to be observed more commonly in sun-exposed areas and in darker skin types.

Vitiligo may develop at any age. Onset has been reported from birth to 81 years of age. Congenital vitiligo is however very rare.[15] The peak age of onset in all series was between 10 and 30 years. In 50% of cases the Onset is before 20 years of life.

AETIOPATHOGENESIS

Although vitiligo is generally recognized as a single entity, the etiology is complex. There appears to be a certain genetic predisposition and a number of potential precipitating causes.

GENETIC FACTORS and INHERITANCE

A positive family history has been observed in 30-40% of vitiligo patients suggesting a genetic basis for this disorder.[16] Monozygotic twins have been seen to have vitiligo with a similar or dissimilar mode of onset, type, extent and course of the disease.[17]

It is likely that vitiligo is not transmitted in a simple Mendelian autosomal dominant or recessive pattern. The transmission is more complex, polygenic with variable expression. No definite human leukocyte antigen (HLA) association is established for vitiligo although increased incidence of HLA DR4 in black people, HLA B13 in Morocean Jews and HLA BW35 in Yemenite Jews with vitiligo have been reported.

An association between the Catalase gene (CAT) and vitiligo has been suggested.[18] A novel gene named VITI, possibly associated with vitiligo, has been identified recently by differential display.

Increased incidences of Diabetes mellitus, thyroid diseases and atopic diathesis in the family members have been reported.

Although no definite precipitating factor is ascertained, many factors have been incriminated which include local trauma, itching, friction, infections, infestations, gastrointestinal disturbances, emotional upset, pregnancy, parturition and surgery. However no precipitating factors can be identified in 50% of cases.

THEORIES ON THE PATHOGENESIS:

Theories on the pathogenesis of vitiligo centered on mechanisms for the destruction of melanocytes as there are no melanocytes present in the fully evolved white macules. Traditionally there have been three hypothesis to explain vitiligo.[19]

1. Neural Hypothesis
2. Self destructive Theory
3. Autoimmune Theory

1. NEURAL HYPHOTHESIS [20, 21]

Evidences in favour of neural hypothesis include

A. Stress and severe emotional trauma is a known initiating or precipitating factor in vitiligo.
B. The common embryologic origin of melanocytes and the nervous system.
C. Dermatomal distribution of segmental vitiligo.
D. Demonstration of direct contact between cutaneous free nerve endings and epidermal melanocytes in vitiligo macules.
E. Demonstration of neuropeptides in the skin and their ability to regulate melanocyte differentiation has given more strength to this hypothesis.
F. An increased immunoreactivity of neuropeptide (NYP) or an altered balance of nerve growth factor receptors and calcitonin gene related peptide has been observed in vitiligo.
G. Alteration of the catecholamine pathway, increased catechol-o-ethyl transferase and monoamine oxidase activities and increased expression of B_2-adrenoreceptors has been described in vitiligo.

These alterations are said to induce melanocyte dysfunction and melanocyte injury by promoting the production of melanocytotoxic compounds and by decreasing the natural detoxification.

At present, however, the role of nervous system in vitiligo, if any, is poorly understood.

2. SELF DESTRUCTIVE THEORY by A.B.LERNER [22]

Lerner put forth that melanocytes in vitiligo have lost an intrinsic protective mechanism that eliminates toxic intermediates or metabolites in the melanogenesis pathway.

This theory stems from the belief that cytotoxic precursors to melanin synthesis accumulate and result in the death of melanocytes. Melanocytes synthesize melanin by oxidation of tyrosine to dihydroxyphenylalanine (DOPA) and to dopaquinone, which by a multistep reaction forms indoles. All the intermediates in the biosynthesis of melanin are phenols: excessive production or accumulation of phenolic radicals or intermediates within the melanocyte could damage the cell.[23]

It is well known that workers in rubber and plastic industries who are exposed to large quantities of phenols and catechols can acquire depigmentation that resembles vitiligo.

It has been suggested that melatonin receptor and melatonin could play a key role in vitiligo. Melatonin is known to stimulate the melanogenic pathway without the production of melanins, leading to an accumulation of

toxic intermediates which causes injury to keratinocytes and melanocytes with release of specific cellular proteins that initiate a secondary autoimmune reaction.

The presence of high levels of Hydrogen peroxide (H_2O_2) and low levels of catalase[24] in epidermis of vitiligenous skin suggests that there is an increased oxidative stress in vitiligo patients.[25] Several pathways could be involved in overproduction of H_2O_2 in vitiligo.

A. An abnormality in tetrabiopterin metabolism leading to over production of metabolites in this pathway, $6BH_4$ and $7BH_4$. The defective recycling of $6BH_4$ lead to formation of H_2O_2.[26]

B. Over production of H_2O_2 is claimed to result also from increased catecholamine biosynthesis in association with increased levels of monoamine oxidase A from inhibition of thioredoxin / thioredoxin reductase by calcium and increased nitric oxide synthase activities.

3. AUTOIMMUNE THEORY [27]

The association of vitiligo with autoimmune diseases suggested an immunologic basis for vitiligo.

A. Humoral immunity

1. There is an increased frequency of organ-specific auto antibodies in patients with vitiligo, even in the absence of any associated disease in up to 30% of patients. Antibodies to thyroid tissue, gastric parietal

cell, adrenal cytoplasm and pancreatic islet cell have been demonstrated.

2. Circulating antibodies to various melanocyte antigens, including tyrosinase, tyrosinase-related protein 1 and protein 2 have been demonstrated.
3. More recently, autoantibodies to a transcription factor called SOX10 have been found in vitiligo associated with APECED.

B. Cell mediated immunity

Evidence for the role of cellular immunity is even stronger. In marginal skin from progressive lesions of generalized and inflammatory vitiligo, an infiltrate of skin-homing (CLA+) cytotoxic T cells expressing granzyme/perforin is often found close to the remaining melanocytes. This infiltrate is composed of CD8 T cells, CD4 T cells and subsets of macrophages, and this correlates with the increased number of CLA+ MART-1 reactive CD8 T cells in the peripheral blood of patients with progressive vitiligo. These specific cytotoxic T cells react against the melanocyte differentiating antigens in vitiligo patients.[28]

It is not known whether these specific immune abnormalities are a cause or an effect of the disease, whether they damage melanocytes or aggravate melanocyte injury initiated by other causes, or are an irrelevant epiphenomenon.

4. OTHER HYPOTHETICAL THEORIES

A. Convergence theory suggests that genetic factors, stress, accumulation of toxic compounds, infection, autoimmunity, mutations, altered cellular environment, and impaired melanocyte migration and proliferation can all contribute to this disease.[29]

B. An intrinsic defect of the structure and function of rough endoplasmic reticulum in vitiligo Melanocytes.[30]

C. deficiency of Melanocyte growth factor

D. viral origin

E. dysregulation of Melanocyte apoptosis

F. Primary disturbance of T lymphocytes resulting in the development of "forbidden" clones of autoreactive lymphocytes in the epidermis.

CLINICAL FEATURES[31]

A typical lesion is a well defined depigmented (milky white or chalky) macule, round to oval in shape, has slightly brushed to fairly distinct, often with scalloped margins, measures from few mm to many cms in diameter, showing a variable number of depigmented (white) hairs and without any change in the skin texture.

The number, size, shape, and location of individual macules vary widely. Frequently the initial macule occurs on the exposed areas (such as the dorsal surface of hands, elbows, feets, legs, knees, neck and face), body folds (such as axillae, groin, and sub mammary region in women), lips or genitalia.

Of the usually covered areas, the initial lesion is often noted on the chest wall, lower back or areola. In general, with extensive involvement the distribution is similar to that of hyperpigmentation in Addison's disease (i.e., exposed areas, body folds, periumblical region, mucous membranes, and external orifices, sites of recent trauma or pressure or naevi). When lesions occur bilaterally, distribution is generally more or less symmetrical. The initial lesion may be one or more macules widely varying in size and shape. In some cases almost total depigmentation of body surface may develop slowly or rapidly with only a few or no islands of normal pigmentation. The lesions enlarge by invading the normally pigmented surrounding skin, which assumes a concave shape at the border. Virtually no area of skin is exempted.

Common sites of vitiligo lesions include the extensor surfaces of the body such as the pretibial regions, sides of ankles, knees, elbows and skin overlying the digits, periorificial areas such as periorbicular & circumoral, anogenital areas (glans penis, prepuce, vulva) and also flexor aspect of wrists, axillae, groins, lower back, loin, palms, soles, toe and finger tips and scalp. Involvement of pretibial region, palms and sole are quite common in India.[32]

The initial unifocal lesion may be followed by the appearance of new lesions elsewhere. In less than 25% of cases the onset may be multifocal. Onset of the lesions is usually insidious. The disease is progressive in nature as a rule and course is virtually unpredictable and may be quite erratic, it may be jerky, indolent or rapid. While some lesions may show signs of repigmentation, new

lesions may develop on other parts of the body simultaneously. There is an episodic phase of rapid extension of lesions after remaining quiescent over a long period of time.

Although no definite precipitating factor is ascertained, many factors have been incriminated which include local trauma, itching, friction, infection, infestations, gastrointestinal disturbances, emotional upset, pregnancy, parturition and surgery. However precipitating factor can be suspected in 50% of cases.

Emotional trauma and repression have been noted to be responsible for very sudden onset, rapid extension and spread of lesion. Such cases are referred to as 'valeceo' type vitiligo.

Koebner's phenomenon is observed in 6-20% of cases of vitiligo vulgaris.[33] Minor trauma such as scratch mark, laceration, or stitches on the skin results in the development of a corresponding linear depigmented macule, usually in 2-4 weeks. This isomorphic phenomenon indicates an abnormal pattern of cutaneous response to trivial physical trauma.

A positive family history, fewer lesions, less than 5% of body surface area involvement, frequent segmental involvement, and greater difficulty in treatment but relatively better prognosis are the hallmarks of childhood vitiligo.[34]

Morphological variations on the typical vitiligo macule[35]

1. Trichrome vitiligo refers to the presence of intermediate colour; this is a uniform tan coloration that is narrow to broad interface between the normally pigmented skin and central depigmented macule. It naturally evolves to a typical vitiligo macule later.
2. Quadrichrome refers to the fourth colour; this is macular perifollicular or marginal hyperpigmentation seen in some cases of repigmenting vitiligo.
3. Pentachrome vitiligo also may be observed. This includes a depigmented macule, tan, brown hyperpigmentation, blue-gray hyperpigmentation and normal pigmentation.
4. Blue vitiligo corresponds to vitiligo macules occurring in sites of post inflammatory hyperpigmentation.
5. Inflammatory vitiligo has an erythematous, raised border.
6. Confetti macules, which are typical in colour but only 1 to 2 mm in diameter, may occur randomly or may be perifollicular

Clinical Types of Vitiligo[36]

1. Focal vitiligo / Vitiligo areata is an isolated macule or a few scattered macules: the macules are limited in both size and number. 20% of children with vitiligo have the focal pattern.
2. Segmental / Dermatomal / Zosteriform Vitiligo: Segmental vitiligo is characterized by unilateral vitiligenous macules and patches in a dermatomal or quasi-dermatomal distribution. It has an earlier onset, slower

progression, stable course, non association with other diseases, non familial and it is resistant to treatment.[37]

Koebnerization is absent. 5% of adults and 20% of children with vitiligo are found to have this pattern.

- Trigeminal area involved in > 50% of cases
- Neck involvement 23% of cases
- Trunk involvement 17% of cases
- Multiple site involvement 13% of cases

Nearly half of these cases are associated with white hairs.

3. Generalized vitiligo / Vitiligo vulgaris: This is the most common type of vitiligo and is characterized by few to many widespread macules. These macules are often symmetrically placed and involve extensor surfaces of the trunk, extremites, periorificial areas and mucous membranes.
4. Acrofacial vitiligo involves distal digits and periorificial facial areas.
5. Lip – tip vitiligo: periungual involvement occurring with involvement of mucous membranes like lips, distal penis and nipples.
6. Vitiligo universalis / Universal vitiligo describe such widespread vitiligo that there are few remaining normal macules of pigmentation; this type has been associated with the multiple endocrinopathy syndrome.
7. Combination Vitiligo: very rarely vitiligo vulgaris and segmental vitiligo are seen in the same patient.

Vitiligo area scoring index[38]

The percentage of vitiligo involvement is calculated in terms of hand units. One hand unit (which encompasses the palm plus the volar surface of all digits) is approximately equivalent to 1% of the total body surface area. The degree of pigmentation is estimated to the nearest of one of the following percentages:

100% - complete depigmentation, no pigment is present;

90% - specks of pigment present;

75% - depigmented area exceeds the pigmented area;

50% - pigmented and depigmented areas are equal;

25% - pigmented area exceeds depigmented area; and

10% - only specks of depigmentation present.

The VASI for each body region is determined by the product of the area of vitiligo in hand units and the extent of depigmentation within each hand unit measured patch.

$$\text{Total Body VASI} = \sum_{\text{All body sites}} [\text{hand units X residual depigmentation}]$$

Vitiligo disease activity score (VIDA) [39]

The VIDA is a six-point scale for assessing vitiligo activity. Scoring is based on the individual's own opinion of the present disease activity over time. Active vitiligo involves either expansion of existing lesions or appearance of new lesions. Grading is as follows: VIDA Score

+4 – Activity of 6 weeks or less duration;

+3 – Activity of 6 weeks to 3 months;

+2 – Activity of 3 - 6 months;

+1 – Activity of 6 - 12 months;

0 - Stable for 1 year or more; and

-1 - Stable with spontaneous repigmentation since 1 year or more.

A low VIDA score indicates less activity.

Depending upon the activity of the disease process vitiligo classified as

1. Active – V1
2. Quiescent – V2
3. Improving – V3

Depending on the etiopathogenesis vitiligo classified as[40]

1. Immune (Progressive Vitiligo)
2. Neural (Segmental Vitiligo)
3. Chemical (Contact Vitiligo)

Other associated cutaneous abnormalities

1. Leucotrichia: Depigmented hairs are found commonly in isolated vitiligo macules, it has been reported in 9-45% of vitiligo patients. Presence of white hair may be a marker of poor prognosis in repigmentation.
2. Premature gray hair occurs in up to 37% of patients.[41]
3. Alopecia areata in 16% of cases
4. Halo naevi[42]
5. Psoriasis[43]
6. Lichen planus
7. Bullous pemphigoid
8. Dermatitis herpetiformis[44]
9. Atopic dermatitis[45]
10. Ichthyosis
11. Chronic actinic dermatitis[46]
12. Twenty nail dystrophy[47]
13. Connective tissue disorders: Morphoea, Lichen sclerosis, Lupus Erythematosus, DLE
14. Malignant melanoma[48]

Ocular Abnormalities

1. Iritis in 5% of patients.[49,50]
2. careful examination revealed depigmentation in choroid and retina in up to 30% of cases.[51,52]
3. Visual acuity is usually normal.
4. Vogt-Koyanagi-harada syndrome: a rare multisystem disease characterized by vitiligo, poliosis, uveitis, dysacousia and alopecia.

Otic abnormalities

1. Sensory neural deafness has been reported in a very few patients with vitiligo.[53]
2. Alezzandrini's syndrome: facial vitiligo, poliosis, deafness and unilateral tapetoretinal degeneration.

Systemic disease associations

1. Thyroid abnormalities: either hypothyroidism or hyperthyroidism[54]
2. Diabetes mellitus: occurs in 1 to 1.7% of vitiligo patients and conversely, vitiligo occurs in 4.8% of diabetic patients[55, 56]
3. Addison's disease in 2% of cases[57]
4. APECED: Increase in incidence (13%) of vitiligo in patients with autoimmune polyendocinopathy, candidiasis, ectodermal dystrophy has been established. This is particularly seen in patients with extensive vitiligo.[58]
5. Pernicious anaemia[59]

6. Hypoparathyroidism
7. Myasthenia gravis and Thymoma
8. Autoimmune hemolytic anaemia
9. Lymphomas and Leukemias
10. HIV infection[60]
11. Chronic active HCV infection
12. Rheumatoid arthritis

DIAGNOSIS

1. Usually clinical
2. Wood's lamp examination may be required to visualize macules in fair skinned individuals and macules in sun protected areas.
3. Histology.

HISTOPATHOLOGY:

Histopathology as a means of diagnosis is rarely employed in vitiligo but it useful when other cause of hypopigmentation needs to be excluded.

The histopathological changes classically associated with vitiligo are a complete absence of melanocytes in the basal layer of the epidermis with loss of melanin content of the epidermis. The upper dermis often has sparse superficial perivascular infiltrate of lymphocytes with a few melanophages.

On H& E stained sections melanocytes are recognized as randomly dispersed cells within the basal layer having a small rounded darkly staining nucleus and a clear cytoplasm as a result of shrinkage artifact. They are found

wedged between the basal cells of the epidermis and tend to protrude beneath the level of the basal cells appearing to hang down into the papillary dermis.

Histopathology of vitiligo in sections stained with H & E[61]

It varies according to the type of the lesion that is biopsied as well as the biopsy site.

1. Early evolving lesion: These are inflammatory lesions, histologically showing sparse to moderate dense lympho-histiocytic infiltrate that is usually present around the superficial blood vessels as well as around the adnexal structures and even around dermal nerve twigs. Focal interface changes with vacuolization of basal cells with few lymphocytes in the basal cell layer close to melanocytes are also seen in early lesions. Melanin is slightly reduced in the basal layer but with an almost normal complement of melanocytes.
2. Fully developed vitiligo of short duration: The epidermis is almost devoid of melanin but otherwise normal. Melanocytes are usually absent but an occasional melanocyte may be present. Such lesions often show the presence of several clear cells in the upper dermis, which have been identified as Langerhans cells. Inflammatory infiltrate is sparse, superficial and perivascular with few melanophages.
3. Long standing lesions of vitiligo: This shows complete absence of melanin from the epidermis and total absence of melanocytes. The epidermis is flattened with loss of normal rete ridge pattern and often

shows hyperkeratosis. The papillary dermis shows moderate thickening with increased number of fibrocytes with thin elongated nuclei and thickened collagen.

4. Pigmented margins of vitiligo lesion: Hyperpigmented margin may show increased melanin in the basal layer, increased number of melanocytes often with large dendrites, and large melanocytes that contain abundant melanin in their cytoplasm.
5. Repigmenting vitiligo: These lesions show an epidermis that is flat and thin with absence of melanin and melanocytes, a testimony to the depigmented lesion it was. The epidermis at places shows normalization of its architecture with reappearance of the rete ridge pattern, presence of melanin in the basal cells with few melanocytes that show heavily melanized dentritic process.

Special stains for melanin

1. Fontana-Masson stain:[62] Melanin is argyrophilic and use of silver stains indicates the presence of melanin. Argyrophilia is based on the ability of melanin to be impregnated with silver nitrate solution that on reduction with hydroquinone turns black. Melanin is also argentaffin and can reduce ammoniated silver nitrate in the absence of an external reducing agent forming black silver precipitate.
2. The DOPA Reaction:[63] This demonstrates functionally active melanocytes and although not of much practical importance in

diagnostic dermatopathology is instructive with regards to the biochemistry of melanization. Unfixed tissue sections of enzymatically separated epidermal sheets are incubated in a 0.01% of solution of 3-4 dihydroxyphenylalanine (DOPA). This stains functionally active melanocytes dark brown or black. Its role in vitiligo is restricted by the usual absence of melanocytes in this disease.

On the basis of DOPA reaction vitiligo may be classified as follows:[64]

A. Absolute : No DOPA positive melanocytes

B. Relative type 1: Weak DOPA reaction but normal number of melanocytes

C. Relative type 2: Reduced number of DOPA positive melanocytes

Immunohistochemical staining for melanocytes:

Immunoperoxide stains using S-100 protein and HMB 45 are used to identify melanocytes.

Differential Diagnosis:

1. Post inflammatory hypopigmentation
2. Pityriasis alba
3. Indeterminate Hansen's disease
4. Pityriasis versicolor
5. Post kala azar dermal leishmaniasis
6. Naevus depigmentosus
7. Chemical leukoderma

8. Piebaldism
9. Idiopathic guttate hypomelanosis
10. Albinism
11. Chediak-Higashi syndrome
12. Lupus Erythematosus
13. Ash leafy macule
14. Waardenburg's syndrome
15. Woolf's syndrome
16. Ziprokowski-Margolis syndrome
17. Incontinentia pigmenti
18. Halo naevus
19. Syphilis and Yaws.

COURSE OF THE DISEASE

The natural course of the disease is unpredictable and uncertain, most often showing tendency towards slow progression. Spontaneous repigmentation is noted in about 10-20% of patients, most frequently in sun-exposed areas and in younger patients.

Focal vitiligo, although stable for a time, may be a precursor of generalized vitiligo. Spontaneous resolution is possible. The natural course of vitiligo vulgaris is often abrupt onset, followed by progression for a time, then a period of stability follows and may last for some time, even decades. This may be followed later by a period of rapid progression. Total

spontaneous regression is unusual. The most common course is one of gradual progression of existing macules and periodic development of newer lesions.

Segmental vitiligo slowly progress for a relative period of one year and remains stable with little extension or regression. Tendency towards spontaneous pigmentation is rare.

PROGNOSIS

There is no reliable indicator of good prognosis, but the following factors usually indicate a poor prognosis:

1. Lesions on the resistant sites, such as bony prominences, non-fleshy areas, non-hairy areas and mucosal areas.
2. Higher percentage of white hairs in the patch
3. Extensive long standing cases
4. Associated with systemic diseases
5. Family H/O vitiligo
6. Old age
7. Iatrogenic factors, injudicious administration of topical and systemic medications.

MEDICAL MANAGEMENT OF VITILIGO

The various medical therapeutic options available today are able to give 60 -90% results either singly or in combination.

General principles:

1. Patient should be explained the nature of the disease and its unpredictable course and prognosis.
2. Reassurance is essential.
3. Balanced nutritious diet with good quality proteins, vitamin B complex, Vitamin E, and minerals such as copper, iron and zinc should be supplemented.
4. Avoidance of precipitating factors and drugs like Alpha interferon,[65] beta blockers,[66] chloroquine.[67]
5. Avoidance of soaps, detergents and substances containing phenolic compounds.
6. Avoidance of sunlight exposure, if necessary sunscreens are prescribed.

SYSTEMIC THERAPIES

Photochemotherapy :

This is the most widely employed systemic therapy for vitiligo. Treatment using a combination of psoralen derivatives or any other photosensitizing agent, orally or topically, followed by irradiation with Ultraviolet A constitutes photochemotherapy.[68]

Psoralens:

Photochemotherapy using the psoralen group of drugs in conjugation with UVA from an artificial source is called PUVA therapy.[69] When sun-exposure is utilized as a source of UVA, it is called PUVASOL therapy.[70] 5-methoxy psoralen and 8-methoxy psoralen are naturally occurring, whereas 4, 5, 8 trimethylpsoralen is synthetic.

Psoralens (8-MOP or TMP) are given in the dose of 0.6mg/kg/day followed by exposure to UVA after 1-3 hrs for 2-3 times a week. The treatment is started with a dose of 4J/cm^2 of UVA. Subsequently increments of 0.5J/cm^2 are made till a uniform erythema occurs over the lesions or a total dose of 8J/cm^2 is reached.

Exposure time in minutes is calculated by the formula:

$$\text{Exposure time in minutes} = \frac{16.7 \times \text{Dose in J/cm}^2}{\text{Irradiance in W/cm}^2}$$

Irradiance is the amount of UVR liberated from a given source measured by photometers in mW/cm^2. If sunlight is utilized as a source of UV light the TMP is preferred as it is less phototoxic when compared to 8-MOP[71]. If there is no response after 6 months or 50 treatments, PUVA should be terminated.

Khellin [72, 73, 74, 75]

Khellin is a furanochrome used as a photosensitizer in the treatment of vitiligo along with UVA irradiation (KUVA). It is administered orally at a dose of 100mg, two hours before UVA exposure or applied topically as a 5%

cream. The major advantage is that it does not induce phototoxic erythema and thus considered safe for home treatment or treatment with natural sunlight.

Phenylalanine: PAUVA [76, 77, 78]

Phenylalanine has been used orally and/or topically with UVA exposure in treating vitiligo. A 5% aqueous solution of L-phenylalanine (50-100 mg/kg) was orally administered one hour before UVA radiation. Supplementation with 10% cream 20min before exposure yielded better results.

Other photochemotherapeutic agents:

1. Clofazamine
2. Griseofulvin
3. Sulphonyl ureas and Phenothaizines have dubious value.

Systemic corticosteroids:

Systemic steroids are mainly used in controlling the activity of the disease. It can be used alone or in combination with other immunomodulators like levamisole or topical agents for vitiligo. In order to reduce the side effects of systemic steroids it has been advocated in the pulse form, Oral Mini Pulse therapy (OMP) .[79]

This regimen comprises of administering 5mg of betamethasone with breakfast on two consecutive days in a week. For children, the dosage is 0.5mg for every 5kg of body weight is prescribed.

Systemic therapy with ACTH[80] (25-40 IU IM twice a week) and Oral prednisolone 10-40 mg/day for a period of 3months to 1 year has been tried in vitiligo with better results.[81]

Immunomodulators:

1. Levamisole is used alone or in combination with systemic steroids for control of active disease. It is useful if the patient has mild disease (a few lesions which are spreading slowly).[82] It is recommended in a dose of 150mg on two consecutive days per week for a period of 16 weeks. For children between 6-12 years the recommended dose is 100mg and 50 mg for children between 3-6 years.
2. Pentoxyphylline[83] has been documented to work as an immunomodulator by directly acting on cytokine production. In a dose of 400mg thrice daily with local and or systemic steroids gave excellent results in patients unable to take levamisole.
3. Isoprinosine[84] in a dose of 500mg/kg for a period of six months can be tried as a immunomodulator.
4. Recently a new immunomodulator, Suplatast tosilate[85] is found to be effective in patients with vitiligo.
5. Oral Zinc has been traditionally claimed to be effective in vitiligo.

Nutritional agents [86, 87]

Multivitamin therapy with folic acid, Vitamin B12 and Vitamin C has been reported to show repigmentation, particularly in children. Antioxidants given for a prolonged period has a beneficial effect in vitiligo patients.

Other systemic agents

Dapsone[88] in a dose of 100mg/day for a prolonged period claimed to be useful in segmental vitiligo. It probably acts by immunomodulation.

Nialamide, [89] a monoamine oxidase inhibitor in a dose of 150mg/day has been shown improvement in 50% of cases with segmental vitiligo.

Intramuscular Injection of aqueous extract preparation of human placenta (Placentrex) [90] has been advocated by some to achieve good results in vitiligo. It is considered as a biogenous stimulator.

Penicillamine[91] has been found to repigment vitiligo in patients with Rheumatoid arthritis.

Azathioprine[92] in dose of 50mg/day is effective in repigmenting vitiligo in patients with Air Born Contact Dermatitis.

Cyclosporine[93] and cyclophospamide[94] has been tried in vitiligo.

Canthaxanthin[95] a naturally occurring carotenoid, used as a food colouring agent and sun tanning agent, is useful in vitiligo of Type 1 and 2 skin as a orally effective cosmetic camouflage.

Combination of Calcium pantothenate 100mg with Para amino benzoicacid (PABA) 500mg twice daily may help repigmentation of vitiligo patches and leukotrichia associated with vitiligo.[96]

Recently a pineal gland hormone, Melatonin[97] has been found effective in vitiligo.

TOPICAL THERAPIES

Topical photochemotherapy :

1. Topical PUVA

Topical psoralen solutions are available as 0.75% and 1% 8-MOP and 0.2% TMP. These are diluted in isopropyl alcohol to achieve a 0.1% solution of 8-MOP and 0.01% of TMP. This is then applied on the lesion and exposed to UV radiation after 2-3 hours, initially weekly and later twice or thrice weekly. It can be used in patients having very few lesions and it is a safe option in children. An acute phototoxic reaction is an expected regular side effect.

PUVA bath is a safer alternative to oral Psoralens. 50ml of 0.75% of 8-MOP or 37.5ml of 1%8-MOP is added to 100 litres of water in a bathtub to obtain a concentration of 3.75mg/lt. The patient soaks in this solution for 15 minutes and is then exposed to UV radiation.

PUVA Bath suit: [98] 2 ml of 0.75% or 1.5ml of 1% of 8-MOP is added to 4 litres of water. Then a suit made up of absorbable cotton that is tailored to that individual is soaked in the solution, gently squeezed and the patient then puts it on for 15 min followed by UV exposure.

2. Topical PAUVA:

Topical Phenylalanine 10% cream application followed by UV exposure has been tried in few patients.

3. Topical KUVA:

Khellin 5% cream along with UVA proved to be effective in vitiligo.

4. Pseudocatalase:

This treatment consisted of application of Pseudocatalase and calcium chloride in a cream base to the vitiligo lesions twice daily and the patients were subjected to short term sub-erythemogenic UVB exposure twice weekly, one hour after application of the cream.[99]

Narrow band UVB therapy[100]

Narrow band UVB therapy is presently considered a treatment of choice for vitiligo. It is safe in children over 6 years of age. Narrow band fluorescent tubes (Philips TL-01/100W) with an emission spectrum of 311 nm are used for this therapy. It is said to be less erythemogenic than broad band UVB. It is given at a starting dose of 0.075 J/cm^2, biweekly, with 20% increments until erythema was achieved.

Topical steroids

Topical steroids have virtually become the first line of treatment in patients with a few localized lesions. Topical steroids like mometasone, fluticasone or prednicarbate that are less likely to produce atrophy would be preferred. Early lesions, facial lesions, flexural lesions, focal lesions in children comparatively showed better results. It may be used in combination with other therapies.

Topical Tacrolimus and Topical Pimecrolimus

Tacrolimus is a macrolide lactone produced by Streptomyces tsukubaensis. Tacrolimus by its immunomodulating activity found to be useful

in treating vitiligo. It is available as 0.03% and 0.1% ointment and it has to be applied twice daily for a period of 3 to 6 months, and recent reports claimed it as a success even when used alone as a monotherapy. Pimecrolimus is available as 1% cream.

Topical Placentral extract[101]

Human placentral extract, Placentrex lotion is an aqueous extract of fresh human placenta which gives clinical improvement in some cases when applied locally. 1 to 2 ml of the lotion depending on the size of the patch is rubbed gently for one minute, three times daily. Affected area was exposed to sunlight for 5 minutes for better results.

Melagenina[102]

Melagenina plus is a hydro alcoholic extract at 50% of human placenta obtained from healthy pregnant women in aseptic conditions, after normal deliveries. It has to be applied over the affected part once daily. Some studies in Cuba claimed that repigmentation occurred in 84% of cases treated with Melagenina.

Basic fibroblast growth factor[103]

Recent studies indicate that basic fibroblast growth factor is a putative growth factor for the melanocytes and is produced by keratinocyte in normal individuals. Recent experiments suggest that topical application of 0.5% B-FGF gel or injection weekly or biweekly along with sunexposure show evidence of repigmentation both clinically and histopathologically in humans.

Miscellaneous topical agents

Topical 5-Fluorouracil:[104] 5-FU cream has been used successfully in Japan for non-dermatomal vitiligo. Dermabrasion is followed by daily application of the cream under occlusive dressing for 7-10 days.

Minoxidil:[105] Topical Minoxidil with PUVA has been reported to repigment vitiligo patches better than those treated with PUVA alone. Minoxidil may retain the hair in the anagen phase during which the melanocytes are active and proliferate.

Topical crude coal tar in combination with topical steroids when given to vitiligo patients showed 50% repigmentation after 10-20 weeks of therapy.[106]

SURGICAL MODALITIES FOR VITILIGO

The various surgical procedures are designed with any of the following 4 aims.

1. Introduction of artificial pigments into the lesion for permanent camouflage.
2. Removal of the depigmented areas for ever.
3. Repopulation of the depleted Melanocytes by various grafts.
4. Therapeutically wounding the lesion so as to stimulate the Melanocytes from the periphery and the normal hair follicles to proliferate, migrate and repigment the lesion.

Patient selection:

1. Patients not responding to medical line of management.
2. Vitiligo lesions should be stable for minimum of two years.
3. Psychologically stable patients with realistic expectations.

Various surgical modalities include: [107]

1. Cosmetic tattooing
2. Excision and closure
3. Thin Thiersch's graft
4. Suction blister technique
5. Miniature Punch grafting
6. Therapeutic wounding: Dermabrasion, Laser ablation, LN cryosurgery, needling, Phenol or TCA application
7. Ultra thin grafting

8. Grafting of non-cultural epidermal suspension
9. Skin cultures -- autologous, allologous or foetal: either epidermis containing both Melanocytes and keratinocytes (or) pure Melanocytes alone.
10. Other Modalities – Trypsinized autograft injection, single hair transplant homologous grafting.

Depigmenting therapy for extensive vitiligo[108]

In extensive vitiligo which is refractile to the repigmenting therapies, bleaching or removing the remaining islands of pigments to achieve an uniform appearance may be cosmetically desirable.

20% Monobenzyl ether of hydroquinone in a cream base is applied to the remaining areas of pigmentation twice daily for 3-6 months. It produces a permanent depigmentation.

Nothing conclusive has really happened in the Medical management of vitiligo during the past few years. Researchers are looking for a more effective therapy for this common depigmentary disorder.

MELAGENINA PLUS LOTION

Melagenina lotion is a hydro alcoholic extract at 50% of human placenta obtained from healthy pregnant women in aseptic conditions, after Cesarean section.[109] It is used as a topical agent for repigmentation in vitiligo patients.[110]

COMPOSITION OF MELAGENINA PLUS

EACH 100 ML contain:	
50% Alcoholic extract from human placenta	-100 ml
Calcium chloride	-100 mg
Phospholipids	-40 mg
Lipids	-30 mg
Proteins	-93 mg
Total Cholesterol	-20-45mg/dl
Total nitrogen	-10-20mg/dl

Tests for identification of

- Amino acids: positive
- LIPOPROTEIN: positive
- L-Dopa oxidation: positive

Calcium content: 0.2-0.5mg/ml

Residue by evaporation: 0.004 – 0.008 g/ 100 ml

Product dispensed in 235ml amber coloured bottles.

MECHANISM OF ACTION

Melagenina plus is a 50% hydroalcoholic extract of human placenta with the addition of calcium chloride. It is obtained from healthy pregnant women in aseptic conditions, after Caesarean section.

The active principle of this extract is alpha lipoprotein with a molecular weight of 1500-4000 Dalton.[111]

This lipoprotein stimulates the melanocytes reproduction and the synthesis of the melanin pigment.[112] It also accelerates the oxidation of the L-DOPA amino acid in presence of sunlight, favoring its transformation to melanin after internal chemical processes.

A.Meyer Y S Nagishi (1986) described the role of calcium in the pigmentation process of the skin by means of stimulating the secretory activity of melanocytes which is related to the concentration of calcium.[113, 114]

It is stated that the calcium added to this product increases the permeability of the cell and therefore allows a better activity of the active principle in the extract and an increase in number of melanocytes in the vitiligo lesions.

APPLICATION GUIDELINES

Melagenina plus is a nontoxic biological product used as topical lotion. It should not be swallowed or injected. It is recommended to shake the bottle before using it.

The lotion has to be applied by rubbing it with the fingertips on the depigmented areas, once daily, at the same time. It should remain on the skin for at least one hour. Exposure to sunlight or ultraviolet radiation, 30 min after application seems to be beneficial in large number of patients.

It is preferable to take bath before application and it is ensured that the lesion is dry before application. Fans should be switched off before application. Cotton, gauze, towels, fabrics should not used for applying the lotion.

Melagenina plus can be applied to any body surface. By its alcohol content it can produce a bit of burning or itching sensation in mucous membranes like lips, genital organs and perianal areas. The drug should not enter the eyes.

Melagenina plus lotion should be stored in dark amber coloured bottle. It should be stored in a cool, dark place. There is no need of refrigeration.

It should not be used together with other drugs like psoralens, corticosteroids prescribed for vitiligo, since they can equally interfere with the effect caused by the lotion.

Ideally cosmetics, creams, deodorants and perfumes should not be used during the therapy. Bath soaps should be as neutral as possible.

Safety of the product: Regarding the safety of the lotion, despite the fact that the Melagenina lotion has been used for more than 25 yrs, no serious adverse reactions have been reported. Erythema in and around the depigmented areas may appear during the application of the drug, which would be an expected adverse reaction and would not require any treatment. All placenta donors were screened for HIV and HBsAg infection.

Melagenina plus is a nontoxic substance, which does not have any interactions with other drugs that the vitiligo patient may be using for the treatment of other medical problems like diabetes, hypertension and asthma and can be safely applied to any area in the human skin surface without any serious adverse effects.

It can be safely used during menstruation, pregnancy and lactation and women undergoing treatment to aid conception.

Efficacy of the product: [115] This product has an efficacy rate of 84% in patients suffering from vitiligo, 2yrs after initiation of therapy.

AIM OF THE STUDY

The Aim of the study is to evaluate of the rate of repigmentation in 6 months with Melagenina plus lotion and Melagenina plus lotion along with UVA in the treatment of localized stable vitiligo.

MATERIALS AND METHODS

Study Design:

Melagenina plus lotion

V/s

Melagenina plus lotion along with Ultraviolet A irradiation

This was a six month, randomized, open, prospective, parallel group, comparative study conducted in vitiligo patients attending the Vitiligo Clinic, Department of Dermatology, Government General Hospital, Chennai. This study was conducted from April 2005 to September 2006. (1 ½ years)

Forty patients, both men and women, 13-60 years of age with clinical diagnosis of vitiligo were eligible for enrollment in the study.

During the initial visit the patient's demographic details including the name, age, sex, marital status, occupation, and residential address were noted. A detailed history regarding the onset, duration and course of the disease, presence or absence of precipitating factors, family history, associated skin and systemic problems, treatment taken so far and its outcome were recorded. Dermatological assessment of the disease was carried out noting down the sites of involvement, total body surface area involved, total number of patches, size and distribution of the patches, presence of white hair in the patch.

Details regarding the margin of the patch, skin texture, presence or absence of perifollicular pigmentation, Koebner's phenomenon, associated other skin and systemic problems were noted. Focal sepsis was ruled out by referring the patient to ENT and Dental OPD for check up.

After collecting the preliminary reports the patient was assessed for eligibility for randomization. Randomization was performed according to computer generated random code. Treatment was identified by a code number either A or B according to treatment group.

Patients with code A received treatment with Melagenina plus lotion alone and patients with code B received treatment with Melagenina plus lotion along with Ultraviolet A irradiation from PUVA chamber in our department. The patients were asked to stick only to the study treatment as per the randomization code.

Table of Randomization

Group A	Group B
4	1
5	2
8	3
9	6
10	7
11	12
13	15
14	18
16	19
17	20
23	21
27	22
28	24
29	25
30	26
31	32
36	33
37	34
39	35
40	38

Inclusion Criteria:

1. Patients of both sexes.
2. Age group between 13-60 years.
3. Patients suffering from vitiligo on face, trunk & extremities.
4. Depigmentation not more than 10% of total body surface area.
5. Stable patches of vitiligo more than 1 year duration.
6. Patient not on any form of therapy for vitiligo for the previous 1 month.

Exclusion Criteria:

1. Mucosal vitiligo, unstable vitiligo, actively spreading vitiligo.
2. Vitiligo less than 1year duration.
3. Vitiligo more than 10% of body surface area.
4. Children less than 12 years of age.
5. Pregnant and lactating women.
6. Patient having associated systemic abnormalities (both endocrine and non endocrine)
7. Patient currently under other topical or systemic therapy for vitiligo.
8. History of photosensitivity or presence of photosensitive dermatoses.

After fulfilling the inclusion criteria, the patients were assigned the study treatments. Group A patients were asked to apply Melagenina plus lotion topically, whereas, Group B patients were subjected to Ultraviolet A radiation after application of Melagenina plus lotion topically.

Administration of the Study Treatment

- Melagenina plus lotion was applied (after shaking the bottle well), with fingertips for about 5 minutes ensuring skin is dry, once daily at the same time.
- Patients were advised to have a bath before applying Melagenina plus lotion.
- Patient should switch off the fan before applying Melagenina plus lotion.
- Clothes should not be worn for 30 minutes after application.
- Patients on study group B were exposed to Ultraviolet A radiation, one hour after application of Melagenina plus lotion, twice weekly for a period of 6 months.
- The initial dose of UVA is 4joules/cm^2 for a period for 6 min which was gradually incremented depending upon the erythema response.
- At the time of UVA exposure patient's eyes were protected using UVA blocking goggles and genitals were protected using a genital shield.

Melagenina plus lotion is available in 235ml amber coloured bottles commercially. Since it is impossible in our setup to give an entire bottle to one patient, we used a 60 ml amber coloured glass bottles with screw cap and inner plastic cap to dispense the Melagenina plus lotion. We used a measuring cup and plastic funnel to refill the glass bottle. During the visits the patients were

supplied with Melagenina plus lotion, 20 or 30 ml depending upon their body surface area involvement.

Total duration of study was 6 months. During the study period of 6 months medications either local or systemic, intended for other illnesses were allowed to be taken. But no other systemic medications or topical applications for vitiligo were allowed.

The visits on day 3 and day 7 ensured that the patients have understood and were following the protocol. This was checked during every subsequent visit by asking the patient about the method of application of the study medication. This ensured the compliance of the protocol.

Subsequently the patients were followed up once in two weeks for a total period of 6 months and once a month during the next 3 months to watch for any clinical relapse after using the medication for 6 months.

If the patient was on any other form of topical therapy for vitiligo, before commencing the study a wash out period of one month was given before prescribing the Melagenina plus lotion.

Any adverse effects during the study were recorded with importance to erythema and systemic side effects. If any adverse effects are observed then its severity, onset, course, action taken and relationship to study drug were recorded.

EFFICACY PARAMETERS

The primary efficacy variable was the percentage change in depigmentation from baseline to the end of study period. (i.e. 6 months)

The secondary efficacy parameters include the Physician's Global Improvement assessment and Patient's Global Assessment which was computed at the end of 6 months of the study.

During the initial assessment, estimation of body surface area (BSA) involvement was assessed using Vitiligo Area Scoring Index (VASI). The body was divided into five separate and mutually exclusive regions: Face and Neck, Upper extremities (excluding Hands), Lower extremities (excluding Feet), Hands and Feet and Trunk. Buttocks were included with the lower extremities.

One hand unit, which encompasses the palm plus the volar surface of all the digits is approximately 1% of total body surface area and was used as a guide to estimate the baseline percentage of vitiligo involvement of any body region. To eliminate variation in hand size, we defined a hand unit to be the volar hand, including the fingers of single investigator.

All the patients were followed up once in every two weeks, to look for any macular repigmentation and presence of adverse effects. At each follow up assessment the extent of residual depigmentation within each affected patch that had been present at baseline was estimated to the nearest of one of the following percentages: 0, 10%, 25%, 50%, 75%, 90%, or 100%. Any new

depigmented patches that developed during the study were also estimated using the hand unit method and were included in the VASI calculation.

For each body region the VASI was determined by the product of the area of vitiligo in hand units (which were set at 1% per unit) and the extent of depigmentation within each hand unit measured patch.

Standardized assessment for estimating the degree of pigmentation to derive the VITILIGO AREA SCORING INDEX (VASI).

At 100% depigmentation, No pigment is present.

At 90%, specks of pigment are present.

At 75%, the depigmented area exceeds the pigmented area.

At 50%, the depigmented area and the pigmented area are equal.

At 25%, the pigmented areas exceed the depigmented area

At 10%, only specks of depigmentation are present.

Total body VASI was then calculated using the following formula by considering the contributions of all body regions (possible 0-100)

$$\text{Total Body VASI} = \sum_{\text{All body sites}} [\text{hand units X residual depigmentation}]$$

Clinical photographs were taken at baseline and at each monthly follow up visits as an aid to the Global clinical scoring. They were not used to derive the VASI, which was instead determined by direct clinical examination.

The Physician's Global improvement assessment evaluated the overall change from baseline in VASI score on a 5 point scale.

Score	Improvement in %	Comments
1	76%-100%	Excellent improvement
2	51%-75%	Marked improvement
3	26%-50%	Definite improvement
4	1% -25%	Minimal improvement
5	0%	No change

Patient's Global assessment was evaluated based on the response to the treatment as perceived by the patient with or without comparison to previous treatment if any. This was assessed in a 4 point scale.

Score	
1	Much better
2	Slightly better
3	Same
4	Worse

Primary efficacy parameter, VASI was assessed at baseline and then once in a month during follow up for 9 months and Secondary efficacy parameters Physician's Global assessment and Patient's Global assessment were evaluated only at the end of 6 months.

The investigator performed all the efficacy evaluation except the patient's Global assessment.

STATISTICAL ANALYSIS

It was calculated that a sample size of 20 patients per group would provide 90% power to detect a 40% difference between treatments, assuming a common standard deviation of 40%.

Efficacy Analysis included all randomized patients either in Group A or Group B who completed the study period of 6 months.

Safety Analysis included all randomized patients who applied the medication at least once during the study period, irrespective of whether they completed the study or not.

The primary efficacy criterion was the reduction in percentage of depigmentation from the baseline as evaluated using VASI scoring.

Between groups comparison of the primary efficacy criterion was performed using analysis of covariance (ANCOVA) with the VASI score at baseline as Covariate. P-values less than 0.05 were considered significant.

Between groups comparisons for secondary variables were evaluated using the analysis of variance (ANOVA). The change from baseline of efficacy parameters within each treatment group was evaluated using the student t-test.

Descriptive statistics were used to evaluate baseline characteristics and adverse effects.

OBSERVATION

A total of 40 patients were enrolled in the study, with 20 patients randomized to receive treatment with Melagenina plus lotion and 20 patients to receive treatment with Melagenina plus lotion and Ultraviolet A irradiation (MUVA). All 40 patients were included in the safety population.

	Melagenina			**MUVA**		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
Total enrolled	10	10	**20**	8	12	**20**
Completed	10	9	**19**	6	10	**16**
Drop out	0	1	**1**	2	2	**4**

Out of these 40 patients, 35 patients completed the study for the period of 6 months. Five of these patients, 1 (5%) in the Melagenina group and 4 (20%) in the MUVA group, discontinued the study and their efficacy data was not included in the analysis. The higher percentage of patient dropouts in MUVA group is probably due to inability of the patients to come twice weekly for a period of 6 months. None of the drop outs are due to adverse effects. Most of the drop outs are between 4 to 12 weeks after commencing the treatment

Age and Sex distribution:

Melagenina plus			
Age	**Male**	**Female**	**Total**
13-20 yrs	4	3	7
21-30 yrs	3	5	8
31-40 yrs	2	1	3
41-50 yrs	0	1	1
51-60 yrs	1	0	1
	10	10	20

Melagenina plus + UVA			
Age	**Male**	**Female**	**Total**
13-20 yrs	1	3	4
21-30 yrs	4	6	10
31-40 yrs	3	3	6
41-50 yrs	0	0	0
51-60 yrs	0	0	0
	8	12	20

Out of the 20 patients enrolled for group A treatment plan (M), 10 were Males and other 10 were Females. 40% of them are in the age group of 21 to 30 yrs. Out of the 20 patients enrolled for group B treatment plan (MUVA), 8 were Males and 12 were Females, predominantly in the age group of 21 to 30 yrs (50%).

The mean age of patients in group A was 26.6 yrs and in group B it was also 26.6 yrs.

Disease duration in years:

	Melagenina	Melagenina + UVA
Total in years	2.6375 yrs (n=20)	2.0000 yrs (n=20)
Male	2.7000 yrs (n=10)	1.3750 yrs (n= 8)
Female	2.5750 yrs (n=10)	2.4200 yrs (n=12)

The mean duration of vitiligo was 2.64 yrs in patients enrolled for Melagenina group and 2.00 yrs in the MUVA group.

Type of vitiligo:

	Melagenina			Melagenina + UVA		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
Segmental	3	0	3	0	0	0
V.Vulgaris	0	3	3	8	8	16
Acral	3	1	4	0	1	1
Focal	4	6	10	0	3	3
	10	10	20	8	12	20

10 patients with focal vitiligo, 3 with segmental vitiligo, 3 with vitiligo vulgaris and 4 with acral vitiligo were included in Melagenina in treatment group. 16 patients with vitiligo vulgaris, 3 with focal vitiligo and 1 with acral vitiligo were included in the MUVA group.

Patients with mucosal involvement alone were not included in the study.

Total body VASI reduction:

Total body VASI	0 mon	2 mon	4 mon	6 mon
M	0.9976 (100%)	0.9763 (97.86%)	0.8668 (86.90%)	0.7793 **(78.12%)**
MUVA	2.3356 (100%)	2.1781 (93.26%)	1.9203 (82.22%)	1.5281 **(65.43%)**

The mean total body VASI in Melagenina group at baseline was 0.9976 (Taken as 100%) and in MUVA group it was 2.3356(100%) with a P value 0.005.

The mean total body VASI at the end of 2 months in Melagenina group was 0.9763 (97.86%) and in MUVA group was 2.1781 (93.26%). It shows that VASI reduction was earlier in patients belonging to MUVA group.

After the end of 6 months the mean total body VASI in Melagenina group was 0.7793 (78.12%) and in MUVA group was 1.5281 (65.43%) with a P value 0.02. The P value was statistically significant.

The total body VASI reduction in percentage after 6 months of therapy with Melagenina alone was 21.88% and 34.57% in MUVA group.

Area wise VASI reduction	Face& neck	Upper limb	Lower limb	Hands& Feet	Trunk
MELAGENINA					
0 mon	100 %	100 %	100 %	100 %	100 %
2 mon	98.23 %	88.38 %	98.70 %	100 %	100 %
4 mon	90.40 %	63.76 %	85.55 %	100 %	83.33 %
6 mon	86.11 %	48.61 %	68.89 %	90.58 %	55.56 %
MUVA					
0 mon	100 %	100 %	100 %	100 %	100 %
2 mon	96.97 %	85.56 %	91.70 %	100 %	92.50 %
4 mon	71.21 %	85.56 %	83.54 %	100 %	71.25 %
6 mon	40.91 %	61.50 %	67.44 %	100 %	50.62 %

The area wise VASI reduction in Melagenina group is as follows:-

Upper limb 51.39%, Trunk 44.44%, Lower limb 31.11%, Face & Neck 13.89% and Hand & Feet 9.42%.

The area wise VASI reduction in MUVA groups is as follows:-

Face & Neck 59.09%, Trunk 49.38%, Upper limb 38.50%, Lower limb 32.56% and Hands & Feet 0%.

The VASI reduction in vitiligo types of Melagenina group was noted as follows: - segmental vitiligo 48.38%, focal vitiligo 38.78%, vitiligo vulgaris 8.89% and acral vitiligo 9.42%.

The VASI reduction in vitiligo types of MUVA group was as follows: - focal vitiligo 43.55%, vitiligo vulgaris 34.24% and acral vitiligo 0%.

VASI reduction in vitiligo types	V.Vulgaris	Focal	Segmental	Acral
MELAGENINA				
0 mon	100 %	100 %	100 %	100 %
2 mon	100 %	94.46 %	94.24 %	100 %
4 mon	96.67 %	72.80 %	66.03 %	100 %
6 mon	91.11 %	61.22 %	51.62 %	90.58 %
MUVA				
0 mon	100 %	100 %	0	100 %
2 mon	92.55 %	96.77 %	0	100 %
4 mon	82.18 %	79.03 %	0	100 %
6 mon	65.76 %	56.45 %	0	100 %

The overall efficacy of Melagenina alone was 33.74% and MUVA was 37.01% after the end of 6 months.

Total reduction in VASI: Physician's Global assessment:

			Melagenina		MUVA	
Score			**No of Patients**	**(%)**	**No of Patients**	**(%)**
5	No change	0%	4	21.05%	3	18.75%
4	Minimal improvement	1% - 25%	6	31.58%	4	25.00%
3	Definite improvement	26%-50%	5	**26.31%**	6	**37.50%**
2	Marked improvement	51%-75%	2	10.53%	2	12.50%
1	Excellent improvement	76%-100%	1	10.53%	1	06.25%

The mean physician's global assessment score was 3.421 in Melagenina group and it was 3.375 in MUVA group (P value 0.911). Physician's global assessment clearly showed that only 26.31% of patients treated with Melagenina alone had definite improvement whereas 37.50% of patients treated by MUVA had definite improvement.

The mean of patient's global assessment in Melagenina group was 2.69 and in MUVA group it was 2.53 (P value 0.66).The P value in both assessment was statistically insignificant.

No serious cutaneous or systemic adverse effects were noted in the study. 1 patient (5%) in the Melagenina group and 3 patients (15%) in MUVA group developed erythema which did not warrant discontinuation of therapy.

94.44ml of Melagenina plus lotion per body surface area was utilized by patients in Melagenina study group and 91.46 ml per body surface area was utilized by patients in MUVA group.

This roughly works out to 1 bottle for 6 months in a patient with 2.5% of body surface area involvement of vitiligo.

The average cumulative joules per patient is 171.5 j/cm^2 in patients received UVA along with Melagenina plus lotion.

DISCUSSSION

This clinical comparative trial explored the efficacy of Melagenina plus lotion when used alone in comparison with Melagenina plus lotion with Ultraviolet A irradiation in patients suffering from vitiligo less than 10% of body surface area involvement.

This is a unique study in that it was the first of its kind in INDIA.

The baseline demographic data and baseline characteristics in both study groups, when compared were similar. The total number of patients with vitiligo vulgaris is more in MUVA group is probably due to the higher number of patients consented for undergoing Ultraviolet therapy. The overall efficacy of MUVA is slightly greater than Melagenina plus lotion alone after the end of 6 months. This shows that Melagenina alone can be effectively used as topical monotherapy in patients with localized stable vitiligo.

However patients in MUVA group showed significant reduction in total body VASI score in 2 months when compared to Melagenina plus lotion alone. The reduction in MUVA group is 6.74% when compared to 2.14% in Melagenina group in the first two months of study period. This clearly shows that significant repigmentation occurs earlier in patients treated with MUVA.

Patients with focal vitiligo and segmental vitiligo showed better clinical response to Melagenina plus lotion, when compared to vitiligo vulgaris. The clinical response to Acral Vitiligo was very poor.

Vitiligo affecting the sun exposed area of the body showed better response to MUVA, when compared to Melagenina plus lotion alone.

The secondary efficacy parameters like Physician's Global Assessment and the Patient's Global Assessment shows results slightly in favour of MUVA group.

Apart from transient erythema, no serious adverse effects were noted. There was no increase in pigmentation of surrounding normal skin, which shows that Melagenina has no effect on normal skin.

During the follow up period of three months after completion of the study, none of the patients in both study groups showed clinical signs of relapse evidenced by the fact that VASI remains the same as that of 6 months. This shows that pigmentation achieved by Melagenina plus lotion may be permanent.

If Melagenina plus lotion is continued for a prolonged time there is greater chance of achieving complete repigmentation of vitiligo.

Small sample size, lack of placebo control and shorter duration of study were the major pitfalls in this study.

Nevertheless Melagenina plus lotion when used alone or in combination with Ultraviolet radiation there was a definite improvement in repigmentation of localized stable vitiligo.

CONCLUSION

1. Melagenina plus lotion can be effectively used as topical monotherapy for treating localized stable Vitiligo. When used alone its overall efficacy is 33.74% after a period of 6 months.

2. When Ultraviolet A radiation was combined with Melagenina plus lotion (MUVA) there was significant reduction in VASI score at an earlier date, when compared to Melagenina plus lotion monotherapy.

3. The overall efficacy of MUVA is 37.01%, which was only slightly higher than Melagenina plus lotion monotherapy.

4. Localized Vitiligo in sun exposed areas like face and upper limbs showed better response to MUVA.

5. Melagenina plus lotion was most effective in focal and segmental Vitiligo than in Vitiligo vulgaris.

6. Melagenina plus lotion was safe as there were no serious systemic or cutaneous adverse effects apart from transient erythema in a few patients.

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PROFORMA

Serial NO :

Name of the patient :

O.P./I.P. NO :

Name of the Parent / Guardian :

Date of enrollment :

Age :

Sex :

Occupation :

Marital Status :

Residential Address :

HISTORY

H/O Presenting Compliants :

Duration of illness

days weeks months years

H/O Itching yes no

H/O drug intake before the onset of skin lesions yes no

H/O external application yes no details

H/O contact with chemicals yes no details

H/o Photosensitivity yes no

H/o Physical and emotional stress yes no

H/O trauma yes no

H/O GIT disturbances yes no

Family History present absent

H/O other systemic problems

PAST HISTORY

Diabetes mellitus		Hypertension		CAD	
TB		Epilepsy		Asthma	

PERSONAL HISTORY

H/O exposure to STD		present		absent	
Smoker		alcoholic			
Veg		Non Veg			

Menstrual History

Treatment details

GENERAL EXAMINATION

DERMATOLOGIC ASSESSMENT

1. Site of involvement

Face & Neck		Upper extremities	
Trunk		Lower extremities	
Hands & Feet			

2. Total Body surface involvement in percentage

3. Total number of patches

4. Size of the patches X cms

5. Presence of white hairs in the patch yes no

6. Texture of the Skin normal abnormal

7. Margin of the patch

	Hyperpigmented		Trichrome
	inflammatory		Quadrichrome

8. Presence of perifollicular pigmentation yes no

9. Koebner's phenomenon yes no

10. Associated skin disease if any

11. Associated systemic disorders if any

PAST HISTORY

Diabetes mellitus ☐ Hypertension ☐ CAD ☐

TB ☐ Epilepsy ☐ Asthma ☐

PERSONAL HISTORY

H/O exposure to STD ☐ present ☐ absent

Smoker ☐ alcoholic ☐

Veg ☐ Non Veg ☐

Menstrual History ☐ ☐

Treatment details ☐ ☐

GENERAL EXAMINATION

DERMATOLOGIC ASSESSMENT

1. Site of involvement

Face & Neck	☐	Upper extremities	☐
Trunk	☐	Lower extremities	☐
Hands & Feet	☐		

2. Total Body surface involvement in percentage ☐

3. Total number of patches ☐

4. Size of the patches ☐ X ☐ cms

5. Presence of white hairs in the patch ☐ yes ☐ no

6. Texture of the Skin ☐ normal ☐ abnormal

7. Margin of the patch

☐	Hyperpigmented	☐	Trichrome
☐	inflammatory	☐	Quadrichrome

8. Presence of perifollicular pigmentation ☐ yes ☐ no

9. Koebner's phenomenon ☐ yes ☐ no

10. Associated skin disease if any ☐

11. Associated systemic disorders if any ☐

	Baseline	2 wks	4 wks	6 wks	8 wks	10 wks	12 wks
Size of the patch							
No of patches							
Erythema							
Perifollicular repigmentation							
Pigmentation over margins							
Hair pigmentation							

	14 wks	16 wks	18 wks	20 wks	22 wks	24 wks
Size of the patch						
No of patches						
Erythema						
Perifollicular repigmentation						
Pigmentation over margins						
Hair pigmentation						

Physician's Global Evaluation

	VISIT II 2 weeks	VISIT III 4 weeks	VISIT IV 6 weeks	VISIT V 8 weeks	VISIT VI 10 weeks	VISIT VII 12 weeks
Grade						

	VISIT VIII 14 weeks	VISIT IX 16 weeks	VISIT X 18 weeks	VISIT XI 20 weeks	VISIT XII 22 weeks	VISIT XII 24 weeks
Grade						

Grade	Improvement in %	comments
1	76%-100%	Excellent improvement
2	51%-75%	Marked improvement
3	26%-50%	Definite improvement
4	1% -25%	Minimal improvement
5	0%	No change

Patient's Global Evaluation

	VISIT II 2 weeks	VISIT III 4 weeks	VISIT IV 6 weeks	VISIT V 8 weeks	VISIT VI 10 weeks	VISIT VII 12 weeks
Grade						

	VISIT VIII 14 weeks	VISIT IX 16 weeks	VISIT X 18 weeks	VISIT XI 20 weeks	VISIT XII 22 weeks	VISIT XIII 24 weeks
Grade						

Grade	
1	Much better
2	Slightly better
3	Same
4	Worse

Adverse Events

Adverse Event	Severity	Onset	Course	Action	Relationship to study drug