"A HOSPITAL BASED PROSPECTIVE RANDOMIZED HALF BODY COMPARATIVE STUDY TO DETERMINE THE EFFICACY OF TRIPLE COMBINATION TREATMENT WITH FRACTIONAL CARBON DIOXIDE LASER PLUS TOPICAL TACROLIMUS 0.1% OINTMENT AND NARROW BAND UVB FOR REFRACTORY VITILIGO."

Submitted by

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Under the guidance of

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LIST OF ABBREVIATIONS

NBUVB	-	Narrow band ultra violet B therapy
SV	-	Segmental vitiligo
NSV	-	Non segmental vitiligo
WHO	-	World health organization
VAS	-	Visual analogue scale
VIDA	-	Vitiligo disease activity scoring
PUVA	-	Psoralen with ultra violet A
TCI	-	Topical calcineurin inhibitors
IL	-	Interleukin
TNF-**α**	-	Tumour necrosis factor alpha
IFN-**γ**	-	Interferon-gamma
UV	-	Ultra violet
MED	-	Minimal erythema dose
OPD	-	Outpatient section of the department

ABSTRACT

Background

Vitiligo is most common depigmenting disorder that affects both skin and mucosa. The exact pathogenesis of the disease remains unknown origin and has significant effect on quality of life. As few lesions remain recalcitrant and might take longer duration to re-pigment the quest for combining safest treatment modalities that would address the causes for attributed refractoriness continues.

Objective

To determine the efficacy of triple combination treatment with fractional carbon dioxide laser, topical tacrolimus 0.1% ointment and narrowband ultraviolet B in refractory vitiligo.

Method

It is a hospital based, randomized, half body, prospective comparative study. Forty four patients suffering from symmetrical recalcitrant vitiligo attending the OPD of a tertiary care hospital were included in this study. Detailed history and clinical examination were performed and recorded. Lesions on one side of the body were assigned randomly to weekly dose of fractional carbon dioxide laser followed by twice weekly NBUVB therapy plus twice daily topical 0.1% tacrolimus ointment (group A) and other side lesion was treated with twice weekly NBUVB therapy plus twice daily application topical 0.1% tacrolimus (group B). Pictures and adverse effects were noted during every visit. Results regarding repigmentation are calculated by calculating mean between percentage of repigmentation given by 2 experienced dermatologists by assessing the pictures.

Results

Out of 44 patients enrolled, 38 patients completed the study successfully.

Out of these 38 patients, 15 group A lesions achieved more than 50% or more than grade 3 repigmentation which was statistically higher (P=0.021) than 6 group B lesions that

showed similar response. The mean percentage of repigmentation noted among group A lesions was 33.6 was found to be significantly higher (P =0.032) than 21.6 noted in group B. The mean VAS scores of 3.8 in group A lesion was statistically greater (P =0.001) than 2.8 noted in group B lesions.

Though statistically not significant among group B lesions both treartment sites showed better repigmentation in lesion with early onset.

Most common adverse effect among laser group was pain followed by erythema. Erythema and itching were noted among group B lesions. None of these were serious enough to discontinue treatment.

Conclusion

Triple combination treatment with fractional carbon dioxide laser, topical tacrolimus and NBUVB is better modality for treatment of refractory vitiligo when compared to combination of NBUVB and tacrolimus.

Keywords: Fractional carbon dioxide laser, recalcitrant vitiligo, triple combination.

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INTRODUCTION

INTRODUCTION

Vitiligo is a common acquired pigmentary disorder, which is caused due to loss of melanocytes in the affected skin. Though exact cause remains unknown, many theories like autoimmune theory, neuronal theory, cytotoxic theory, oxidative stress theory, and melano-cytorrhagy theory have been put forward which are individually inconclusive but each of them partially contributes to the pathogenesis.[1]

The global prevalence of vitiligo is estimated to be 1% out of which highest incidence was recorded in India, followed by Mexico and Japan.[2] Although there is no sexual preponderance, greater numbers have been reported among females as they frequently seek medical attention early due to social stigma. Majority of the affected population belongs to the age group below 30 years.[3]

Vitiligo is characterized by depigmented macules/ patches with varying size, shape and number. Usually lesions are round to oval, with convex margins and may also involve mucosa. Itch / burning sensation are rare but may precede or accompany the onset of lesions.[2]

The treatment of vitiligo can be broadly classified as medical, physical, and surgical therapies and the choice of treatment modality depends on the extent, activity, and refractoriness of the disease. On occasions, monotherapy may not be effective completely when combinations of different modalities can be used to generate better outcomes.[4]

Refractory vitiligo can be arbitrarily defined as a vitiligo lesion most commonly over the extremities and / or bony prominences showing no signs of improvement with more than a year of conventional treatment.[4]

Out of the many implicated reasons for treatment resistance fewer active melanocytes within the lesion, less number of pilo-sebaceous units and poor absorption of the topical drug absorption are widely accepted.[5] Therefore an effective anti-inflammatory drug alone may not always be sufficient in all cases to induce re-pigmentation.

The above-mentioned causes may also be a reason for ineffectiveness of many systemic immunosuppressive therapies compared to other inflammatory skin disorders. Identifying and understanding and the subset of patients with these hurdles will help to formulate a better plan of treatment.[6]

Hence the quest of combining multiple effective treatment modalities to overcome the reasons of refractoriness and thereby improving the outcome of vitiligo are always in need.

Fractional carbon dioxide laser system is a recent advancement in the treatment of vitiligo which works on the concept of fractional photothermolysis. This method not only acts by chemostimulation of melanocytes but creates microscopic treatment zones that help in increasing the penetration of topically applied agent which indirectly improves drug efficacy leaving the surrounding skin unaffected. Compared to other ablative techniques this modality has better efficacy with minimal side effects.[4]

Another safest and effective topical treatment modality for the treatment of vitiligo is topical tacrolimus. This calcineurin inhibitor is nearly as efficacious as a mid-potent steroids and provides a advantage of using for longer periods with minimal and acceptable side effects unlike steroids. Moreover, some studies showed the synergistic activity that occurs when topical tacrolimus and ultra violet B (UVB) phototherapy are combined.[7]

In a study conducted by *Lepe et al*., to compare the efficacy of topical tacrolimus and clobetasol in 20 children with vitiligo, tacrolimus led to a mean percentage of re-pigmentation of with mild and transient adverse effects in 2 (10%) patient, whereas clobetasol resulted in a mean percentage of 49.3% but along with good re-pigmentation clobetasol showed adverse effects like atrophy and telangiectasia in 3 (15%) patients.[8]

The narrow band ultraviolet B (NBUVB) therapy has been postulated to act not only by melanocyte stimulation and migration but also suppressing autoimmune activity by depleting T lymphocytes.[7] Fractional carbon dioxide as an adjuvant with NBUVB also resulted in significant better outcomes compared to NBUVB alone. Moreover, extension of ablative laser treatment from the border of the lesion into the adjacent normal skin which is done in our study may probably trigger melanocyte migration from normal skin into the lesion.[1]

As the above mentioned modalities are currently existing state of the art methods with good safety profile, this study is conducted to determine the efficacy of triple combination treatment with fractional carbon dioxide laser plus topical tacrolimus 0.1% ointment and narrowband ultraviolet B for refractory vitiligo.

OBJECTIVE

OF THE STUDY

OBJECTIVE OF THE STUDY

1. To determine the efficacy of triple combination treatment with fractional carbon dioxide laser, topical tacrolimus 0.1% ointment and narrowband ultraviolet B in refractory vitiligo.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Vitiligo is a benign depigmentary disorder that occurs due to loss of melanocytes in affected area. It can occur in all individuals irrespective of age, sex, and race. Due to the obviousness of the disease, it has a significant effect on social, mental, and emotional patient's life as severe as psoriasis and chronic eczema.[2]

The history of vitiligo is dates back to 1200 - 1500 years by its record in early Hindu, Egyptian and Japanese literature. *Ebers Papyrus*, an Egyptian collection of writings from 1500 to 3000 BC was the first to differentiate leprosy from vitiligo. In 100AD, *Charaka Samhita*, the first ayurvedic text mentions the term *svitra* (whiteness) for vitiligo. Other traces were also found among the old *Koran* and Buddhist scripts. In few parts of India, the disease was considered as a punishment for the sins committed in previous life by few communities and the diseased women were not allowed to get married.[9]

Epidemiology:

Vitiligo is the commonest de-pigmentary disorder, with a worldwide prevalence of about 0.5–1%. The highest incidence has been recorded in India, followed by Mexico.[10] It is estimated that about 3-4% of India's total population suffer with this disease, and the world's highest prevalence (8.8%) was documented in Gujarat state.[11]

Though both the sexes are equally affected, females are believed to seek early medical attention due to the obvious stigma. Disease can manifest at any age but usually presents by the age of 30 years. Family history of vitiligo has been associated with in early disease onset.[2,12]

In general, childhood vitiligo shows more female preponderance and segmental distribution, whereas autoimmune or endocrine disorders association are more common in adults .[4]

Etiopathogenesis:

The exact pathogenesis of vitiligo remains unknown till date. Many theories have been proposed which partially contribute to the pathogenesis of disease but none of them are conclusive. Hence the disease is believed to be polygenic or can be considered as a common phenotypical manifestation for a spectrum of disorders. [2]

The theories/ mechanisms that have been proposed are: -

1) Genetic hypothesis
2) Autoimmune hypothesis
3) Neurohumoral hypothesis
4) Auto-cytotoxic hypothesis
5) Oxidative stress hypothesis
6) Biochemical theory of vitiligo
7) Melanocytorrphagy hypothesis
8) Decreased melanocyte survival hypothesis

Among all these theories, autoimmune hypothesis is widely accepted because of the numerous genetic studies in association with immune defects. Following the autoimmune theory, neurohumoral, oxidative stress, and cytotoxic theories have moderate evidence. Recently proposed theories like melanocytorrhagy and decreased melanocyte survival, have just began to ensue data. [2]

Classification:

Vitiligo can be broadly classified based on the type of distribution of depigmentation as segmental, non-segmental, unclassified and mixed types. These types and their subtypes are presented in table 1.[13]

Table 1: Classification of vitiligo based on type of distribution of depigmentation:

<table>
<tr><th>Segmental (SV)</th><th>Non-segmental (NSV)</th><th>Unclassified</th><th>Mixed</th></tr>
<tr><td>Uni-segmental</td><td>Acrofacial</td><td>Focal</td><td rowspan="4">Features of both SV and NSV</td></tr>
<tr><td>Bi-segmental</td><td>Mucosal (at least two sites involved)</td><td rowspan="3">Mucosal (either oral or genital)</td></tr>
<tr><td rowspan="2">Pluri-segmental</td><td>Generalised</td></tr>
<tr><td>Universal</td></tr>
</table>

[SV- Segmental vitiligo, NSV- Non segmental vitiligo]

Behl *et al.,* introduced another classification which categorises the disease activity and aids in assessing the treatment response and management. This clinical classification is presented in table 2.[13]

Table 2: Classification of vitiligo based on activity of disease.

Activity	Clinical features
Progressive vitiligo	• Developing new lesions • Increase in the size of existing lesions • Ill-defined borders of the lesion(s)
Quiescent vitiligo	• No appearance of new lesions • Stationary old lesions
Improving vitiligo	• Well-defined, hyper pigmented borders • Decreasing and / or disappearing lesions

Clinical features:

Vitiligo is characterized by the appearance of depigmented macules or patches which are usually round and / or oval shaped, often with scalloped or convex margins. The size of the lesions varies from person to person. Skin, hair and mucous membranes are affected either alone or in combination. The lesions are usually asymptomatic but rarely may be itchy or may have burning sensation during their onset. The disease is usually progressive and may exhibit exacerbations and remissions correlating with triggering events. Although any part of the skin and mucous membrane can be involved, the disease has a predilection for regions like face, axillae, areolae, genitalia, palms and soles. Furthermore, in an active disease lesions may exhibit Koebner's phenomenon.[3]

Vitiligo can present with varied morphological forms like:

- **Trichrome vitiligo:** It is characterised by the presence of intermediate colour zone between a vitiligo macule and normal pigmented skin.
- **Quadrichrome vitiligo:** It is a vitiligo lesion with fourth colour in it, which is usually perifollicular or marginal hyperpigmentation and is sign of repigmenting disease. It is usually seen in darker skin types.
- **Pentachrome vitiligo:** It is a rare variant of vitiligo in which there is a sequential display of white, tan, brown, blue gray hyperpigmentation and the normal skin.[3]

Stability of disease:

Determining the stability of vitiligo plays a crucial role in choosing the treatment modalities like oral immunosuppressive medications and suitability for surgical procedures. Features that are to be assessed for determining the stability are:

1. No change in the size of lesions
2. Absence of new lesions during this period
3. No signs of koebnerization
4. No increase in vitiligo disease activity scoring (VIDA)
5. Negative mini graft testing

Other criteria based on biochemical, serological, microscopic and ultrastructural correlation have also been described which are not routinely used in day to day life though they are significant. Recently dermoscopic criteria are being widely explored due its effectiveness and ease. This criterion focuses on perifollicular pigment as an important dermoscopic marker of vitiligo activity. [14]

Associated diseases:

World Health Organization, in its 10th international statistical classification of diseases has given a diagnosis code of L80 which was assigned to other conditions like acne and alopecia aerata which were once considered as cosmetic diseases.[15]

Vitiligo is associated with multiple autoimmune diseases. Approximately 15% to 25% of patients with vitiligo have autoimmune thyroid diseases like Grave's disease and Hashimoto's thyroiditis that present with hypothyroidism or hyperthyroidism. [15,16]

A study by Hann *et al.*, on 227 vitiligo patients has shown presence of anti-smooth muscle, antinuclear and anti-microsomal antibodies among 25.7%, 12.4% and 7.1% patients respectively.[17]

Sixty percent of patients with vitiligo were found to have cochlear dysfunction and 12% to 38% have sensorineural hearing loss, vision changes and abnormal tear production have also been reported.[15]

There is higher prevalence of pernicious anemia, myasthenia gravis, systemic lupus erythematosus, rheumatoid arthritis, atopic dermatitis, Sjogren syndrome, Addison disease, type 1 diabetes, scleroderma, chronic urticaria, ichthyosis, psoriasis, and alopecia areata among the vitiligo patients.[15]

Psychiatric illnesses have also shown a close association as the disease has a significant effect on quality of life. Patients with more emotional stress and low self-esteem are prone to suffer from anxiety and depression disorders.[16]

Diagnosis of vitiligo

When disease presents classically, the diagnosis is usually made on clinical grounds. In patients with an atypical presentation, diagnosis is more difficult and should be done using histopathological examination and using Wood's lamp. Histopathologically, vitiligo is characterized mainly by typical findings at the dermal epidermal junction. Lesional skin shows absence of melanin and loss of melanocytes and occasionally, there may be presence of lymphocytic infiltrate in the outer active part of the lesion.[18]

Treatment:

There are multiple modalities of treatment for vitiligo which are outlined in table 3.

Though recent biologicals like tofacitinib and ruxolitinib have given good results, there are no long term controlled trials regarding their safety. Despite numerous treatment modalities available for vitiligo, response to treatment may not be satisfactory at times. Hence, new treatment modalities and combination approaches are required for better outcome.[19]

Table 3: Common treatment modalities for vitiligo

Medical Therapy		**Physical Therapy**	**Surgical Therapy**	
Topical	**Systemic**	**Phototherapy**	**Tissue grafting**	**Cellular grafting**
Corticosteroids	Corticosteroids	PUVA	Punch grafting	Non cultured epidermal suspension
Tacrolimus/ Pimecrolimus	Cyclophosphamide	NBUVB	Split thickness skin grafting	Cultured melanocyte transplantation
Calcipotriol	Azathioprine	Excimer laser	Suction Blister epidermal Grafting	
Tofacitinib	Levamisole	Helium neon laser	Hair follicle grafting	
Ruxolitinib		Fractional carbon dioxide Laser		

[PUVA- psoralen with ultra violet A, NBUVB- narrow band UVB therapy]

Topical Calcineurin inhibitors

Topical calcineurin inhibitors (TCI) like tacrolimus and pimecrolimus are used in the treatment of vitiligo. Tacrolimus is a macrolide produced by the bacteria called *Streptomyces tsukubaensis.* Its structure and mechanism of action are similar to cyclosporine. Food and Drug Association approved dermatological indication of topical tacrolimus is atopic dermatitis. Vitiligo is one of the off labeled indications. Tacrolimus achieves better re-pigmentation over the face and neck than on other body areas.[20]

TCI act by inhibiting the activation and maturation of T cells by blocking the transcription of several cytokines, including tumour necrosis factor alpha (TNF-α), interleukin (IL)-2, IL-3, IL-4, IL-5, and interferon-gamma (IFN-γ). It also enhances T-cell apoptosis in vitro and melanoblast proliferation.[21]

Lan *et al.,* reported that the proliferation of melanocytes was significantly enhanced and there increase in concentration of stem-cell factor and matrix metallopeptidase-9 activity in keratinocyte supernatant treated by tacrolimus.[22]

Though both tacrolimus and pimecrolimus are being studied in detail regarding their use in vitiligo, tacrolimus holds an upper hand in studies regarding safety and efficacy, since its first use by Smith *et al.,* and Grimes *et al.,* in 2002.[23,24]

A comprehensive literature review which included 29 studies and 709 patients to determine the safety and efficacy of topical tacrolimus as monotherapy in treating vitiligo by Sisti *et al*., in 2016 has stated some interesting finding which are as follows:

1. Majority of the patients had 50% repigmentation only after 6 months of treatment and not usually before 2 months.
2. Best results were seen in the facial and neck lesions.

3. Lesions with twice daily application had better improvement than the lesions treated with single time.
4. In adult patients, treatment with tacrolimus 0.03% yielded similar results to tacrolimus 0.1% ointment.[25]

Treatment was generally well-tolerated without any serious adverse effects. The most frequent adverse effects were burning sensation and pruritus, local erythema or irritation, acne or folliculitis like manifestations, dysesthesia, stinging, formication and soreness.[25]

Choi *et al.*, in his retrospective study to evaluate the efficacy of tacrolimus 0.1% ointment in vitiligo among 79 adults and children found tacrolimus to be as efficacious as topical clobetasol and the group with shorter duration of lesions (< 6 months) showed statistically significant response rate than the group with longer duration. One more interesting finding was that the signs of repigmentation were early in TCI group when compared to steroid group.[26]

A randomized, double blind, comparative study conducted by Lepe *et al.*, to compare the efficacy of tacrolimus 0.1% and clobetasol 0.05% in 20 children did not show much statistical difference between both the groups. However, computerized monographic evaluation showed that tacrolimus is significantly more effective treatment with mild side effect of burning sensation among 2 patients (10%) than the clobetasol group in which 3 patients (15%) had lesional atrophy and 2 (10%) had telangiectasia by the end of 8th week.[8]

Similar double-blind, randomized, placebo-controlled trial conducted by Ho N *et al.*, in 100 children concluded that both tacrolimus 0.1% and clobetasol 0.05% ointments were equally efficacious in repigmenting both facial and nonfacial vitiligo. Though facial repigmentation was more striking in both groups.[27]

A review by Dang Y P *et al.*, to compare the efficacy of TCI as monotherapy or combined with phototherapy for vitiligo treatment has stated that TCI had a better therapeutic effect than placebo in the treatment of vitiligo with phototherapy. However, the typical UV resistant sites (i.e., hand and foot) were still difficult to cure even with combined therapy.[28]

Recently published guidelines by the European Dermatology Forum group proposed twice daily topical calcineurin inhibitors for vitiligo lesions as a first-line approach for a period of 6 months with moderate but daily sun exposure to evaluate the response. If results are satisfactory, treatment plan may be prolonged (e.g. longer than 12 months), as side-effects of long-term use of TCI are reassuring in other conditions such as atopic dermatitis.[29]

Narrow band ultra violet B therapy

The use of narrowband ultraviolet B light phototherapy (NBUVB), has become the mainstay of treatment for widespread vitiligo because of its efficacy and favourable side effect profile since first reported by Westerhof W *et al.*, in 1997. In this study, while comparing the treatment of vitiligo with NBUVB phototherapy vs topical psoralen gel PUVA for 4 months, UV-B therapy was more efficient and seems to produce faster repigmentation.[31]

In contrast to topical PUVA therapy, NBUVB showed multiple advantages like:

1. Fewer UV related adverse effects,
2. No lesional hyperkeratosis even after long-term treatment,
3. No difference in the contrast of repigmentation,
4. Safety in pregnancy,
5. Lesser duration of treatment sessions.[31]

The exact mechanism of action by which NBUVB plays a role in repigmentation in vitiligo is not known. It may exert its effects either by stabilizing the depigmentation process or by stimulation of residual melanocytes or both. It has been postulated that NBUVB stimulates amelanotic melanocytes in the outer hair root sheaths to proliferate, produce melanin, and migrate outwards to adjust depigmented skin, resulting in perifollicular repigmentation. Imokawa *et al.*, postulated that post phototherapy induced endothelin-1, interleukin-1, and tyrosinase in human keratinocytes both *in vitro* and *in vivo*, would plays a significant role in melanocyte mitogenesis, melanogenesis and melanocyte migration.[32]

In 2000, Njoo *et al.*, showed NBUVB to be effective and safe even in children. In this open trial authors used twice weekly NBUVB radiation therapy for treating 51 children with generalized vitiligo for a maximum period of 1 year. Among them 53% of patients achieved more than 75% overall repigmentation and the stabilization of disease was seen in 80%.[33]

Similar outcomes were reported in three other studies conducted by Brazzelli *et al.*, and Percivalle *et al.*, using twice- to thrice-weekly NBUVB therapy which showed good response in 50% and 42.9% respectively at the end 6 months. A prospective, open trail by Kanwar *et al.*, on 26 patients showed complete repigmentation in 75% patients who took NBUVB twice weekly for 12 months.[34,35,36]

A meta-analysis and literature review regarding nonsurgical therapies in generalized vitiligo by Njoo *et al*., in 1998 showed higher success rates with NBUVB (63%) than oral PUVA therapy (51%). This study was an important attempt to develop evidence-based guidelines for the treatment of vitiligo.[36] Parsad *et al.*, in a similar retrospective comparative study on 69 patients found NBUVB to be more

effective than PUVA and repigmentation induced with NBUVB was statistically significant and more stable.[37]

An open uncontrolled trail by Anbar *et al*., on 150 patients showed marked response in 64 patients (48%) out of 135 patients of non-segmental vitiligo whereas the other 15 segmental vitiligo patients showed very mild improvement. They also correlated duration of the disease and treatment response and noted that the extent of repigmentation decrease with increase in the duration of the disease.[38] Similar finding was also observed by Scherschun *et al.,* and Hallaji *et al.,* who analysed thrice weekly NBUVB as monotherapy for treating vitiligo.[39,40] An open trail, on 84 Asian patients by Nicolaidau *et al.,* added more evidence for the same finding and also stated that better outcome was seen in patients with skin types III-V.[41]

A retrospective, open study by Natta *et al.,* involving 60 recalcitrant adult and pediatric patients with recalcitrant vitiligo demonstrated effectiveness of NBUVB in 25 (42%) patients without serious side effect. The only clinical parameter that could differentiate nonresponders from responders was previous exposure to oral PUVA therapy, stating that failure to respond to oral PUVA therapy probably denotes a population of treatment-resistant vitiligo.[42]

Although widely used, there is no universally accepted protocol for NBUVB, so treatment protocols differ from study to study. Narrow band fluorescent bulbs of Philips TL-01, which emit a wave length 311 nm, are used for the treatment. Treatment is given performed twice or thrice weekly on non-consecutive days. The initial dose ranges from 100 to 280 mJ/cm^2. The dose is subsequently increased in most studies by 10% to 20% per session. In many studies, the dose is stabilized at 70% of minimal erythema dose (MED).[43]

Safety of NBUVB therapy was debated since its discovery. A retrospective study done by on 3867 patients Hearn *et al.,* to evaluate the risk of malignancy induction with NBUVB therapy has failed to show any significant relation.[44]

Recently published guidelines by the European Dermatology Forum group proposed NB-UVB as first line of therapy to stabilize and treat a case of non-segmental vitiligo for at least 3 months. If response there is clinical response, treatment can be continued for a optimal duration of at least 9 months.[18]

Ablative fractional carbon dioxide laser

Fractional carbon dioxide is a new addition to the armamentarium for treating vitiligo. The exact mechanism by which ablative laser plays a role in repigmentation is unknown. Kumar R *et al.,* proposed that during the inflammation and the healing phase, matrix metalloproteinase-2 is released, which in turn, may induce the migration of melanocytic stem cells from hair follicles, contributing to re-pigmentation.[45] A study by Ross EV *et al.,* proposed that immediate tissue retraction or shrinkage by ablative carbon dioxide lasers causes tissue narrowing of the treated vitiligo.[46]

As monotherapy with lasers showed limited benefits in repigmentation, several studies were conducted using combination therapies based on the concept of fractional photothermolysis proposed by Manstein D *et al.,* which states that fractional ablation causes selective tissue injury at required depth sparing the surrounding skin.[47] These micro channels might facilitate efficient delivery of topical agents. Epidermal barrier that has been impaired by lasers might also amplify the delivery and response of phototherapy.[48]

In 2012, Shin *et al*., compared combination of fractional carbon dioxide laser and NB-UVB therapy with NBUVB alone in 10 patients with recalcitrant vitiligo

among them only one patient (10%) had more than 50% improvement after four months of treatment with fractional carbon dioxide and NBUVB.[49]

A similar comparative study by El-Zawahry *et al.,* in 2017 studied two different fractional carbon dioxide treatment protocols combined with NBUVB therapy and NBUVB therapy alone. This study did not find any significant advantage of adding fractional carbon dioxide laser along with NBUVB therapy over NBUVB alone. Both these studies had different laser protocols.[50]

Hélou *et al.,* tried fractional carbon dioxide laser followed by sun exposure to treat 10 vitiligo patients. In this study, 6 patients (60%) had more than 50% repigmentation by the end of 3 months.[51]

In 2015, Li L *et al.,* tried a novel triple combination by using fractional carbon dioxide laser plus topical betamethasone solution and NB-UVB therapy for treating 25 cases of refractory vitiligo. By the end of 3rd month, more than 50% improvement was seen in 10 patients (44%) and remained stable at 6 months of follow up. The patient's satisfaction scores were better than the control group with minimal and tolerable adverse effects.[4]

In a pilot, parallel group trail by Yaun J *et al.,* in 2016, authors compared the efficacy of triple combination among 2 different ablative fractional carbon dioxide lasers and the same combination with a non-ablative laser and NBUVB alone in 20 patients. Authors found triple combination group to show better pigmentation rate than NBUVB group and the outcome with ablative fractional carbon dioxide lasers was better than the non-ablative fractional carbon dioxide laser. There was also no significant variation in results among 2 different ablative laser used in this study.[5]

Vachiramon *et al.,* in 2016 replaced betamethasone with clobetasol in above mentioned triple combination and also changed the monthly laser treatment protocol

to weekly sessions to treat the vitiligo lesions at refractory sites. In this comparative study involving 26 patients, clobetasol triple combination group showed good to excellent outcomes in 6 lesions (23.1%) which was significantly higher than control group treated with clobetasol and NBUVB which showed improvement in only 1 lesion by the end of 3 months. This may be explained by the difference in protocol of treatment used in that study as every patient subjected to fractional carbon dioxide laser at 1-week interval for 10 sessions combined with narrowband and 0.05% clobetasol propionate.[52]

In 2018, Chen W *et al.,* conducted a preliminary study involving 45 patients to treat vitiligo with fractional carbon dioxide laser and tacrolimus and inferred that this combination treatment was significantly effective in treating active vitiligo. Though there is risk a of isomorphic response, lesions in progressive phase repigmented better than the stable lesions.[53]

With available literature on fractional carbon dioxide laser and its combination therapies with NBUVB and topical medication, it is evident that it is relatively safe and promising tool in re-pigmentation of vitiligo. The present study is undertaken to determine the efficacy of triple combination treatment with fractional carbon dioxide laser plus topical tacrolimus 0.1% ointment and narrowband ultraviolet B for refractory vitiligo.

METHODOLOGY

METHODOLOGY

Source of data

Patients suffering from refractory, symmetrical vitiligo attending Department of Dermatology, Venereology and Leprosy of B.L.D.E.U's Shri B. M. Patil Medical College Hospital and Research Centre, Vijayapur, Karnataka, were recruited from the outpatient section of the department (OPD). The study was conducted between September 2016 and August 2018.

Method of data collection

Male and female patients of all ages, suffering from refractory, symmetrical lesions on extremities and/or bony prominences were taken into the study subjects. A total of 44 subjects were recruited into the study. Detailed history, examination and informed written consent was taken from all the study subjects.

Inclusion criteria:

1) All patients with symmetrical vitiligo lesions without improvement despite more than a year of conventional treatment including NB-UVB phototherapy, topical steroids and calcineurin inhibitors over extremities and/or bony prominences were taken into the study subjects.

Exclusion criteria:

1) Patients with active skin infections.
2) Patients who are pregnant and nursing.
3) Patients who are currently taking isotretinoin or have taken isotretinoin within the previous 12 months.
4) Patients with new, spreading lesions and positive Koebner's phenomenon within the preceding 1 year are excluded.

Method

Detailed history with respect to the onset and duration of symptoms, any treatment received, and pre-existing medical conditions were recorded from the patients/ parents in scheduled proforma.

Initial clinical examination of the patient was done and all the details were recorded on a body chart (1^{st} visit record). Before initiating the treatment, each patient was explained about the study and procedure in a simpler manner in local language (kannada/hindi).

The vitiliginous area was randomly assigned into two study groups.

1. Group A: treated with fractional carbon dioxide laser followed by NBUVB therapy plus topical tacrolimus (Study site)
2. Group B: treated with NBUVB therapy plus tacrolimus (Control site)

For patients assigned to group A the skin was cleansed using a mild cleanser before treatment. The study treatment side was randomly assigned among these patients. Local anaesthetic cream (2.5% lidocaine + 2.5% prilocaine) was applied under occlusion for 30-45 minutes to treatment site. The site was treated with fractional carbon dioxide laser with 10,600nm pulse. Immediately after the laser treatment, both sides were treated with NB-UVB in a phototherapy unit. The patients received 10-weekly sessions of Fractional carbon dioxide laser. The phototherapy sessions were given twice weekly for 20 sessions on non-consecutive days.

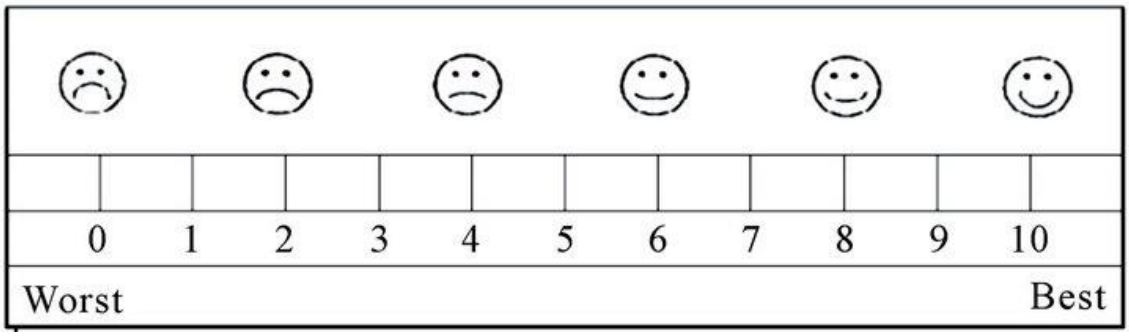
The subjects were then asked to apply topical tacrolimus 0.1% ointment over the lesions (both sides) on the same day of the procedure and continued twice daily throughout the study period. After the last treatment, the patients were followed up after 8 weeks. Standard digital photographs were taken at baseline, before each

treatment session, and 8 weeks after the final treatment. Improvement was scored objectively by two blinded dermatologists using a quartile grading scale.

- Grade 0 : no improvement
- Grade 1 : 1–25% repigmentation (minimal)
- Grade 2 : 26–50% repigmentation (moderate)
- Grade 3 : 51– 75% repigmentation (good)
- Grade 4 : > 75% repigmentation (excellent)

The mean of the scores evaluated by both dermatologists were used in the analysis. In addition, the patients were asked to grade their overall satisfaction using a 10-point visual analogue scale (VAS; 0= not satisfied at all, 10= extremely satisfied). Figure 1 shows visual analogue scale used in this study.

Figure 1: Visual analogue scale



Adverse events and complications were recorded at every follow-up visit.

Investigations

No investigations were performed in this study.

Statistical analysis

All characteristics were summarized descriptively. For continuous variables, the summary statistics of mean± standard deviation (SD) were used. Chi-square (χ^2) test was used for association between two variables by following formula:

The formula for the chi-square statistic used in the chi square test is:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

The subscript "c" are the degrees of freedom. "O" is observed value and E is expected value.

C= (number of rows-1)* (number of columns-1)

In cases of more than 30% cell frequency <5, Freeman-Halton Fisher exact test was employed to determine the significance of differences between groups for categorical data. The difference of the means of analysis variables between two independent groups was tested by unpaired t test.

The t statistic to test whether the means are different can be calculated as follows:

$$t = \frac{(\overline{x_1} - \overline{x_2}) - (\mu_1 - \mu_2)}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}}$$

where $\bar{x}_1$ = mean of sample 1

$\bar{x}_2$ = mean of sample 2

n_1 = number of subjects in sample 1

n_2 = number of subjects in sample 2

s_1^2 = variance of sample 1 = $\frac{\Sigma(x_1 - \bar{x}_1)^2}{n_1}$

s_2^2 = variance of sample 2 = $\frac{\Sigma(x_2 - \bar{x}_2)^2}{n_2}$

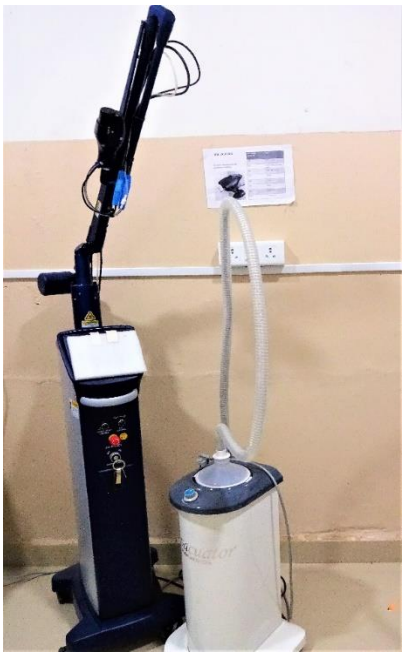
If the p-value was < 0.05, then the results were considered to be statistically significant otherwise it was considered as not statistically significant. Data were analysed using SPSS software v.23.0. and Microsoft office 2007.

Ethical clearance

Institutional ethical clearance was undertaken for the study

Figure 2: Whole body narrow band ultra violet B machine

Figure 3: Fractional carbon dioxide laser



RESULTS

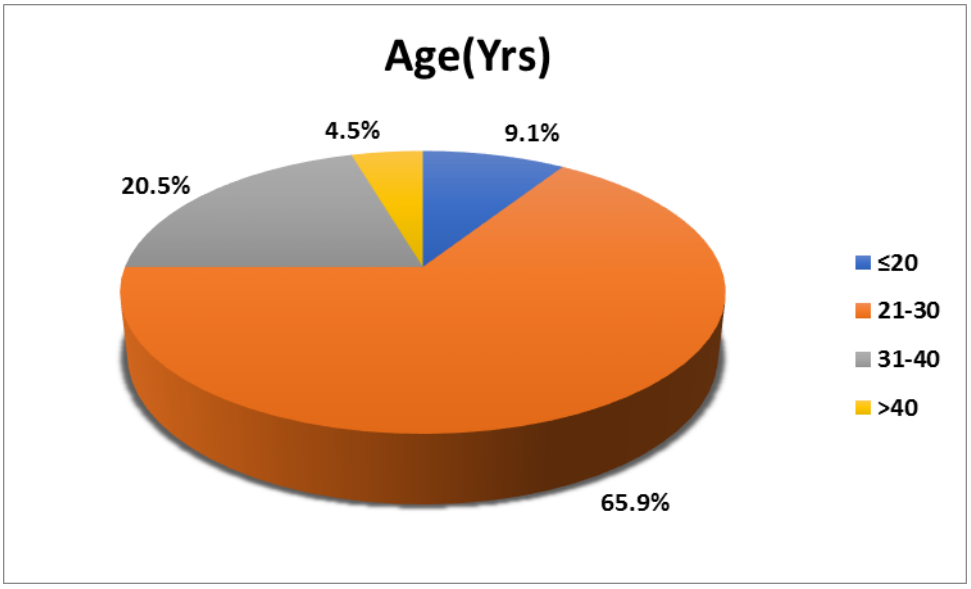
RESULTS

A hospital based prospective, double blinded, randomized, half body comparative study was conducted from September 2016 to August 2018. A total 44 cases of vitiligo were included in the study.

Age distribution

The age of the patients enrolled in the study group ranged from 15 to 46 years. The mean age (±SD) of the study population was 27.5 (± 6.4) years. The maximum number of patients were in the age group 21 to 30 years. The age wise distribution of the patients enrolled in this study have been presented in figure 4.

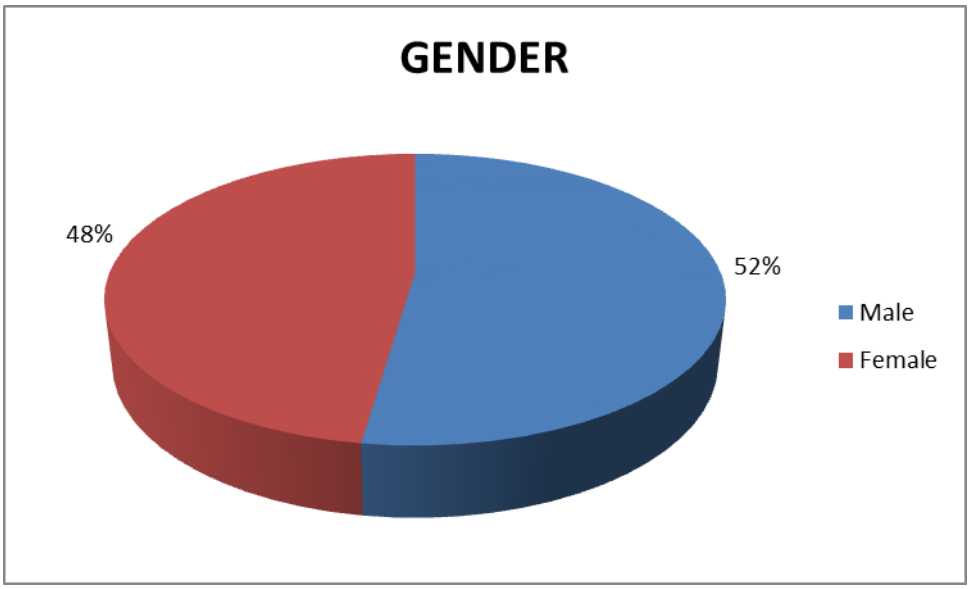
Figure 4: Distribution of cases according to age



Gender distribution

Among 44 cases, 23 (52.3%) were males, and 21 (47.7%) were females. The gender distribution of the patients with vitiligo have been presented in figure 5.

Figure 5: Gender distribution of patients with vitiligo



Duration of disease onset

The mean (±SD) of duration of disease since the onset is 6.2 (± 6.4) years among all the cases enrolled in this study. The mean duration of disease onset has been presented in table 4.

Table 4: Mean duration of disease onset

PARAMETERS	RANGE	MEAN	SD
Duration of disease	1-10	6.2	6.4

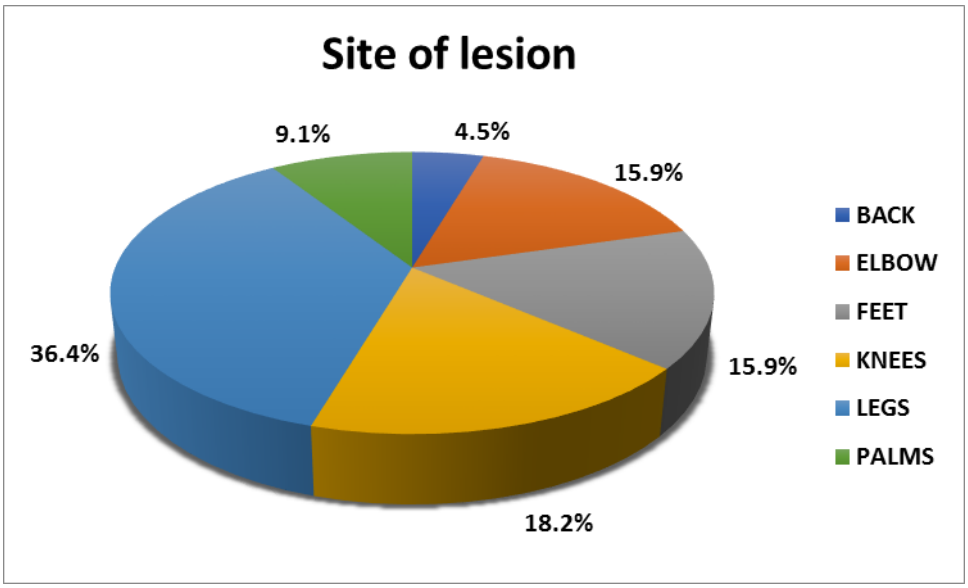
Family history

Family history of vitiligo was present in 6 (13.6%) cases out of 44 cases enrolled.

Site of lesions

Among the cases enrolled 16 (36.4%) cases had symmetrical vitiliginous lesions over the legs, followed by 8 (18.2%) lesions over knees, 7 (15.9%) lesions over elbows and feet each, 4 (9.1%) palmar lesions and 2 (4.5%) on lesions over the back. The percentage distribution of site of vitiligo lesions have been presented in figure 6.

Figure 6: Distribution of cases according to site of lesion



Among 44 vitiligo cases enrolled in the study, 38 (86.36%) patients completed the study at the end of 12 weeks. Six (13.64%) patients were lost to follow-up.

Grade of repigmentation

Among 38 cases who completed the study, group A lesions had better grade of repigmentation which was statistically significant (*P*=0.021). Good to excellent (i.e, ≥ grade 3) repigmentation was seen among 15 (39.5%) lesions in group A whereas similar grade of repigmentation was only in 6 (15.8%) lesions under group B. Lesions which showed nil, mild and moderate grades of re-pigmentation were 23 (60.5%) in group A and 32 (84.2%) in group B. The distribution of grade of re-pigmentation between case and control groups have been presented in table 5.

Table 5: Distribution of grade of re-pigmentation between study groups

Grade of re-pigmentation	GROUP A		GROUP B		*P* value
	N	%	N	%	
>3	23	60.5	32	84.2	0.021*
≥3	15	39.5	6	15.8	
Total	38	100	38	100	

Note: * significant at 5% level of significance (*P* <0.05)

Mean percentage of re-pigmentation

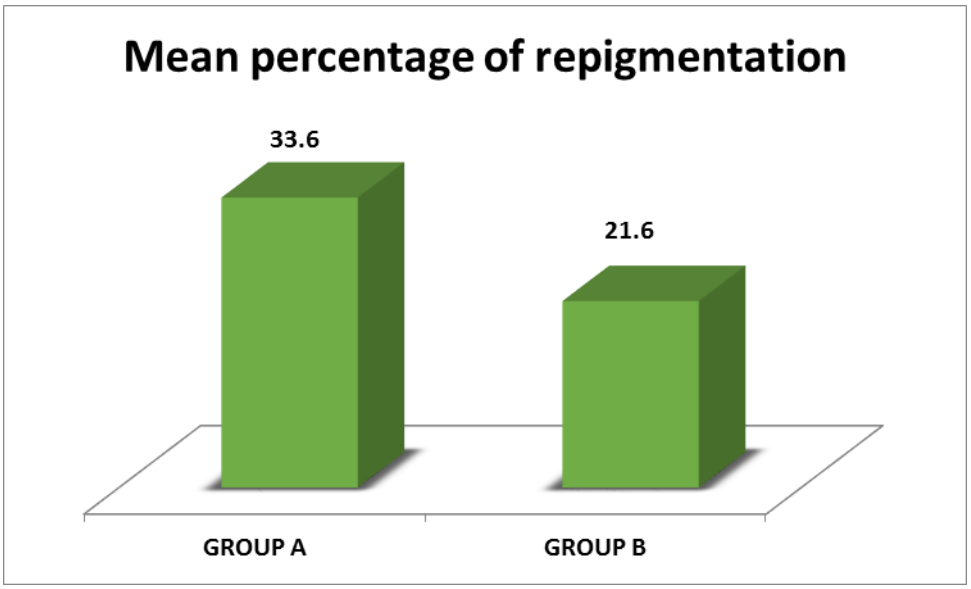
The percentage of repigmentation among group A lesions ranged from 0 – 80 with a mean (±SD) of 33.6 (±26.7). The percentage of repigmentation among group B lesions ranged from 0 – 70 with a mean (±SD) of 21.6 (±23). The repigmentation was statistically significant (*P*=0.032) among group A lesions as compared to Group B lesions. The mean percentage of re-pigmentation among group A and group B lesions have been presented in table 6 and figure 7.

Table 6: Mean percentage of re-pigmentation among study groups.

Mean percentage of re-pigmentation	Group A		Group B		*P* value
	Range	Mean (± SD)	Range	Mean(±SD)	
	0-80	33.6 (±26.7)	0-70	21.6 (±23.0)	0.032

Note: * significant at 5% level of significance (*P* <0.05)

Figure 7: Mean percentage of re-pigmentation among study groups



Time taken for initiation of repigmentation

The lesions in group A showed signs of re-pigmentation earlier than the lesions in group B though the difference was not statistically significant. Twelve (31.6%) lesions in group A showed signs of improvement in less than 6 weeks whereas only 5 (13.2%) lesions in group B showed improvement in the same duration. Nineteen (50%) lesions in group A and 22 (57.9%) lesions in group B took more than 6 weeks to show evidence of pigmentation. The number of lesions which showed no signs of re-pigmentation were 7 (18.4%) in group A which was comparatively less than 11 (28.9%) lesions in group B. The time taken for initiation of re-pigmentation among lesions in group A and group B have been presented in figure8.

Re-pigmentation according to site

The mean percentage of repigmentation (±SD) over the back, elbows, feet, legs and palms are 60 (±7.1), 20 (±25.9), 43 (±25.9), 31 (±29.6) and 13.8 (±4.8) respectively among the lesions in group A.

The mean percentage of repigmentation (±SD) over the back, elbows, feet, legs and palms are 47.5 (±10.6), 11.7 (±24), 26 (±27.2), 21.3 (±26.8) and respectively among the lesions in group B.

The values of mean percentage of repigmentation were higher in group A than in group B at all the sites. A statistically higher (*P* =0.025) value was noted at knees where the mean percentage of group A was 50 (±18.2) and group B was 26.7 (±12.1). The mean percentage of repigmentation among group A and group B lesions have been presented in figure 9.

Figure 8: Distribution of rate of repigmentation with respect to duration of treatment in study groups.

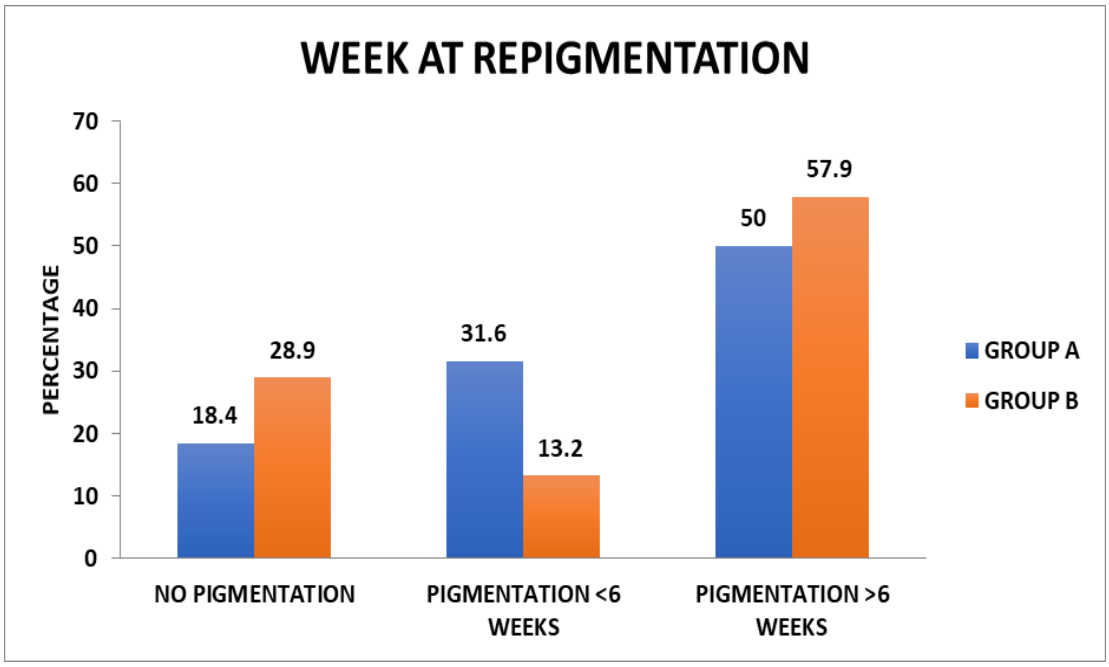
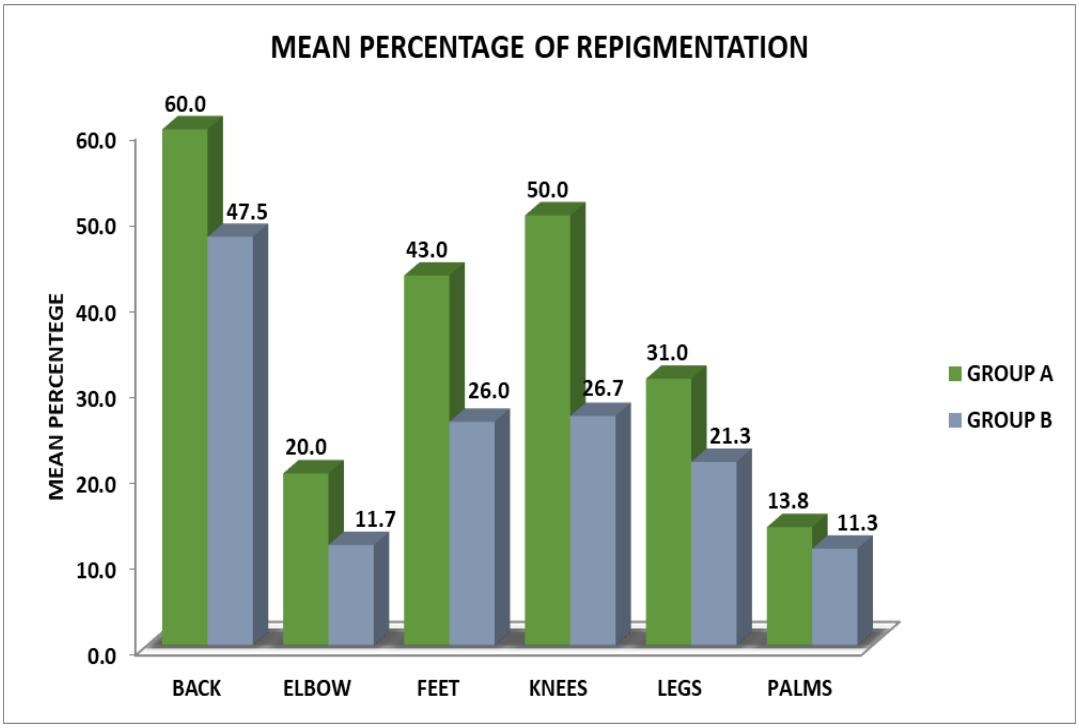


Figure 9: Mean percentage of repigmentation among Group A and Group B lesions



Relation between family history and mean grade of repigmentation

The mean grade of re-pigmentation was lower among the cases with family history of vitiligo than in those without. This was statistically significant (*P* =0.045). Cases with positive family history had mean (±SD) grade of re-pigmentation of 0.67 (±0.25) and cases without family history had a mean (±SD) of 1.63(±1.11). The mean grade of re-pigmentation in relation to family history in group A and group B have been presented in table 8.

Table 7: Distribution of mean grade of repigmentation in relation with family history in study groups.

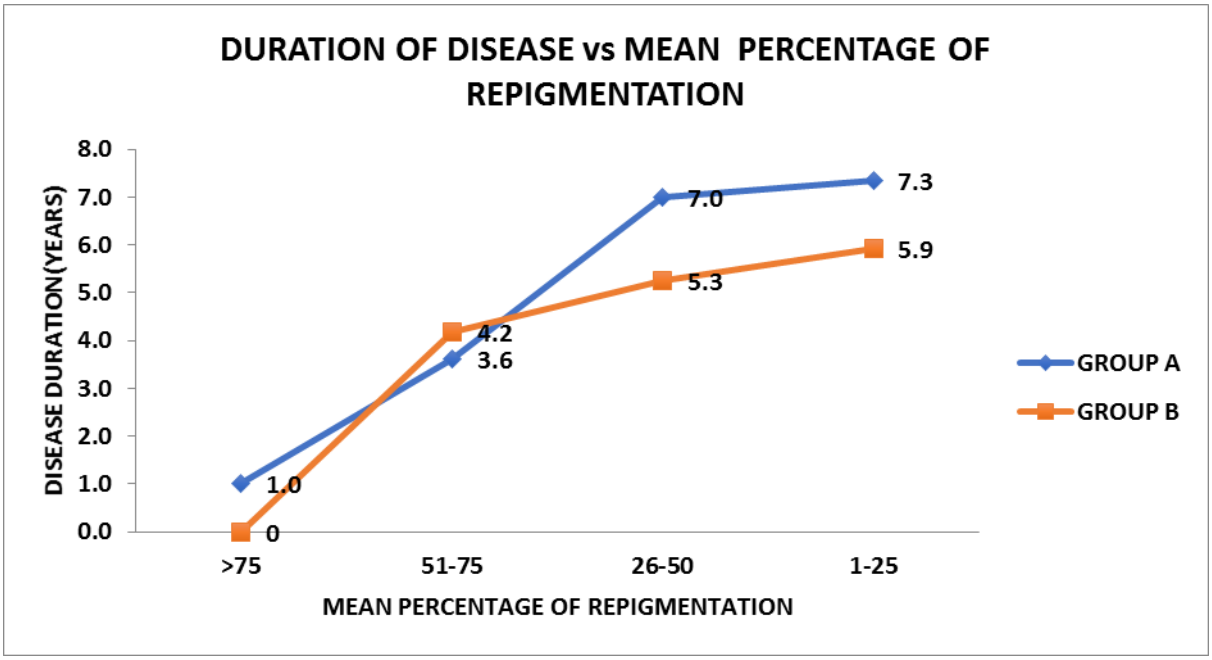
Mean grade of repigmentation	Negative family history	Positive family history	*P* value
	Mean (±SD)	Mean (±SD)	
	1.63 (±1.11)	0.67 (±0.26)	0.045*

* significant at 5% level of significance (*P* <0.05)

Evidence of repigmentation in relation to duration of disease

In group A lesions, duration of the disease showed declining trend with higher mean percentage of repigmentation. This was statistically significant(*P* <0.01). Although group B lesions also showed similar pattern it was not statistically significant. The mean percentage of repigmentation with respect to disease duration have been presented in figure 10.

Figure 10: Distribution of mean grade of re-pigmentation in relation with duration of disease in both the groups.



Type of re-pigmentation

In group A, marginal and diffuse re-pigmentation was seen among 12 (31.6%) lesions followed by marginal in 8 (21.1%), marginal and perifollicular in 6 (15.8%) lesions, perifollicular pigmentation in 3 (7.9%) and diffuse and perifollicular re-pigmentation in 2 (5.3%).

In group B, perifollicular pigmentation was seen in 10 (26.3%) lesions follwed by marginal pigmentation in 6 (15.8%) of lesions, mariginal and perifollicular in 5 (13.2%) lesions, diffuse pigmentation in 3 (7.9%) lesions and marginal along with diffuse in 2 (5.3%) lesions. The distributuin of type of repigmentation among group A and group B have been presented in table 9.

Table 8: Distributuin of type of repigmentation among group A and group B

Type of repigmentation	Group A		Group B	
	N	%	N	%
Diffuse	0	0.0	3	7.9
Perifollicular	3	7.9	10	26.3
Marginal	8	21.1	6	15.8
Marginal + Diffuse	12	31.6	2	5.3
Marginal + Peri follicular	6	15.8	5	13.2
Diffuse + Peri follicular	2	5.3	0	0.0

Visual Analogue Scale (VAS)

The VAS showed a mean (±SD) score of 3.8 (±2.2) in group A lesions and mean (±SD) score of 2.8 (±1.9) in group B lesions .A statistically significant (*P* <0.001) difference was noted between the two groups. The mean VAS scores among group A and group B have been presented in table 10.

Table 9: Mean VAS score between group A and group B

	Group A	Group B	*P* Value
VAS	Mean (±SD)	Mean (±SD)	
	3.8 (±2.2)	2.8 (±1.9)	<0.001*

Note: * significant at 5% level of significance (*P* <0.05)

Adverse effects

Out of the 38 cases who completed the study, adverse effects were noted in 6 patients . The most common adverse effect noted in group lesions A was pain during laser procedure in 8 (21.1%) cases followed by erythema at laser site in 4 (10.5%) cases. Although the number of cases who had adverse effects were less in group B, 2 (5.3%) cases experienced itching over the lesions post phototherapy and 2 experienced erythema following the treatment.

There was no serious adverse effect noted that need discontinuation of treatment. The adverse effects noted in group A and group B have been presented in figure 11.

Figure 11: Distribution of adverse effects noted in group A and group B

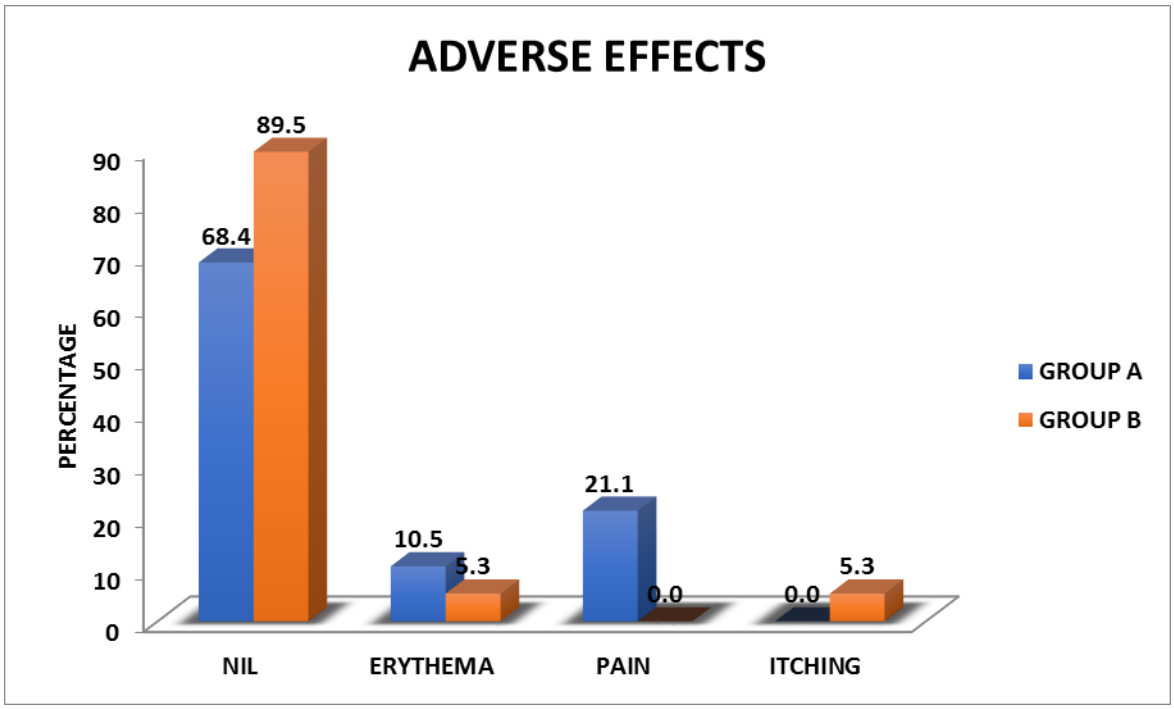


Figure 12a and 12b are the clinical photographs showing grade 3 re pigmentation at test site (Left side) and grade 2 at control site (Right side) based on mean percentage of repigmentaiton.

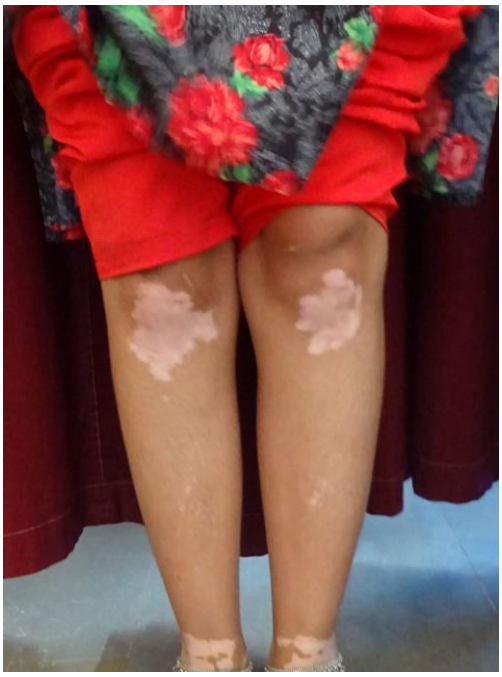


Figure 12a

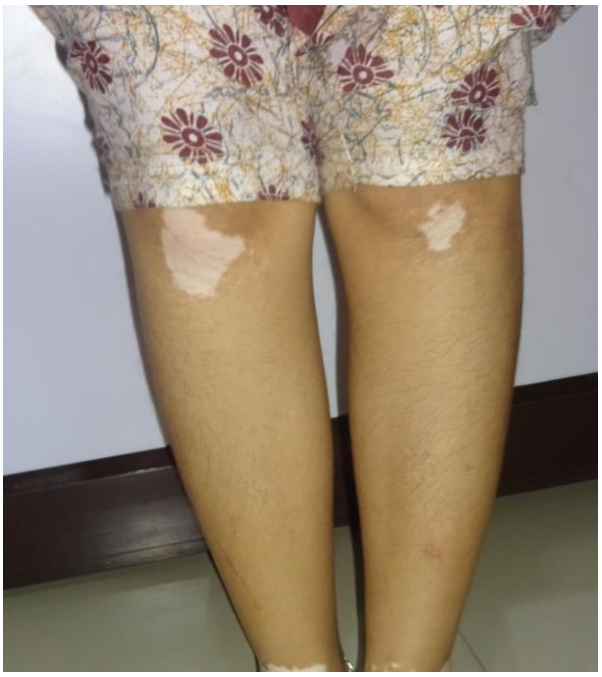


Figure 12b

Figure 13a and 13b are the clinical photographs showing grade 3 repigmentation at test site (Left side) and grade 2 control site (Right side) based on mean percentage of repigmentaiton.

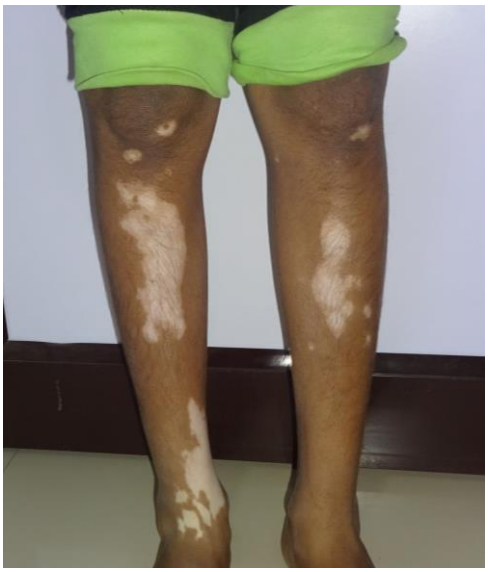


Figure 13a

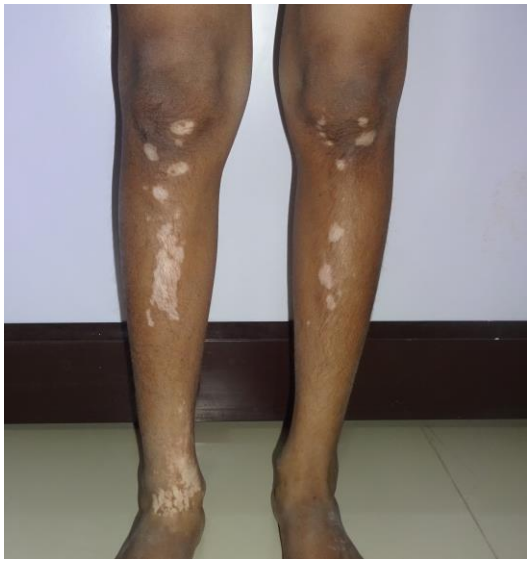


Figure 13b

Figure 14a and 14b are the clinical photographs showing grade 2 repigmentation at both test site (Right side) and control site (Left side) based on mean percentage of repigmentaiton.

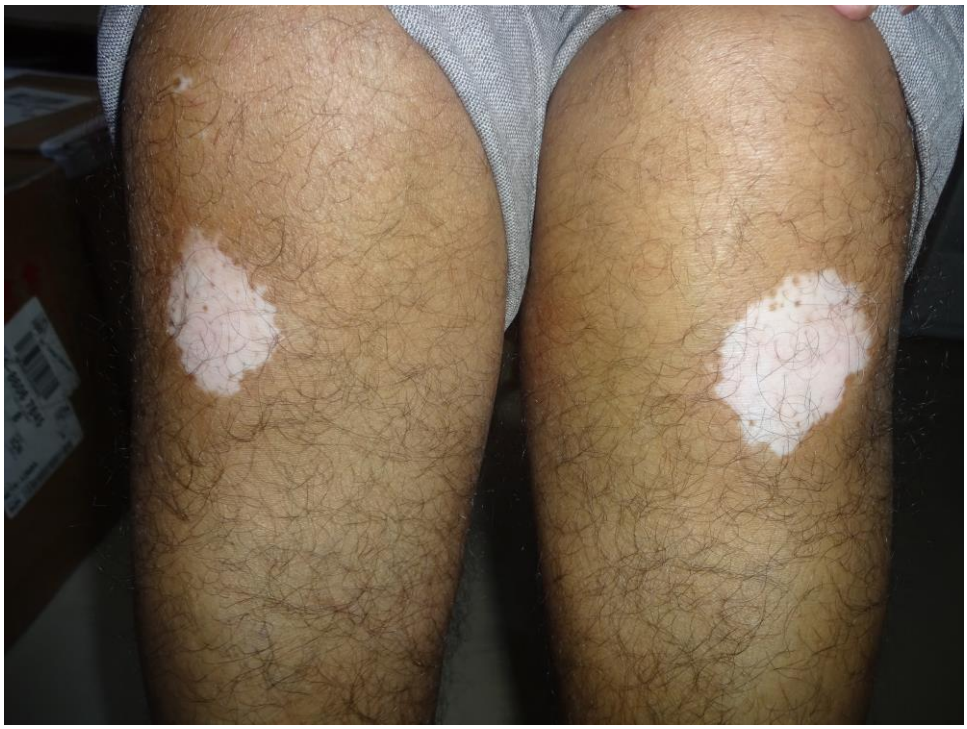


Figure 14a

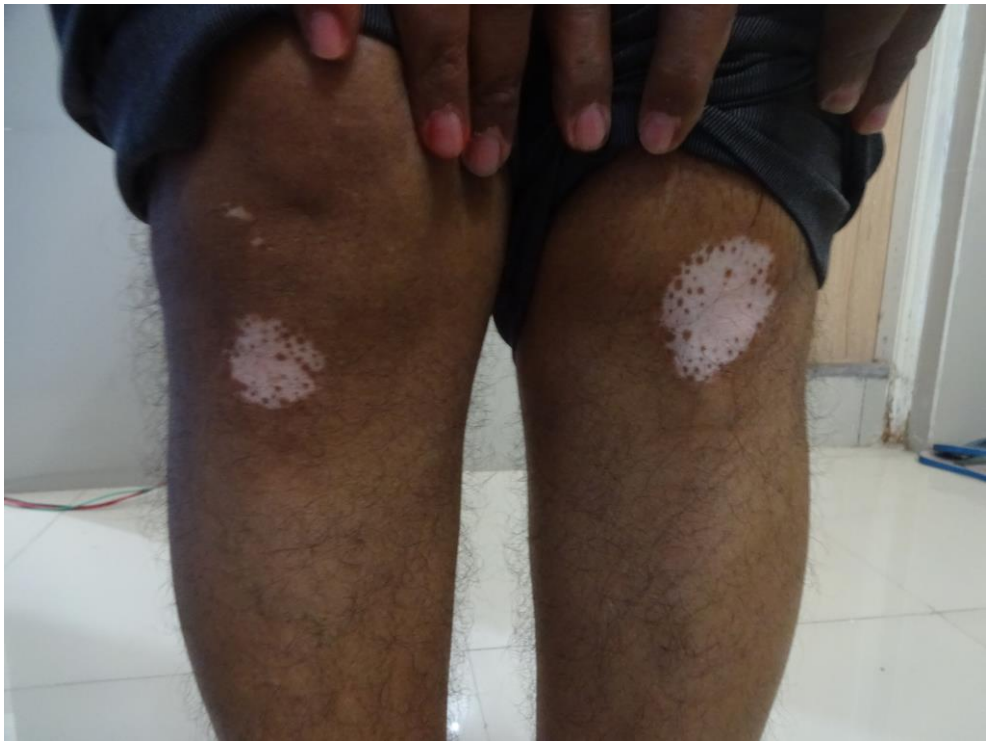


Figure 14b

Figure 15a and 15b are the clinical photographs showing grade 1 repigmentation based on mean percentage of repigmentaiton at test site (Right side) and no change at control site (Left side) by the end of 12 weeks of study.

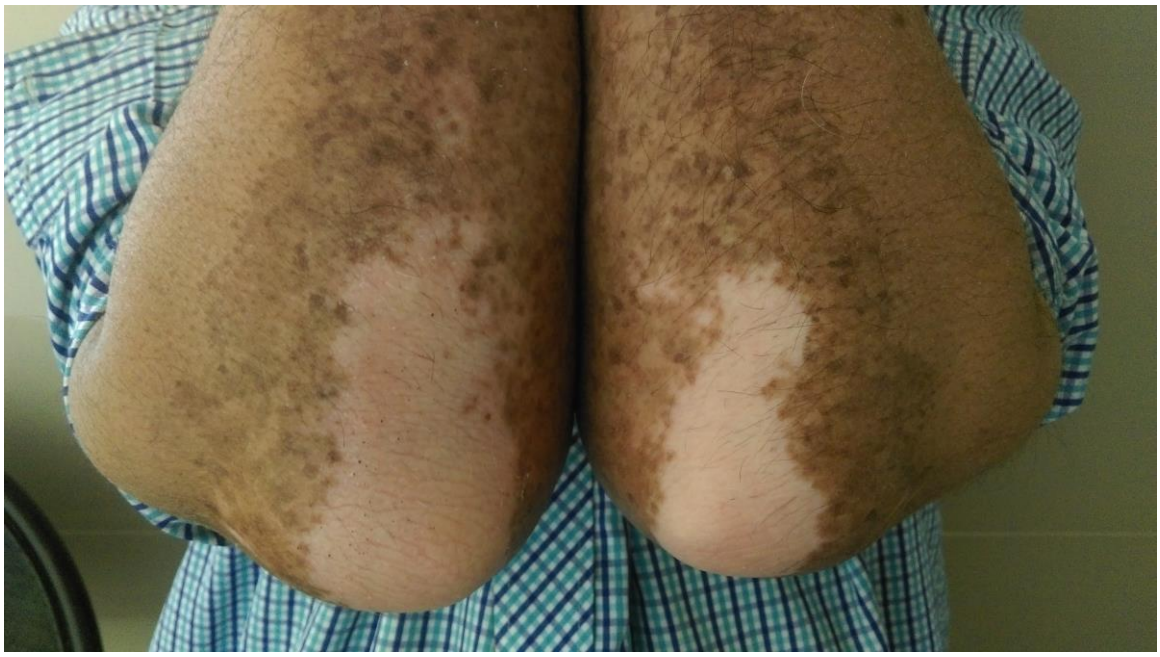


Figure 15a

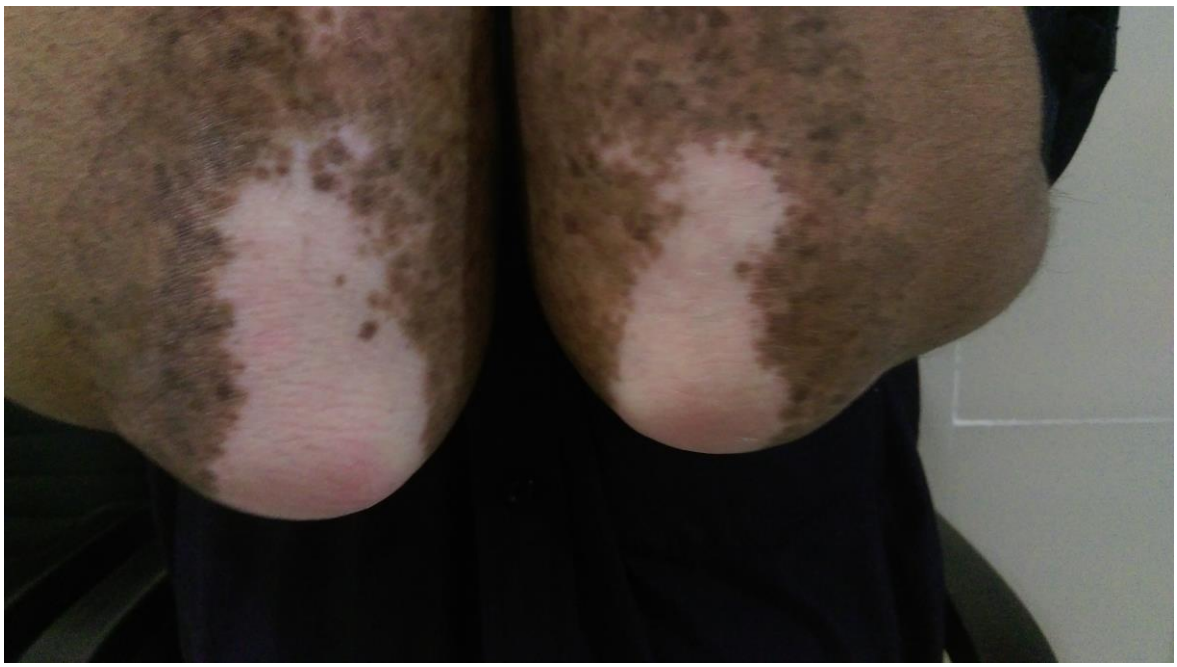


Figure 15b

DISCUSSION

DISCUSSION

Vitiligo is a common depigmenting disorder with unknown origin. It affects both skin and mucosa. This disease has a significant impact on quality of life of the patients often affecting personal relationships and hindering their participation in day to day activities. In spite of advances in the treatment modalities the response may often remain poor in day to day practice as few lesions remain recalcitrant and other might take longer duration to repigment.[7] Monotherapy may not be always successful in obtaining desired response both to practitioner and the patient. Combining the safer and effective treatment modalities might yield better grades and early repigmentation that would improve the treatment compliance.[1]

Narrow band ultra violet B therapy (NBUVB) is a well-known modality with good safety profile and has been widely studied in the past decade in combination with multiple topical modalities. In multiple studies, NBUVB therapy was found to been more efficacious as an adjuvant.[40]

Topical tacrolimus came into use as a treatment option in vitiligo since in 2002. It was found to be equally effective as topical clobetasol with signs of early repigmentation and better safety profile.[26] Recently the European Dermatology Forum group proposed twice daily topical calcineurin inhibitors for head and neck lesions as a first-line approach.[29]

Lasers were used for treating vitiligo since 1980's. The Q- switched ruby laser was initially used but was not popular because of its unwanted tissue destruction. Since then the search for adding a safe and effective laser for benefiting treatment response has continued.

Ablative fractional carbon dioxide laser is new addition in this category. It varies from other lasers by creating microscopic thermal zones at specific distance in desired depths without damaging the surrounding skin that would play a major role in cytokines release, melanocyte transfer and tissue repair. The use of this modality became popular when Shin *et al*., in 2012 used it in combination with NBUVB therapy for refractory non-segmental vitiligo lesions effectively.[49]

The concept of triple combination using fractional carbon dioxide laser was introduced in year 2015 by Li L *et al*. The authors conducted a comparative study by combining the fractional carbon dioxide laser at the interval of 2 weeks with NBUVB phototherapy with topical betamethasone solution for treating refractory vitiligo and on one half of the body. The results were compared by treating the lesions on other half of the body with a combination of laser and NBUVB therapy.[4]

Following this, in the same year Vachiramon *et al.*, studied treatment response by treating difficult to treat lesions of vitiligo with weekly fractional carbon dioxide laser prior to NBUVB therapy and topical 0.05% clobetasol propionate cream by comparing them with combination of NBUVB therapy and topical 0.05% clobetasol propionate cream. Authors found better grade of repigmentation, overall mean improvement and patient scores in group treated with triple combination than the comparison group.[1]

In the present study, efficient techniques from both the studies have been adopted, that is concept of triple combination and half body comparison from Li L *et al*., dosing and treatment schedule from Vachiramon *et al*., . The topical corticosteroid has been replaced with topical tacrolimus which has similar efficacy and lack of side effects that were encountered with topical steriods.[1]

A total of 44 patients were recruited in the present study, 38 of whom completed the treatment duration of 12 weeks. Six patients failed to follow up for the treatment sessions due to their personal and professional commitments.[1]

Out of 44 cases who enrolled our study 23 were males and 21 were female and belonged to mean age group 27.5 (± 6.4) years. In a similar manner, males outnumbered female in the study conducted by Vachiramon *et al.*, in which out of 27 patients 15 were males and 11 were females with mean age 51.2 (± 8.5) years.[1]

The mean duration of disease was 6.2 (±6.4) years in the present study which was similar with Vachiramon *et al.*, where the mean duration of disease was 70 (±25.69) months.[1]

In the present study more than 50% of mean percentage of re-pigmentation (grade 3 or good- 50-75%, grade 4 or excellent – 75-100%) was seen among 15 lesions in group A and 6 lesions in group B. This was statistically significant (*P*=0.021) and higher than compared to the results of Vachiramon *et al.*, who noted good to excellent repigmentation in 6 patients of laser group and 1 in NBUVB group.[1] The reason for this drastic variation might be due restricting their study to lesions on hands alone. The mean (±SD) of mean percentage of repigmentation in the present study was 33.6(±26.7) in group A which was found to statistically higher (P=0.032) than group B which was about 21.6(±21.6).

Shin *et al.*, compared fractional carbon dioxide laser with NBUVB and NBUVB therapy alone. The study showed improvement more than 50% in 1 patient in both the treatment groups.[49] This variation may be due to longer duration between laser sessions and additional effect of topical tacrolimus which may have played a role in immune suppression and melanocyte transfer after reaching the desired depth with ease.

The site specific mean (±SD) percentage of repigmentation are 60 (±7.1) and 47.5(±6) over the back, 20 (±25.9) and 11.7(±24) over the elbows, 43 (±25.9) and 26 (±27.2) over the feet, 50(±18.2) and 26.7 (±12.1) over the knees, 31 (±29.6) and 21.3 (±26.8) over legs, 13.8 (±4.8) and 11.3(±8.5) over the palms among group A and group B lesions respectively. These values are not comparable with the mean percentage of pigmentation based on site by Vachiramon *et al*., due to the obvious site variation among the study groups.[1]

The mean (±SD) VAS score of patients in group A was 3.8 (±2.2) and is statistically significant (*P*= 0.001) than the VAS score of Group B with mean (±SD) of 2.8 (±1.9). Shin *et al*., in his study noted similar statistically significant (*P*=0.023) variation between mean (± SD) VAS scores of 1.7 (± 1.6) in combination group of fractional carbon dioxide laser with NBUVB and 0.4 (±0.7) in only NBUVB group. [49] The probable reason for higher values in the present study may be due to addition of tacrolimus and weekly interval between laser session that promoted drug penetration continuously. Li *et al.,* had similar patient satisfaction mean (±SD) scores of 4.08 (± 2.89) on the treatment side and 1.52 (± 1.29) on the control by the end of 3rd month.[4]

Among 31 lesions in group A of present study that showed response, 20 lesions showed mixed type of re pigmentation. This was similar to the finding noted in the study by Vachiramon *et al*., in which 11 out of 15 responders showed mixed types of pigmentation.[1]

Most of the lesions in group B in the present study i.e., 10 out 26 responded with peri follicular type of pigmentation. This finding differs from the findings of Vachiramon *et al*., in which 4 out of 8 responders in group treated with topical clobetasol and NBUVB showed mixed types of pigmentation. This may also be site

variation between the studies as the compared study restricted its study site to hands itself.[1]

The most common side effect noted among group A lesion in the present study was post laser treatment pain. This was noted in 8 cases and it was transient and did not require any intervention. Similar complaint was noted among 25 out of 26 patients treated by Vachiramon *et al*., and which did not warrant any intervention.[1] The difference among the findings would have been influenced by several factors like quality, quantity, duration of contact and occlusion of the analgesic applied. There is possibility of subjective variation in pain perception as none of patients in our study experienced any kind of pain on group B lesions, whereas pain was noted in 12 out of 16 patients in study by Vachiramon *et al*.

Followed by pain, erythema at laser site was seen among 4 patients in group A in the present study. Itching and erythema was complained by 2 patients each under group B. There were no serious adverse effects noted that lead to discontinuation of the treatment.

Apart from the above mentioned comparable findings with previous studies present study adds few interesting findings to the data.

Although the present study had only 6 cases with positive family history these cases had statistically significant (P=0.045) lower mean (±SD) grade of repigmentation of 0.67 (±0.25) when compared to the cases without family history 1.63(± 1.11). The exact cause for this variation remains unknown. It can be speculated that the association of family history with systemic involvement, the underlying autoimmune factors would have played their role in the recalcitrant nature of the disease.

The mean percentage of repigmentation in both the groups showed inverse relation with respect to the duration of disease onset i.e., as the lesions with lesser disease duration had a better repigmentation. This finding was statistically significant (*P*=0.001) only among the lesion in group A although group B lesions showed a similar pattern. The reason for this pattern might be due to role of NBUVB therapy, which was an integral part both the study groups and similar findings are noted in studies conducted by kanwar *et al*., Scherschun *et al*., and Hallaji *et al*., with NBUVB.[36,39,40]

CONCLUSION

CONCLUSION

Vitiligo is a common depigmenting disorder. Often the cases with recalcitrant nature throw a therapeutic challenge. Hence the idea of combining different treatment modalities which would accentuate therapeutic efficacy of one another with limited adverse effects are always warranted.

Triple combination with fractional carbon dioxide laser is a new edition in the treatment armamentarium of recalcitrant vitiligo. The fractional laser is known to induce cytokine release which cause the melanocyte migration. Apart from this it creates microscopic treatment channels at required depths and causes partial ablation of stratum corneum. These effects are speculated to play a major role in penetration of topically applied drug and would increase better penetration of phototherapy respectively.

In the present study 38 patients who completed 10 weeks of study period and 8 weeks of follow up with symmetrical recalcitrant vitiligo lesions. Fifteen group A lesions achieved more than 50% or more than grade 3 repigmentation which was statistically higher (P=0.021) than 6 group B lesions that showed similar response. The mean percentage of repigmentation noted among group A lesions was 33.6 and was found to be significantly higher (P =0.032) than mean percentage of repigmentation noted in group B which was 21.6. The mean VAS scores of 3.8 in group A lesion was statistically greater (P =0.001) than 2.8 noted in group B lesions. Among the lesions which responded more number of lesion under group A showed early onset of repigmentation than the lesions under group B which was not statistically significant. With these findings it can be inferred that addition of fractional carbon dioxide laser to NBUVB and topical tacrolimus 0.1% ointment is a

better modality in treated recalcitrant vitiligo lesions when compared to the combination therapy of NBUVB and topical tacrolimus 0.1% ointment.

Adverse effects that were noted were slightly more among the group A lesions than the group B lesion. Although pain was the commonest complaint encountered in group A lesions it always remained transient, bearable and demand any special attention. Erythema which was seen in both groups rarely needed treatment with topical mid potent corticosteroids for 3 days. Post phototherapy itching of group B lesions was treated with a bland emollient and antihistamines. None of these adverse events were severe enough to warrant treatment discontinuation by the doctor or quitting the study by the patient.

Apart from these results this study noted an interesting finding that recalcitrant vitiligo lesions with family history showing poor signs of improvement among both the groups when compared to the lesions without any family history. As the number of subjects with family history were less this finding need further probing and should be studied on a larger scale.

SUMMARY

SUMMARY

A hospital based prospective, randomized, half-body, comparative study to determine the efficacy of triple combination treatment with fractional carbon dioxide laser plus topical tacrolimus 0.1% ointment and NBUVB therapy for refractory vitiligo was conducted between September 2016 to May 2018. Male and female patients of all ages, suffering from refractory, symmetrical lesions on extremities and/or bony prominences were taken into the study. Each side of the body was assigned randomly to fractional carbon dioxide laser followed by NBUVB therapy plus topical tacrolimus (study group A) and NBUVB therapy plus tacrolimus (group B). The lesions were treated with respective protocols for a period of 10 weeks and the results between the 2 groups were compared after 8 weeks of follow up after completion of treatment.

Following are the salient observations of the study:

- The mean (± SD) of age distribution among the patients enrolled in the study was 27.5(± 6.4) years.
- Gender distribution among the patients who enrolled into this study was almost same though males outnumbered females.
- The mean (±SD) of duration of the disease since the onset was 6.2 (± 6.4) years among the cases enrolled in this study.
- Good to excellent (i.e., ≥ grade 3) repigmentation was seen in 15 (39.5%) lesions of group A which was statistically higher (P=0.021) than in group B with similar grade of repigmentation seen in 6 (15.8%) lesions.
- The mean(±SD) percentage of re-pigmentation among the group A lesions was 33.6 (±26.7) which was statistically significant ($p<0.05$) than group B lesions with a mean (±SD) of 21.6 (±23).

- The number of lesions that showed initiation of repigmentation under a time duration of less than 6 weeks was 12 (31.6%) lesions in group A and only 5 (13.2%) lesions from group B.
- The degree of repigmentation varied inversely with the duration of the disease in both the groups.
- The mean (±SD) score of VAS in group A lesions was 3.8 (±2.2) and group B lesions was 2.8 (±1.9).
- Pain during laser therapy was noted in 6 patients in the group A which was transient and bearable and did not require any intervention.
- Erythema was the most common adverse effect noted among both the groups and rarely needed application of mid potent steroid for a duration of 3 days.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Vachiramonramon V, Chaiyabutr C, Rattanaumpawan P, Kanokrungsee S. Effects of a preceding fractional carbon dioxide laser on the outcome of combined local narrowband ultraviolet B and topical steroids in patients with vitiligo in difficult to treat areas. Lasers Surg Med 2016; 48:197-202.
2. Alikhan A, Felsten LM, Daly M, Petronic-Rosic V. Vitiligo: a comprehensive overview Part I. Introduction, epidemiology, quality of life, diagnosis, differential diagnosis, associations, histopathology, etiology, and work-up. J Am Acad Dermatol 2011; 65:473-491.
3. Sehgal VN, Srivastava G. Vitiligo: compendium of clinico-epidemiological features. Indian J Dermatol Venereol Leprol 2007; 73:149.
4. Li L, Wu Y, Sun Y, Lu L, Qiu L, Gao XH, et al. Triple combination treatment with fractional CO_2 laser plus topical betamethasone solution and narrowband ultraviolet B for refractory vitiligo: a prospective, randomized half- body, comparative study. Dermatol Therapy 2015; 28:131-4.
5. Yuan J, Chen H, Yan R, Cui S, Li YH, Wu Y, et al. Fractional CO2 laser contribute to the treatment of stable non-segmental vitiligo. Eur J Dermatol 2016; 26:592–298.
6. Speeckaert R, Speeckaert MM, van Geel N. Why treatments do(n't) work in vitiligo: an autoinflammatory perspective. Autoimmun Rev 2015; 14:332-340.
7. Fai D, Cassano N, Vena GA. Narrow-band UVB phototherapy combined with tacrolimus ointment in vitiligo: a review of 110 patients. J Eur Acad Dermatol Venereol 2007;21(7):916–20.
8. Lepe V, Moncada B, Castanedo Cazares JP, Torres-Alvarez MB, Ortiz CA, Torres-Rubalcava AB, *et al.* A double-blind randomized trial of 0.1% tacrolimus vs 0.05% clobetasol for the treatment of childhood vitiligo. Arch Dermatol 2003; 139:581-5.

9. Tahir MA, Pramod K, Ansari SH, Ali J. Current remedies for vitiligo. Autoimmun Rev 2010; 9:516-20.
10. Ezzedine K, Eleftheriadou V, Whitton M, et al. Vitiligo. Lancet 2015; 386:74–84.
11. Prasad D, Kumaran SM. Depigmentary and hypopigmentary disorders. In: Sacchidanand S, Oberai C, Inamadar AC, editors. IADVL Textbook of dermatology, 4th edn. Mumbai: Bhalani Publishing Home; 2015. p -1308 – 1322.
12. Iannella G, Greco A, Didona D, Didona B, Granata G, Manno A, Pasquariello B, Magliulo G. Vitiligo: pathogenesis, clinical variants and treatment approaches. Autoimmun Rev. 2016; 15:335-43.
13. Behl PN, Aggarwal A, Srivastava G. Vitiligo *In*: Behl PN, Srivastava G, editors. Practice of Dermatology. 9th ed. CBS Publishers: New Delhi; 2003. p. 238-41.
14. Sahni K, Parsad D. Stability in Vitiligo: Is there a Perfect Way to Predict it? J Cutan Aesthet Surg 2013;6:75-82.
15. Ezzedine K, Sheth V, Rodrigues M, Eleftheriadou V, Harris JE, Hamzavi IH, Pandya AG. Vitiligo is not a cosmetic disease. J Am Acad Dermatol 2015; 73:883-5.
16. Lotti T, D'Erme AM. Vitiligo as a systemic disease. Clinics in dermatology. 2014; 32:430-4.
17. Hann SK, Lee HJ. Segmental vitiligo: clinical findings in 208 patients. J Am Acad Dermatol 1996; 35:671-674.
18. Gawkrodger DJ, Ormerod AD, Shaw L, Mauri- Sole I, Whitton ME, Watts MJ, Anstey AV, Ingham J, Young K. Guideline for the diagnosis and management of vitiligo. Br J Dermatol 2008; 159:1051-76.
19. Njoo MD, Westerhof W, Bos JD, Bossuyt PM. The development of guidelines for the treatment of vitiligo. Arch Dermatol 1999; 135:1514-21.

20. Andrew N, Lin. Topical calcineurin inhibitors. Wolverton SE. Comprehensive dermatologic drug therapy. Elsevier Health Sciences; 2013:535-42.
21. Felsten LM, Alikhan A, Petronic-Rosic V. Vitiligo: a comprehensive overview: part II: treatment options and approach to treatment. J Am Acad Dermatol 2011; 65:493–514.
22. Lan CC, Chen GS, Chiou MH, Wu CS, Chang CH, Yu HS. FK506 promotes melanocyte and melanoblast growth and creates a favourable milieu for cell migration via keratinocytes: possible mechanisms of how tacrolimus ointment induces repigmentation in patients with vitiligo. Br J Dermatol 2005; 153:498-505.
23. Smith DA, Tofte SJ, Hanifin JM. Repigmentation of vitiligo with topical tacrolimus. Dermatology 2002; 205:301–3.
24. Grimes PE, Soriano T, Dytoc MT. Topical tacrolimus for repigmentation of vitiligo. J Am Acad Dermatol 2002; 47:789–91.
25. Sisti A, Sisti G, Oranges CM. Effectiveness and safety of topical tacrolimus monotherapy for repigmentation in vitiligo: a comprehensive literature review. An Bras Dermatol 2016; 91:187-95.
26. Choi CW, Chang SE, Bak H, Choi JH, Park HS, Huh CH, et al. Topical immunomodulators are effective for treatment of vitiligo. J Dermatol 2008; 35:503-7.
27. Ho N, Pope E, Weinstein M, Greenberg S, Webster C, Krafchik BR. A double-blind, randomized, placebo-controlled trial of topical tacrolimus 0.1% vs. clobetasol propionate 0.05% in childhood vitiligo. Br J Dermatol 2011; 165:626-32.
28. Dang YP, Li Q, Shi F, Yuan XY, Liu W. Effect of topical calcineurin inhibitors as monotherapy or combined with phototherapy for vitiligo treatment: a meta-analysis. Dermatol Ther 2016; 29:126-33.

29. Taieb AV, Alomar A, Böhm M, Dell'Anna ML, De Pase A, Eleftheriadou V, Ezzedine K, Gauthier Y, Gawkrodger DJ, Jouary T, Leone G. Guidelines for the management of vitiligo: the European Dermatology Forum consensus. Br J Dermatol 2013; 168:5-19.
30. Mohammad TF, Al-Jamal M, Hamzavi IH, et al. The vitiligo working group recommendations for narrowband ultraviolet B light phototherapy treatment of vitiligo. J Am Acad Dermatol 2017; 76:879-888.
31. Westerhof W, Nieu Weboer-Krobotova L. Treatment of vitiligo with UV-B radiation vs topical psoralen plus UV-A. Arch Dermatol 1997; 133:1525-1528.
32. Imokawa G, Miyagishi M, Yada Y. Endothelin-1 as a new melanogen: coordinated expression of its gene and the tyrosinase gene in UVB-exposed human epidermis. J Invest Dermatol 1995; 105: 32–37.
33. Njoo MD, Bos JD, Westerhof W. Treatment of generalized vitiligo in children with narrow-band (TL-01) UVB radiation therapy. J Am Acad Dermatol 2000; 42: 245–253.
34. Brazzelli V, Prestinari F, Castello M, Bellani E, Roveda E, et al. Useful treatment of vitiligo in 10 children with UV-B narrowband (311 nm). Pediatr Dermatol. 2005; 22:257–61.
35. Percivalle S, Piccinno R, Caccialanza M, Forti S. Narrowband ultraviolet B phototherapy in childhood vitiligo: evaluation of results in 28 patients. Pediatr Dermatol 2012; 29:160–5.
36. Kanwar AJ, Dogra S. Narrow-band UVB for the treatment of generalized vitiligo in children. Clin Exp Dermatol 2005; 30:332–6.

37. Parsad D, Kanwar AJ, Kumar B. Psoralen–ultraviolet A vs. narrow- band ultraviolet B phototherapy for the treatment of vitiligo. J Eur Acad Dermatol Venereol 2006; 20:175-7.
38. Anbar TS, Westerhof W, Abdel-Rahman AT, El-Khayyat MA. Evaluation of the effects of NB-UVB in both segmental and non-segmental vitiligo affecting different body sites. Photodermatol Photoimmunol Photomed 2006; 22:157–63.
39. Scherschun L, Kim JJ, Lim HW. Narrow-band ultraviolet B is a useful and well-tolerated treatment for vitiligo. J Am Acad Dermatol 2001; 44:999–1003.
40. Hallaji Z, Ghiasi M, Eisazadeh A, Damavandi MR. Evaluation of the effect of disease duration in generalized vitiligo on its clinical response to narrowband ultraviolet B phototherapy. Photodermatol Photoimmunol Photomed 2012; 28:115–9.
41. Nicolaidou E, Antoniou C, Stratigos AJ, Stefanaki C, Katsambas AD. Efficacy, predictors of response, and long-term follow-up in patients with vitiligo treated with narrowband UVB phototherapy. J Am Acad Dermatol 2007; 56:274–8.
42. Natta R, Somsak T, Wisuttida T, Laor L. Narrowband ultraviolet B radiation therapy for recalcitrant vitiligo in Asians. J Am Acad Dermatol 2003; 49:473–6.
43. Nicolaidou E, Antoniou C, Stratigos A, Katsambas AD. Narrowband ultraviolet B phototherapy and 308-nm excimer laser in the treatment of vitiligo: a review. J Am Acad Dermatol 2009; 60:470-7.
44. Hearn RM, Kerr AC, Rahim KF, Ferguson J, Dawe RS. Incidence of skin cancers in 3867 patients treated with narrow-band ultraviolet B phototherapy. Br J Dermatol 2008; 159:931-935.

45. Kumar R, Parsad D, Kanwar AJ, et al. Altered levels of Ets-1 transcription factor and matrix metallo proteinases in melanocytes from patients with vitiligo. Br J Dermatol 2011; 165:285-291.
46. Ross EV, Yashar SS, Naseef GS, et al. A pilot study of in vivo immediate tissue contraction with CO2 skin laser resurfacing in a live farm pig. Dermatol Surg 1999; 25:851-856.
47. AbdelghaniR, Ahmed NA, DarwishHM. Combined treatment with fractional carbon dioxide laser, autologous platelet-rich plasma, and narrow band ultraviolet B for vitiligo in different body sites: a prospective, randomized comparative trial. J Cosmet Dermatol 2017;1–8.
48. Chiu YJ, Perng CK, Ma H. Fractional CO 2 laser contributes to the treatment of non-segmental vitiligo as an adjunct therapy: a systemic review and meta-analysis. Lasers Med Sci 2018; 26:1-8.
49. Shin J, Lee JS, Hann SK, Oh SH. Combination treatment by 10,600 nm ablative fractional carbon dioxide laser and narrowband ultraviolet B in refractory nonsegmental vitiligo: a prospective, randomized half- body comparative study. Br J Dermatol 2012; 166: 658-61.
50. El-Zawahry MB, Zaki NS, Wissa MY, Saleh MA. Effect of combination of fractional CO2 laser and narrow-band ultraviolet B versus narrow-band ultraviolet B in the treatment of non-segmental vitiligo. Lasers Surg Med 2017; 9:1953–1958.
51. Helou J, Maatouk I, Obeid G, Moutran R, Stephan F, Tomb R. Fractional laser for vitiligo treated by 10,600nm ablative fractional carbon dioxide laser followed by sun exposure. Lasers Surg Med 2014; 46:443–448.

52. Doghaim NN, Gheida SF, El- Tatawy RA, Mohammed Ali DA. Combination of fractional carbon dioxide laser with narrow band ultraviolet B to induce repigmentation in stable vitiligo: A comparative study. J Cosmet Dermatol 2018.
53. Chen W, Zhou Y, Huang FR, Luo D, Wang DG. Preliminary study on the treatment of vitiligo with carbon dioxide fractional laser together with tacrolimus. Lasers Surg Med 2018.

ANNEXURE

ANNEXURE-I

ETHICAL CLEARANCE CERTIFICATE

B.L.D.E. UNIVERSITY'S
SHRI.B.M.PATIL MEDICAL COLLEGE, BIJAPUR-586 103
INSTITUTIONAL ETHICAL COMMITTEE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this college met on 04-10-2016 at 03-00pm to scrutinize the Synopsis of Postgraduate Students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected & revised version synopsis of the Thesis has been accorded Ethical Clearance.

Title "A hospital based prospective randomized, Self-body comparative study to determine the efficacy of triple combination treatment with fractional CO_2 laser plus topical tacrolimus 0.1% ointment & narrow band ultraviolet B for refractory vitiligo"

Name of P.G. student Dr Pathuri Ram Sushruth
Dept of Dermatology

Name of Guide/Co-investigator Dr Arun C Inamadar
Prof & HOD of Dermatology

DR.TEJASWINI VALLABHA
CHAIRMAN
INSTITUTIONAL ETHICAL COMMITTEE
BLDEU'S, SHRI.B.M.PATIL
MEDICAL COLLEGE, BIJAPUR.

Following documents were placed before E.C. for Scrutinization
1) Copy of Synopsis/Research project.
2) Copy of informed consent form
3) Any other relevant documents.

ANNEXURE-II

CONSENT FORM

B.L.D.E.U's SHRI B M PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPUR-586103

TITLE OF THE PROJECT :- A HOSPITAL BASED PROSPECTIVE, RANDOMIZED, HALF-BODY, COMPARITIVE STUDY TO DETERMINE THE EFFICACY OF TRIPLE COMBINATION TREATMENT WITH FRACTIONAL CARBON DIOXIDE LASER PLUS TOPICAL TACROLIMUS 0.1% OINTMENT AND NARROWBAND ULTRAVIOLET B FOR REFRACTORY VITILIGO.

PG GUIDE **:-** DR. ARUN C INAMADAR.

PG STUDENT **:-** DR. PATHURI RAM SUSHRUTH

PURPOSE OF RESEARCH: -

I have been informed that this project will determine the efficacy of triple combination treatment with fractional CO_2 laser plus topical tacrolimus 0.1% ointment and narrowband ultraviolet B in treatment of refractory vitiligo.

BENEFITS: -

I understand that my participation in this study will help the investigator to know the effectiveness of triple combination treatment with fractional CO_2 laser plus topical tacrolimus 0.1% ointment and narrowband ultraviolet B in treatment of refractory vitiligo.

PROCEDURE: -

I understand that relevant history will be taken and I will undergo detailed clinical examination after which treatment will be given.

RISK AND DISCOMFORTS: -

I understand there are risks like fibrosis, hypertrophic scar, transient post inflammatory hyperpigmentation, pain involved during the procedure.

CONFIDENTIALITY: -

I understand that medical information produced by this study will become a part of hospital records and will be subjected to the confidentiality and privacy regulation of the said hospital. Information of a sensitive personal nature will not be a part of the medical records, but will be stored in the investigator's research file.

If the data are used for publication in the medical literature or for teaching purposes no names will be used and other identifiers such as photographs and audio or videotapes will be used only with my special written permission. I understand I may see the photographs, videotapes and hear the audiotapes before giving this permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask more questions about the study at any time concerned. Dr. Pathuri Ram Sushruth is available to answer my questions or concerns. I understand that I will be informed of any significant new findings discovered during the course of this study, which may influence my continued participation.

REFUSAL OR WITHDRAWAL OF PARTICIPATION: -

I understand that my participation is voluntary and I may refuse to participate or may withdraw consent and discontinue participation in this study at any time without prejudice. I also understand that Dr. Pathuri Ram Sushruth may terminate my participation in this study at any time after he has explained the reasons for doing so and has helped arrange for my continued care by my own physician, if this is appropriate.

INJURY STATEMENT: -

I understand that in the unlikely event of injury to me resulting directly from my participation in this study and if such injury were reported promptly, then medical treatment will be available to me, but no further compensation will be provided. I understand that by my agreement for my participation in this study, I am not waiving any of my legal rights.

I have explained to (patient's / relevant guardian's name) the purpose of the research, the procedures required, and the possible risks and benefits to the best of my ability in patient's own language. I confirm that ……. (Name of the PG guide / chief researcher) has explained to me the research, the study procedures that I undergo and the possible risks and discomforts as well as benefits that I may experience. I have read and I understand this consent form. Therefore, I agree to give my consent for my participation as a subject in this research project.

_____ _____

Investigator / P. G. Guide Date

_____ _____

Participant / guardian Date

_____ _____

Witness to signature Date

ANNEXURE-III

PROFORMA

B.L.D.E.U's SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, VIJAYAPUR.

Department of Dermatology, Venereology and Leprosy.

Name: SL NO:

Age: Date:

Sex: IP NO/ OP NO:

Occupation:

Address:

1. Chief complaints:
2. Age of onset of disease:
3. Progressive / non progressive:
4. History of kobnerization:
5. Family history:
6. Treatment history:

7. Repigmentation: spontaneous / following treatment

Follicular / marginal / diffuse

8. History of other auto immune diseases:

(Diabetes mellitus / Thyroid disorders)

10.Past history:

History of keloid:

Any treatment history for chronic illness:

Other immunosuppressed states:

11.General Physical Examination:

Weight: BP: Pulse rate:

Pallor: Cyanosis: Icterus:

Clubbing: Lymphadenopathy: Edema:

12.Local examination

13.Systemic Examination

Cardiovascular system :

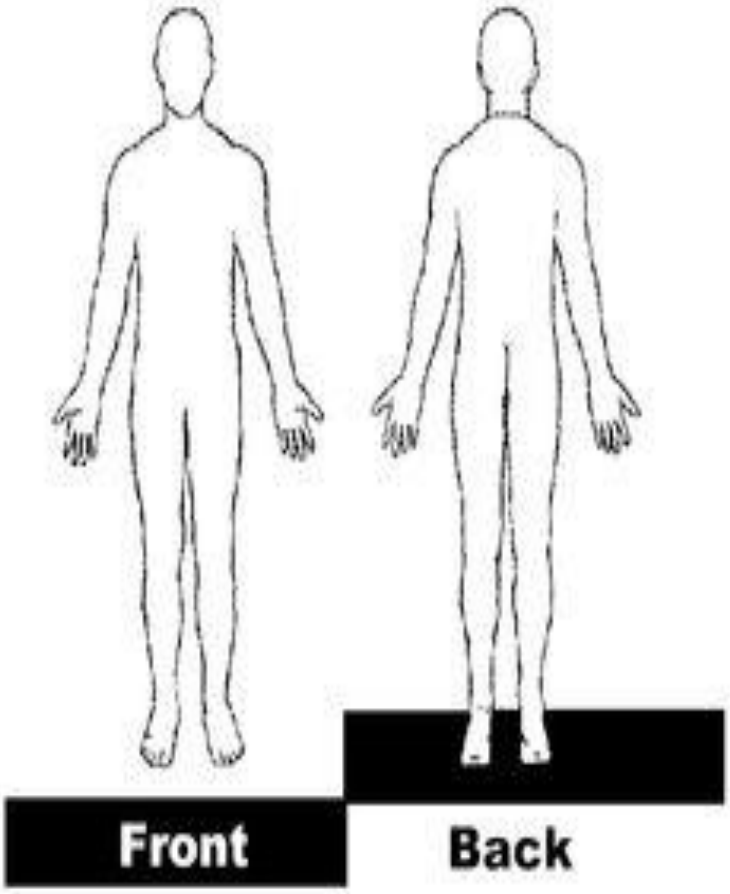
Respiratory system :

Central nervous system :

Abdominal examination :

14.Diagnosis:

FIRST VISIT

Body surface area	
	
No. of lesions	
Study site	
Side of body taken as test	
Side of body taken as control	

TREATMENT

	Laser (session no. & date)	Evidence of repigmentation	Adverse effects	Phototherapy (session no. & date)	Evidence of repigmentation	Adverse effects
Week 1						
Week 2						
Week 3						
Week 4						
Week 5						
Week 6						
Week 7						
Week 8						
Week 9						
Week 10						

FINAL VISIT

	Test site	Control site
Repigmentation (present/ absent)		
Percentage of repigmentation (observer 1)		
Percentage of repigmentation (observer 2)		
Types of repigmentation		

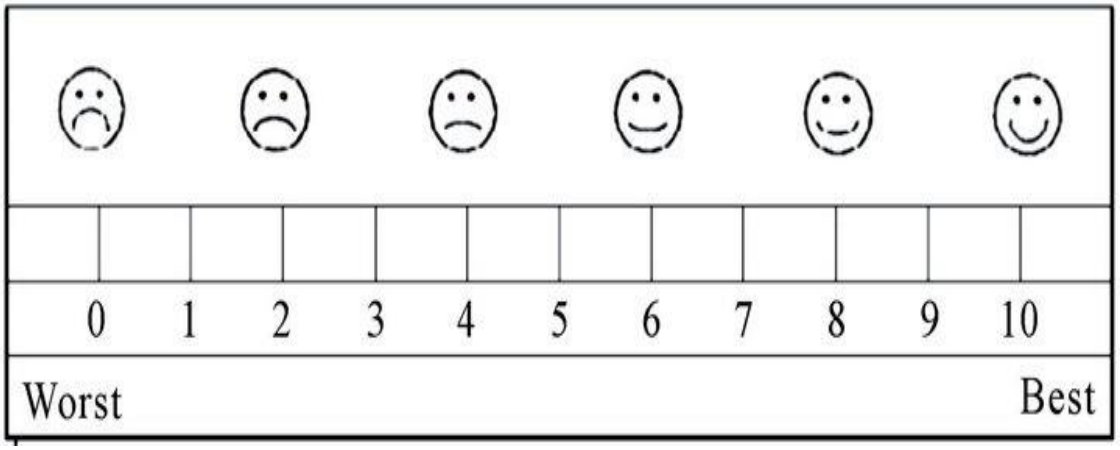
Remarks:-

B.L.D.E. U'S SHRI B. M. PATIL MEDICAL COLLEGE HOSPITAL AND RESEARCH CENTRE, BIJAPUR.

Department of Dermatology, Venereology and Leprosy.

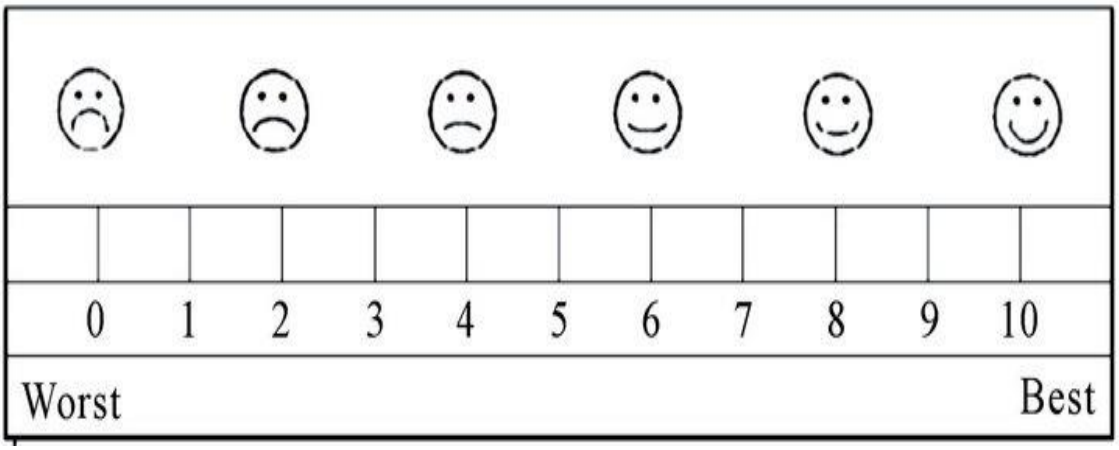
VISUAL ANALOGUE SCALE GROUP A

(VAS; 0= not satisfied at all, 10= extremely satisfied)



VISUAL ANALOGUE SCALE GROUP B

(VAS; 0= not satisfied at all, 10= extremely satisfied)



KEY TO MASTER CHART

NBUVB	-	Narrow band ultra violet B therapy
TCI	-	Topical calcineurin inhibitors
TCS	-	Topical corticosteroids
PUVA	-	Psoralen with ultra violet A
L.F.F	-	Lost for follow up
W.O.R	-	Week of onset of repigmentation
P.R	-	Percentage of repigmentation by observer
M.P.R	-	Mean percentage of repigmentation
T.O.R	-	Type of repigmentation
G.O.R	-	Grade of repigmentation
V.A.S	-	Visual analogue score
A.E	-	Adverse effects
P.F	-	Perifollicular repigmentation
M	-	Marginal repigmentation
D	-	Diffuse repigmentation

MASTER CHART

S.No.	Out Patient No.	Name	Age(Yrs)	Sex	Age of disease onset(Yrs)	Duration of disease(Yrs)	Family history	Previous treatment history	B.S.A(%)	Site of lesion	Side of group A site	Side of group B site	W.O.R in group A	W.O.R in group B	P.R-1 at group A	P.R-2 at group A	M.P.R in group A	T.O.R in group A	P.R-1 at group B	P.R-2 at group B	M.P.R in group B	T.O.R in group B	G.O.R in group A	G.O.R in group B	V.A.S in group A	V.A.S in group B	A.E in group A	A.E in group B
1	4398	Azar Mohammed	24	M	21	3	NEGATIVE	NBUVB	7	LEGS	RIGHT	LEFT	5	7	50	60	55	D+P.F	40	40	40	P.F	3	2	6	5	NIL	NIL
2	29308	Devereddy	26	M	24	2	NEGATIVE	TCI	8	KNEES	LEFT	RIGHT	5	6	60	60	60	M+D	45	55	40	M+D	3	2	7	4	PAIN	ITCHING
3	329043	Bhagyashree	30	F	25	5	NEGATIVE	HOMEOPATHY	12	ELBOW	LEFT	RIGHT	3	6	75	70	70	M+D	60	60	60	P.F	3	3	6	6	NIL	NIL
4	234324	Rama chappar	42	F	35	7	NEGATIVE	PUVA	10	LEGS	RIGHT	LEFT	9	9	5	5	5	M	NIL	NIL	NIL	NIL	1	0	4	0	NIL	NIL
5	94805	Nithin	30	M	22	8	NEGATIVE	UNKOWN	10	KNEES	LEFT	RIGHT	-	-	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F		L.F.F	PAIN	NIL
6	234234	Shrishail	27	M	19	8	NEGATIVE	MIXED	5	ELBOW	RIGHT	LEFT	-	-	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL		0	NIL	NIL
7	355645	Ayesha	26	F	24	2	NEGATIVE	TCI	4	FEET	RIGHT	LEFT	6	8	70	50	60	M+P.F	10	10	10	M	3	1	5	3	PAIN	NIL
8	383987	Sanah	20	F	15	5	NEGATIVE	UNKOWN	6	LEGS	LEFT	RIGHT	4	5	70	70	70	M+D	60	70	65	P.F	3	3	7	5	NIL	NIL
9	498709	Shailender	35	M	42	7	NEGATIVE	NBUVB	9	ELBOW	LEFT	RIGHT	-	-	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F
10	345	Rameeza	20	F	12	8	POSITIVE	AYURVEDA	2	LEGS	RIGHT	LEFT	7	8	30	30	30	P.F	30	30	30	P.F	1	1	3	3	ERYTHEMA	NIL
11	454	M.G.Kotnal	46	M	38	8	NEGATIVE	NIL	7	PALMS	RIGHT	LEFT	9	8	5	15	10	M	20	10	15	M	1	1	2	2	NIL	NIL
12	499	Prabhu	24	M	22	2	NEGATIVE	NIL	4	KNEES	LEFT	RIGHT	6	7	60	70	65	M+D	40	40	40	M+P.F	3	2	6	4	NIL	NIL
13	1335	Khuligawwa	39	F	30	9	NEGATIVE	NBUVB	8	PALMS	RIGHT	LEFT	9	9	10	10	10	M	10	10	10	M	1	1	3	3	PAIN	NIL
14	1578	Geetha	39	F	30	9	NEGATIVE	TCI	12	FEET	LEFT	RIGHT	7	7	30	50	40	M+P.F	30	40	35	M+P.F	2	2	4	3	NIL	ERYTHEMA
15	1909	Khanti	30	M	20	10	NEGATIVE	PUVA	15	ELBOW	LEFT	RIGHT	-	-	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	0	0	NIL	NIL
16	2567	R.A.Kulakarni	32	M	27	6	NEGATIVE	AYURVEDA	15	BACK	RIGHT	LEFT	5	5	55	75	65	M+D	55	55	55	D	3	3	6	4	NIL	NIL
17	2813	Aishwarya	23	F	15	8	NEGATIVE	UNKOWN	9	KNEES	LEFT	RIGHT	7	7	30	40	35	M+D	30	30	30	M+P.F	2	2	5	5	ERYTHEMA	NIL
18	5467	Mahadevi	15	F	14	1	NEGATIVE	NBUVB	10	LEGS	RIGHT	LEFT	3	5	75	85	80	D+P.F	70	60	65	P.F	4	3	6	4	NIL	NIL
19	5988	Usman	23	M	14	9	POSITIVE	UNKOWN	5	LEGS	RIGHT	LEFT	7	9	20	20	20	P.F	5	5	5	P.F	1	1	4	1	ERYTHEMA	NIL
20	6432	Pooja	27	F	20	7	NEGATIVE	UNKOWN	5	FEET	RIGHT	LEFT	-	-	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F
21	10987	Swaroopa	18	F	15	3	NEGATIVE	AYURVEDA	1	FEET	RIGHT	LEFT	5	9	50	50	50	M+P.F	25	15	10	M+P.F	3	1	5	3	NIL	NIL
22	13482	Swathi	23	F	20	3	NEGATIVE	NBUVB	1	BACK	LEFT	RIGHT	5	7	40	50	55	M+D	40	40	40	D	3	2	6	4	NIL	NIL
23	15324	Shantakumar	30	M	22	8	NEGATIVE	UNKOWN	8	LEGS	LEFT	RIGHT	-	-	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F
24	17354	Sindigi	25	M	18	7	NEGATIVE	PUVA	5	LEGS	RIGHT	LEFT	-	-	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	1	1	NIL	NIL
25	20005	Rohit Mohite	24	M	16	8	POSITIVE	AYURVEDA	8	LEGS	LEFT	RIGHT	9	-	15	25	20	M	NIL	NIL	NIL	NIL	1	NIL	2	1	NIL	NIL
26	35555	Amit	30	M	27	3	NEGATIVE	UNKOWN	6	KNEES	LEFT	RIGHT	6	9	60	60	60	M+D	25	15	20	M	3	1	6	4	NIL	NIL
27	39832	Rakshita	28	F	20	8	NEGATIVE	TCS	9	ELBOW	RIGHT	LEFT	9	9	15	15	15	M	10	10	10	M	1	1	3	3	ERYTHEMA	NIL
28	44987	Kiran	25	M	21	4	NEGATIVE	TCS	10	FEET	LEFT	RIGHT	4	4	65	65	65	M+D	70	70	70	M+D	3	3	5	7	PAIN	NIL
29	54135	Shivlingappa	21	M	14	7	NEGATIVE	PUVA	3	LEGS	RIGHT	LEFT	-	-	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	0	0	NIL	NIL
30	64339	Kamalabai	25	F	23	2	NEGATIVE	NBUVB	14	LEGS	LEFT	RIGHT	5	9	70	50	60	M+P.F	10	10	10	P.F	3	1	5	3	NIL	NIL

31	100345	Fathima	37	F	30	7	NEGATIVE	EXTCIMER	15	KNEES	RIGHT	LEFT	-	-	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F
32	113897	V.R.Patil	25	M	17	8	NEGATIVE	UNKOWN	6	FEET	RIGHT	LEFT	-	-	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F	L.F.F
33	149567	Shankamma	25	F	17	8	NEGATIVE	HOMEOPATHY	7	KNEES	LEFT	RIGHT	8	8	20	20	20	M+D	10	10	10	D	1	1	3	3	NIL	NIL
34	178943	Dharani	29	M	22	7	POSITIVE	UNKOWN	5	LEGS	LEFT	RIGHT	8	-	5	15	10	P.F	NIL	NIL	NIL	NIL	1	NIL	2	2	NIL	NIL
35	189345	Gangawwa	34	F	26	8	NEGATIVE	PUVA	5	FEET	RIGHT	LEFT	-	8	NIL	NIL	NIL	NIL	5	5	5	M	NIL	1	1	2	NIL	NIL
36	200394	Pratyusha	31	F	27	4	NEGATIVE	AYURVEDA	4	LEGS	RIGHT	LEFT	4	5	80	60	70	M+P.F	60	70	65	P.F	3	3	6	7	NIL	ERYTHEMA
37	210645	S.S.Patil	27	M	22	5	NEGATIVE	TCS	2	PALMS	LEFT	RIGHT	8	8	10	20	15	M	20	20	20	M+P.F	1	1	2	2	PAIN	NIL
38	230876	Shivappa	22	M	18	4	NEGATIVE	TCI+NBUVB	10	PALMS	LEFT	RIGHT	7	-	20	20	20	M	NIL	NIL	NIL	NIL	1	NIL	3	2	NIL	NIL
39	340897	Lakshmi	32	F	25	7	NEGATIVE	UNKOWN	1	LEGS	RIGHT	LEFT	6	7	50	40	45	M+P.F	45	35	40	P.F	2	2	5	5	NIL	NIL
40	441265	Kantamma	21	F	15	6	NEGATIVE	UNKOWN	1	KNEES	LEFT	RIGHT	5	9	60	60	60	M+D	30	20	20	P.F	3	2	7	3	PAIN	ITCHING
41	552176	Tasleema	27	F	19	8	NEGATIVE	PUVA	2	LEGS	RIGHT	LEFT	-	-	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	1	1	NIL	NIL
42	987	Shivu	32	M	24	8	POSITIVE	TCS	6	ELBOW	RIGHT	LEFT	8	-	20	10	15	M+D	NIL	NIL	NIL	NIL	1	NIL	2	2	PAIN	NIL
43	1345	Bhusnoor	22	M	15	7	NEGATIVE	PUVA	8	LEGS	RIGHT	LEFT	-	-	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	1	0	NIL	NIL
44	2424	kamal	22	M	15	7	POSITIVE	UNKOWN	4	ELBOW	LEFT	RIGHT	8	-	20	20	20	M	NIL	NIL	NIL	NIL	1	NIL	3	1	PAIN	NIL