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ORIGINAL ARTICLE

The Effect of Autologous Non-cultured Melanocyte-Keratinocyte Cell Suspension in Comparison with Split Skin Grafting on Re-pigmentation of Post-burn Leukoderma: A Randomized Control Trial

Chirra Likhitha Reddy,[1] Devi Prasad Mohapatra,[2] Friji Meethale Thiruvoth,[2] Ravi Kumar Chittoria[2]

Keywords:
Burn injuries, Cell suspension, Post-burn leukoderma, Repigmentation, Vitiligo

ABSTRACT

Objectives:

Primary objective To compare the efficacy of Autologous Non- Cultured Melanocyte-Keratinocyte Cell Suspension (MKS) with Split Skin Grafting (SSG) on producing adequate pigmentation among patients with post-burn leukoderma

Secondary objective(s):

i. To compare time to full healing of donor site between the patients treated with non-cultured autologous MKS and SSG

ii. To compare satisfaction scores between patients treated with MKS and SSG

Design: Open-label, non-blinded, single-center, closed sealed envelope, randomized controlled trial.

Setting: Department of Plastic Surgery and Tertiary Burn Care Centre, in South India

Participants: Patients above 12 years of age with previously untreated post-burn leukoderma attending the out-patient department.

Intervention: Participants were randomized by block randomization using a closed-sealed envelope method to receive treatment for post-burn leukoderma (PBL) into two groups. Intervention arm patients were treated with MKS and control arm patients were treated with SSG group.

Main Outcome Measures: Percentage of re-pigmentation after 3 months of intervention, time to donor site healing and patient satisfaction score

Result: Forty-eight patients with post-burn leukoderma were randomly allocated to receive either split-skin graft (Group 1) or autologous non-cultured melanocyte-keratinocyte cell suspension (Group 2). Re-pigmentation was significantly better in a split skin graft group (Group 1) at 12 weeks. Patient satisfaction scores were better in Group 1, and there were no significant differences in the donor site healing time in both groups.

Interpretation: Early (12 weeks) pigmentation is better achieved in post-burn leukoderma patches treated with SSG, while reduced donor skin requirement and reduced donor complications are noted in patients treated with non-cultured MKS

Trial registration: The trial was registered with Clinical Trial Registry of India (CTRI); CTRI/2019/04/018804

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[1]*Department of Plastic Surgery, AIIMS Mangalgiri, Dhanvantri Nagar, Gorimedu, Puducherry, India*
[2]*Department of Plastic Surgery, JIPMER, Dhanvantri Nagar, Gorimedu, Puducherry, India*
***Correspondence:** *Devi Prasad Mohapatra, devimohapatra1@gmail.com*
Professor, Department of Plastic Surgery, JIPMER, Puducherry, India

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INTRODUCTION

The incidence of burns in the world was estimated to be around 11 million in 2004.[1] This number has been reducing in very highly developed, highly developed, and middle-developed countries.[2] In a study done in India, with numbers extrapolated from three major burn centers in India, it was estimated that 6 to 7 million people are affected by burns, of which over 1 million people are moderately or severely burnt every year.[3] There have also been various studies conducted to identify the gender-specific distribution of burns. According to a retrospective analysis done in 2001, the male-to-female ratio of burns in India was found to be 1:3.[4] Another study in 2020 has shown that the ratio of male-to-female burns to be 1:1.36.[5] Even though there is a decline in this ratio, the number of victims of burn injuries is still significant.

Decades earlier, the mortality in patients with second and third-degree thermal burns was quite high. The number of deaths from burns has reduced over the past few years, due to early resuscitation protocols and improved care facilities. This has, however, led to an increase in the number of patients presenting with post-burn deformities which are functionally impairing and resulting in temporary or permanent disabilities. While newer treatment and rehabilitation protocols in such patients have led to the correction of functionally impairing deformities, psychological and aesthetic disabilities have come to the fore. Cutaneous hypopigmentation or depigmentation of burn scars is an emotionally overwhelming sequelae of burn injury, which is a cause for severe psychological burden and social stigma among the affected individuals. This study attempts to compare outcomes of autologous non-cultured melanocyte-keratinocyte cell suspension (MKS) therapy with standard care of split skin grafting (SSG) for post-burn leukoderma with regards to recipient site pigmentation, rate of pigmentation, and donor site healing and patient satisfaction.

AIMS AND OBJECTIVES

The primary objective of the present study was to compare the efficacy of autologous non-cultured MKS with SSG on producing early adequate pigmentation among patients with post-burn leukoderma. As secondary objectives, we wanted to compare time to full healing of donor site between the patients treated with non-cultured autologous MKS and SSG and satisfaction scores between patients treated with MKS and SSG.

MATERIAL AND METHODS

After obtaining institutional ethics committee approval, this randomised control trial was carried out at the Department of Plastic Surgery and Tertiary Burn Care Centre, in South India. The trial was registered with Clinical Trial Registry of India (CTRI); CTRI/2019/04/018804. All patients more than twelve years of age with postburn leukoderma were included in the study after obtaining appropriate consent in accordance to the guidelines of the Declaration of Helsinki. Patients who had undergone surgery or topical management for leukoderma patches in the last one year, patients having ulcers over the leucoderma patches, patients with a history of keloids, autoimmune diseases, and Diabetes mellitus were excluded from the study. The sample size for this study was calculated using the statistical formula for comparing two independent proportions. The samples were collected by convenient sampling among patients attending the Plastic Surgery OPD. Enrolled patients with post-burn leukoderma were randomly allocated to receive either split-skin graft (Group 1) or autologous non-cultured MKS (Group 2). The enrolled patients were divided into two groups based on block randomization with varying block size using an opaque closed-sealed envelope method.

Demographic details, clinical history, and examination findings including, date of burn, previous treatment received, location, duration, number and size of patches were recorded before the start of the procedure assigned to patients in each group.

Operative procedure

Areas of post-burn leukoderma to be treated were identified and measured with a grid. Cleaning and prepping of the donor and the recipient areas were done with povidone-iodine, followed by 70% alcohol. All procedures were carried out under local anesthesia (Lignocaine 2% with adrenaline). The donor site was marked on a suitable area of the thigh on the patient selected according to participant's request.

Group 1: Dermabrasion of the identified leukoderma patch was carried out till uniform pin-point capillary bleeding is seen. A thin split skin graft (0.15 to 0.30 mm thickness) of a size same as the dermabraded area was harvested with a dermatome and applied over the raw area. Vaseline gauze topped with acriflavine-soaked gauze dressing was done which was opened on the 7th post-operative day. The donor area was covered with sterile dry collagen and a transparent dressing was applied.

Group- 2: Dermabrasion of the identified leukoderma patch was carried out till uniform pin-point capillary bleeding is seen. A thin split skin graft (0.15 to 0.30 mm thickness) of a size 1/10th the size of the recipient site as the dermabraded area was harvested with a Silverman handle with blade and applied over the raw area. This graft was converted into MKS as described below and is sprayed over the dermabraded area, covered with vaseline gauze topped with moistened acriflavine-soaked gauze dressing. The donor area was covered with sterile dry collagen, over which a transparent adhesive dressing was applied.

Preparation of melanocyte-keratinocyte cell suspension

1. A skin sample of up to one-tenth of the size of the recipient area is harvested.
2. The skin is washed with phosphate buffer solution [PBS] two times to remove extra hair and blood.
3. The cells are separated by using trypsin solution.
4. Skin specimen with epidermis facing upward is immersed in 8 mL of trypsin 0.25%, in a petri dish for 30 minutes, then trypsin solution is discarded and the sample rinsed with Phosphate buffer solution (PBS).
5. Mechanical separation of the dermis from the epidermis is done and the dermis is discarded. The epidermis is then transferred to a 15 mL centrifuge tube and centrifuged at 2000 RPM for 6 minutes to separate the melanocytes and keratinocytes, which sink to the bottom forming a pellet, while other epidermal cells float.
6. The cell suspension thus formed of keratinocytes and melanocytes is re-suspended in methylcellulose and sprayed onto the dermabraded depigmented recipient area with an insulin syringe.

Post-surgical, adjuvant therapy was given for the repigmentation, where the patients in the MKS group were asked to expose the treated area to direct sunlight for 30 minutes every day. Patients in the SSG group were advised not to get exposed to direct sunlight. An evaluation was done at baseline and post-treatment at POD-7, 14, 28, and 90 days individually by two surgeons with experience in post-burn management. Patient photos were taken preoperatively and at each post-operative visit with the same camera (One plus 6 (2018), specifications: 16 megapixel, f/1.7 aperture, 1.22-micron pixel size), for documentation, to assess the progress at each visit and compare the effect of different treatments. The percentage of the area of repigmentation in the treated lesions relative to the baseline depigmented surface area was recorded to evaluate the efficacy of the intervention. Pigmentation at the recipient site was evaluated at 1, 2, 4, and 12 weeks following surgical intervention in the two groups.

Repigmentation was graded according to the percentage of repigmentation of the treated areas with 90 to 100% pigmentation as excellent, 50.1 to 90% pigmentation as good, 20 to 50% pigmentation as fair and < 20% re-pigmentation graded as poor. Patients' satisfaction was assessed at the end of 3 months, using the VAS (Visual Analogue Scale) scoring system. Clinical information was collected by direct examination as well as photographic evidence on 1st, 2nd, 4th and 12th week post-operative and recorded in an excel sheet.

STATISTICAL ANALYSIS

Statistical analysis was performed using IBM, SPSS Statistics version 26 (IBM Inc.). Independent t-tests were done to compare group similarities at baseline. The association/independence of nominal/dichotomous variables (gender, recipient site repigmentation, color change, donor site complications, recipient site scar formation, and patient satisfaction) between split skin grafting (Group 1) and autologous non-cultured melanocyte-keratinocyte cell suspension (Group 2) were compared with Chi-square tests. Continuous variables were compared using t-test. A paired-sample t-test was used to determine whether there was a statistically significant mean difference in age, duration of burns, and duration of leukoderma patch between the two groups, respectively. Data are mean ± standard deviation unless otherwise stated. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Forty-eight patients (Table 1) with post-burn leukoderma were randomly allocated to receive either split-skin graft (Group1, n= 23) or autologous non-cultured melanocyte-keratinocyte cell suspension (Group 2, n= 25). The assumptions for performing t-tests were met. There were no outliers within 1.5 box-lengths from the edge of the box in a boxplot. The assumption of normality was not violated, as assessed by Shapiro-Wilk's test (p=0.780). There were 17(73.9%) females and 6 (26.1%) males in Group 1 and 19(76%) females and 6(24%) males in Group 2. The mean age of patients in Group 1 was 30.7 ± 6.7 (range, 20 to 41 years), and in Group 2 was 29.4 ± 6.2 (range, 20 to 41 years), respectively. The difference in age and gender between the two groups was not statistically significant. The mean duration between burn injury and intervention in Group 1 was 28.3 ± 19.44 months and in Group 2 was 27.4 ± 16.8 months, respectively. The difference in total body surface area of burns in patients among the two groups was not statistically significant (chi-square tests, p =0.405). The mean duration of leukoderma patch in Group 1 was 25.1 ± 19 months and in Group 2 was 23.8 ± 15.8 months, respectively. Nearly 45% of participants had more than 20% of total burn surface area involved with post-burn leukoderma. Individual lesions treated with either SSG or MKS ranged from minimum of 2 cm^2 to a maximum of 16 cm^2 with nearly 27% of participants having lesion of more than 10 cm^2 (Table 1).

The repigmentation did not statistically differ between the two groups at 1 week and two weeks (Table 2). However, there was a significant difference in recipient site repigmentation at 4 and 12

Table 1: Participant characteristics

Parameters		Group 1	Group 2
Age	20 - 30	13(56.6)	13(52)
	30.1-40	6(26)	8(32)
	40.1-50	4(17.4)	4(16)
Gender	Male	17(73.9)	19(76)
	Female	6(26.1)	6(24)
Duration of burns (in months)	Less than 12	5(20)	4(16)
	12.1-18	4(16)	4(16)
	18.1 -24	5(20)	5(20)
	More than 24	9(36)	12(48)
Total surface area of burns	Less than 10%	2 (8.7)	3(12)
	10 – 20%	3(13)	3(12)
	More than 20%	18(78.3)	19(76)
Mean duration of leucoderma patch (months)		25.1	23.8
TBSA with leucoderma	Less than 10%	8(34.8)	7(28)
	10 – 20%	5(21.7)	6(24)
	More than 20%	10(43.5)	12(48)
Site of lesions	Face & neck	2(8.6)	1(4)
	Chest & abdomen	6(26.3)	8(32)
	Upper limbs	13(56.5)	13(52)
	Lower limbs	2(8.6)	3(12)
Size of lesions (cm2)	Less than 2	0	0
	2.1-5	9(39.1)	8(32)
	5.1-10	9(39.1)	9(36)
	More than 10.1	5(21.7)	8(32)

Table 2: Observed pigmentation in both groups

Pigmentation	1st week			2nd week			4th week			12th week		
	Grp I	Grp II	P value	Grp I	Grp II	P value	Grp I	Grp II	P value	Grp I	Grp II	P value
Excellent (90.1-100%)	22(95.7%)	3 (12%)	0.870	22 (95.7%)	2 (8%)	0.947	21 (91.3%)	1(4%)	0.010	21(91.3%)	3(12%)	0.001
Good (50.1-90%)	1(4.3%)	0		1 (4.3%)	1 (4%)		2 (8.6%)	4(16%)		2(8.7%)	10(40%)	
Fair (20.1-50%)	0	0		0	3(12%)		0	14(56%)		0	10(40%)	
Poor (<20%)	0	22 (88%)		0	19 (76%)		0	6(24%)		0	2(8%)	

Table 3 : Patient satisfaction scores

LEVEL (scores)	GROUP 1 (N, %)	GROUP 2 (N, %)	p-VALUE Chi-square tests
Dissatisfied (0-5)	1(4.3)	3(12)	0.022
Satisfied (6-8)	18(78.3)	20(80)	
Very satisfied (9-10)	4(17.4)	2(8)	
TOTAL	23(100)	25(100)	

weeks (chi-square tests. p=0.010 and 0.001). The pigmentation was significantly better in a split skin graft group (Group 1). The colour change, categorized as normal (same as non-burned skin), hyperpigmented, hypopigmented and absent, did not differ significantly between the two groups over 12 weeks of follow-up.

Donor site complications were categorized into none, hyperpigmentation, hypopigmentation, infection, and hypertrophic scar. The overall difference was not statistically significant between the two groups (chi-square tests, p =.206). Hyperpigmentation was the most common donor site complication. Hyperpigmentation (n=7(30.4%) *versus* n=5(20%), and scarring (n=1(4.3%) *versus* n= 0 (0%), were higher in Group 1 (split skin group) although the difference was not statistically significant.

Higher patient's satisfaction scores of 9-10 (very satisfied) were seen in Group 1 (n=4(17.4%) versus n=2(8%) while scores of 6-8 (satisfied) was more in Group 2 (n=20(80%) versus n=18(78.3%) (Table 3). The difference in satisfaction levels between the groups was statistically significant (chi-square tests, p =0.022).

Time to healing was less than 1-week in 80% of the patients in the MKS group. However, there is no statistical significance in the overall healing time and most patients healed within two weeks.

DISCUSSION

Improved survival of patients following burn injuries largely due to improved resuscitation protocols and the availability of dedicated burn centers has shifted the focus of attention in burn injuries to functional, aesthetic, and psychological post-burn sequelae. Leukoderma or white depigmentation of skin in areas affected by burn is one such sequelae that is of immense psychological burden for the affected individuals.

Regeneration and repigmentation of the epidermis following burn injury occur from the periphery of the wound by the migration of cells in superficial burns (Figure 1).

In deeper burns, the stem cells from the hair follicles can regenerate the epidermis. However, in full-thickness burns, all of the hair follicles are destroyed along with the upper subcutaneous fat, in which case, the stem cells along the hair follicles are also destroyed, and hence regeneration of the epidermis from hair follicles is not possible. In such cases, an epidermal covering forms over the wound, which develops slowly from the wound margins, and eventually spreads out over the entire wound surface.[6] Melanin which is essential in skin for protection against ultraviolet radiation, is produced in melanocytes that are present in the basal layer of the epidermis. Within the melanocytes, melanin is deposited in melanosomes, which is then transferred to keratinocytes via melanocyte processes. Therefore, for maintaining skin color, the communication of melanocytes and keratinocytes is essential. [7] Thermal injuries could result in hypopigmentation in several ways. After the injury, melanocytes may restore slowly and less predictably, leading to potential permanent pigment changes.[8,9] Another possible explanation for post-burn hypopigmentation could

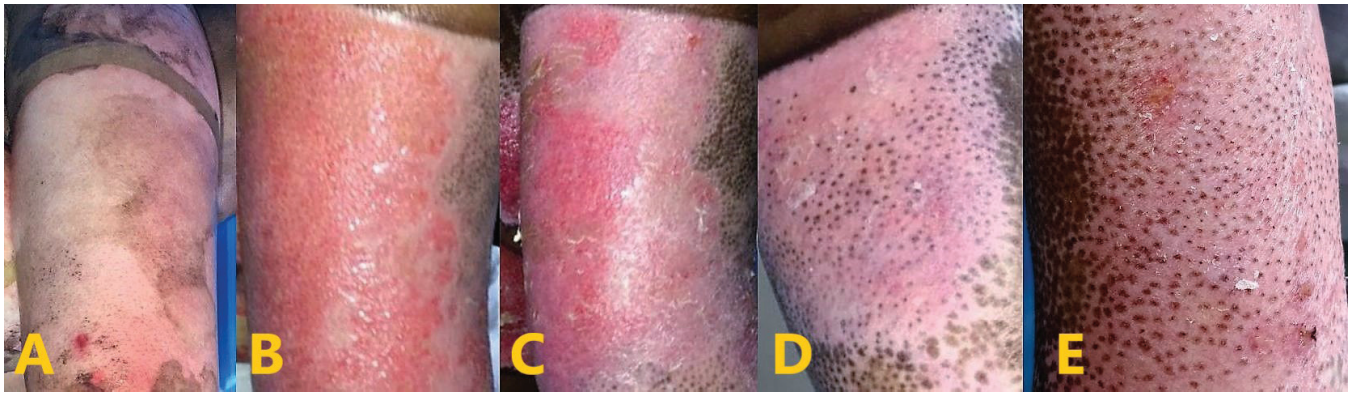

Figure 1: Regeneration and repigmentation of the epidermis occur from the periphery of the wound by the migration of cells in superficial burns.

be that the physical barriers that are produced due to secondary healing and scarring, prevent migration of melanocyte and melanin transfer. Severe thermal injury, resulting in the majority of melanocytes being destroyed could also be a possible etiology. It has also shown that weakened paracrine signaling between melanocytes and other skin cells affect skin pigmentation [10]. Long-term pigmentation in burn scars remains unpredictable.[11]

This loss of pigmentation assumes specific importance

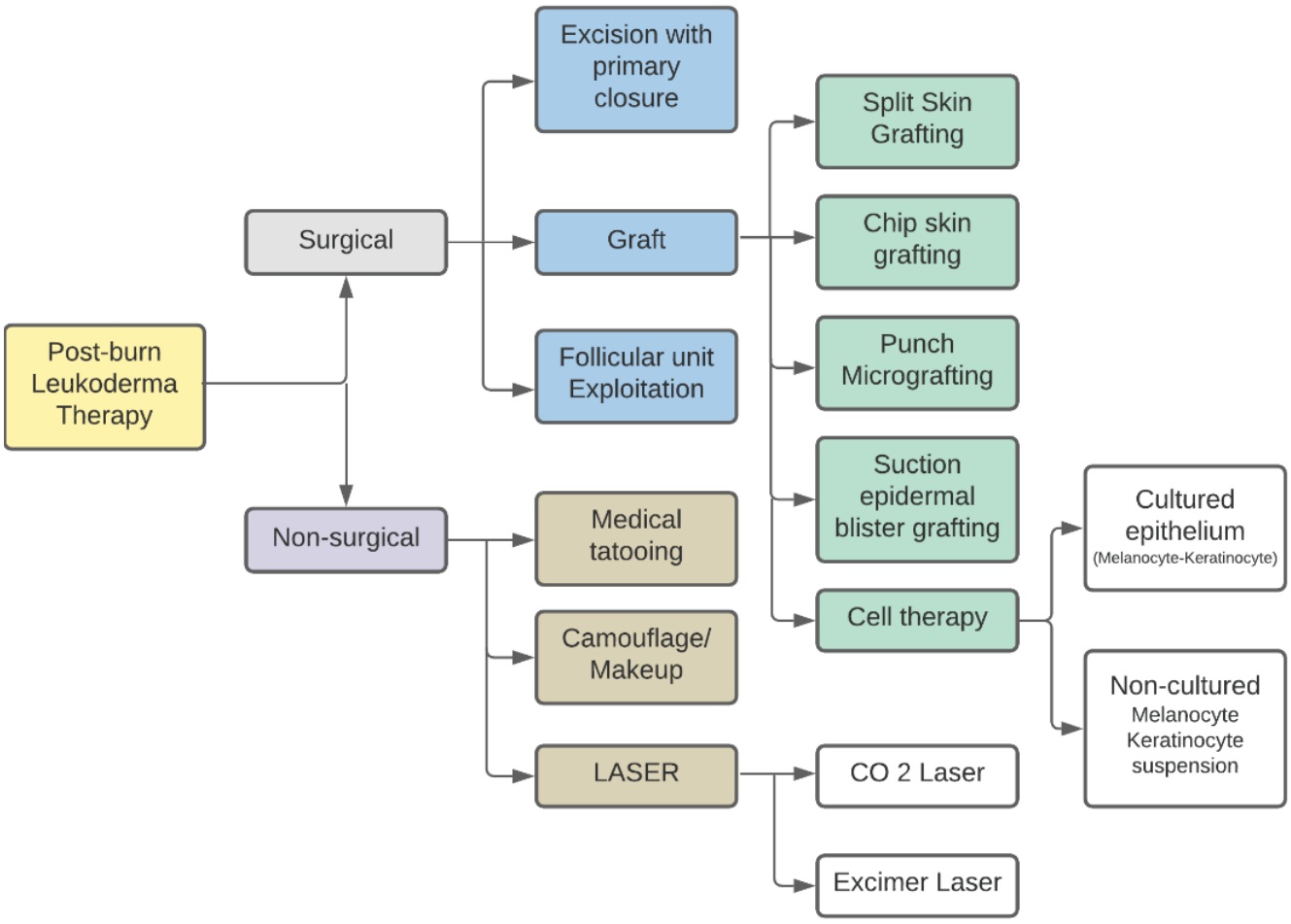

Figure 2: Treatment options for the management of post-burn leukoderma.

Table 4: Comparison of previously done RCTs for melanocytes keratinocyte cell suspensions. (No RCTS showing the use of MKS in PBL could be identified in literature)

Authors	Study type	Sample size	Study population	Results	Follow up
Van Geel (2004)[14]	Double blinded, placebo controlled trial	33, paired	Vitiligo	Ten patients had 70% repigmentation after 12 months	12 months
Back et al (2009)[7]	Double blinded, placebo controlled trial Double	13	Vitiligo	Study done only in Fitzpatrick skin type 1,2 showed poor results	52 weeks
Talukdar et al, (2016)[15]	Double blinded RCT with comparison to SSG	50	Vitiligo	64% had 70% re-pigmentation in MKS group and 52% had 70% re-pigmentation in SSG group	6 months
C Singh et al (2013)[16]	Noncultured root hair sheath cell suspension (NCORSHFS)	47, divided into two groups	Vitiligo	90-100% repigmentation in 83% of lesion in the NCES group and 65% of lesion in the NCORSHFS	16 weeks
Budania et al (2012) [52 17]	Suction blister epidermal grafting	51, divided into two groups	Vitiligo	90–100% repigmentation) in 71% of lesions in the NCES group and 27% of lesions in the SBEG group followed up for 16 weeks	16 weeks

in individuals with darker skin (Fitzpatrick IV, V, VI), where the hypopigmented patches appear more prominent and have an impact on the patient's perception of their image. In addition, hypopigmented skin may be at higher risk of the development of sunlight-induced damage and malignant transformation. Patients with darker skin types also develop areas of hyperpigmentation following burns as well as on skin graft donor sites. This is very unpredictable[12] and the etiopathogenesis remains unclear. Different treatment options have been outlined for the management of post-burn leukoderma (Figure 2).

Since the time Gauthier, Surleve- Bazeille,[13] introduced the concept of non-cultured MKS for vitiligo patches, there have been various studies to understand its efficacy in repigmentation of vitiligo (Table 4) as well as post-burn leukoderma (Table 5).

The mechanism of repigmentation by the autologous melanocytes in stable vitiligo was studied by Lerner *et al.*,[22] where they found that the melanocytes are incorporated into the basal layer of the recipient site and the pigment was transferred to the upper layers in the same pattern as the surrounding skin and the process takes about 6 months. However, patients with active vitiligo have a poor response to autologous melanocyte transfer, as the melanocyte-destroying factors are present and active.[23] The mechanism by which the cells are taken to the basal layer both in vitiligo and in post-burn leukoderma is not yet identified. An important

Table 5 - Studies demonstrating the use of MKS for Post-burn leukoderma

Authors	Study type	Study population	Sample size	Results	Follow up
Mulekar18(2011)	Case series with MKS	Post-burn leukoderma	10 (three lost to follow up)	90-100% repigmentation	3 - 12 months
Henderson19(2011)	Case report with MKS	Post-burn leukoderma	1	90-100% repigmentation	6 months
Busch20 (2016)	RCT, micro-needling vs micro-needling with MKS vs no intervention	Post-burn hypopigmentation	20	50-57.1% improvement in POSAS score	12 months
Iman21 (2013)	MKS, intradermal injection vs spray	Post-burn leucoderma	18	Mild repigmentation to no repigmentation	9-15 months

consideration is the potential loss of cells at the time of transplantation, which would have a bearing on repigmentation. While in stable vitiligo, only one-third of the transplanted cells are taken up, the same may not hold true in post-burn leukoderma which would have scarred dermal and epidermal components. The percentage of take of transplanted melanocytes in post-burn leukoderma scars has not yet been determined. Researchers have tried MKS both in vitiligo and in post-burn leukoderma (Table 4). While some have reported excellent results, few others have reported equivocal or even poor results (Table 5). No consensus on its usefulness and no RCTs comparing autologous non-cultured MKS with split skin grafting in post-burn leukoderma could be found in the literature.

All the patients in this study were from South India, and had similar Fitzpatrick skin type (Type IV, V, VI). There has been no significant difference among both groups, as regards age, gender, and duration of leukoderma patches. All the leukoderma patches covered with SSG had excellent re-pigmentation (Figure 3). with one patient showing partial loss of the graft.

This is in accordance with the study done by Driscoll *et al.*[24] who have used SSG to cover large areas of leukoderma with SSG and found to have less than 5% residual leukoderma. Ninety-five percent (95%) of the patients in Group I demonstrated complete graft take while the remaining patients had patchy graft loss (< 10% grafted area). This could be due to inadequate immobilization and is a potential complication of SSG. Our results closely match the conclusions of a review by Schmidt *et al.*, where they concluded that SSG is beneficial for repigmentation in smaller lesions whereas MKS is more useful in larger lesions. Moreover, SSG carries a possibility of demarcation between skin graft and normal skin.[25] A study conducted by Orecchia *et al.*[25]showed that in tropical countries, sunlight is a good source of UVA light helped in better repigmentation. Adjuvant therapy for the repigmentation was advised to our patients in accordance with Orecchia *et al.*, where the patients were asked to expose the treated area to direct sunlight for 30 minutes every day in the MKS group. Our pigmentation results have not been able to match those shown by Mulekar *et al.* of excellent pigmentation following non-cultured MKS in seven patients whom they could follow up (Figure 4).

This could be due to the shorter follow-up period in our study. Donor site complications are less, with MKS group compared to split skin grafting. This could assume significance when larger areas of post-burn leukoderma need to be re-pigmented.

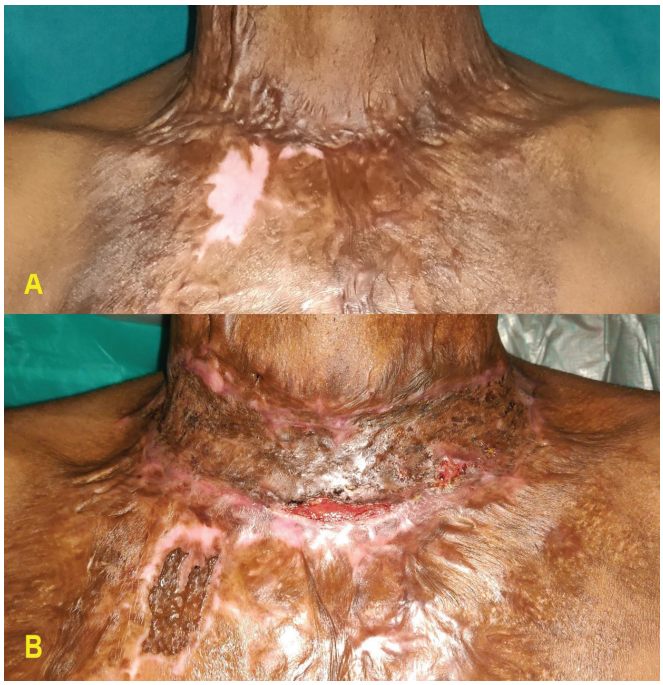

Figure 3: Re-pigmentation on leukoderma patches covered with SSG.

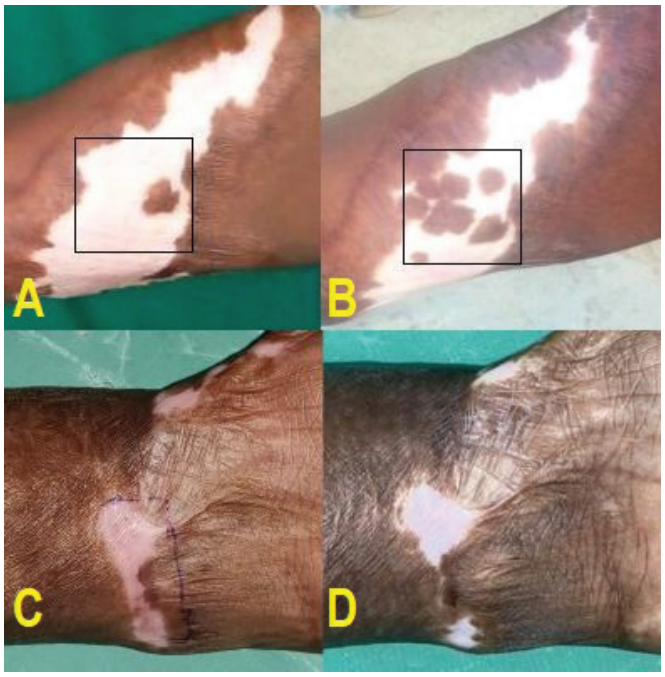

Figure 4: Pigmentation results following non-cultured MKS.

LIMITATIONS

Sample size in this study is small and a subsequent report with additional cases is planned. A longer follow-up period of (one year) may provide better insight into the extent and long-term outcomes of repigmentation procedures.

FURTHER AVENUES

Research into the mechanism of post-burn leukoderma, identification of cellular factors responsible and potential targets for repigmentation techniques, long-term effects of pigmentation with MKS, histomorphological outcomes of repigmentation techniques, and identification of pharmacotherapeutic agents to aid repigmentation, needs to be carried out in the future.

Further studies are also mandated to identify optimum time for MKS therapy following post-burn wound healing, evaluate the number of cells per unit area that are harvested from a donor site, identify the minimum cellular concentration that is needed to achieve satisfactory repigmentation, identify the survival rate of the cells after the transplantation and assess factors which enhance cellular survival and repigmentation.

CONCLUSION

Early (12 weeks) pigmentation with higher patient satisfaction scores is better achieved in post-burn leukoderma patches treated with SSG. Reduced donor skin requirement and reduced donor complications are noted in patients treated with non-cultured MKS suspension which carries significance in patients with paucity of donor areas as well as those requiring early recovery and rehabilitation.

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