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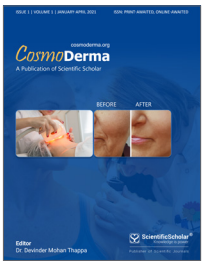

Original Article

Comparison of micro skin grafting and transplantation of non-cultured melanocyte keratinocyte suspension for the treatment of stable vitiligo: A pilot study

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Received : 04-April-2021
Accepted : 14-April-2021
Published : 24-April-2021

DOI
10.25259/CSDM_1_2021

Quick Response Code:

ABSTRACT

Objectives: We conducted this pilot study to compare the outcomes of non-cultured epidermal suspension (NCES) with that of micro skin grafting (MSG) in the treatment of stable vitiligo

Material and Methods: Twenty-nine patients with clinically stable vitiligo lesions (defined as the occurrence of no new lesions and no increase in the size of preexisting lesions for the past six months) and age group between 12 and 70 years were enrolled in the study. The enrolled patients were randomized into two groups using simple randomization using computer-generated random numbers, and allocation concealment was done using opaque sealed envelopes. Group 1 was comprised of 15 patients with 23 stable vitiligo lesions, and group 2 comprised of 14 patients with 22 stable vitiligo lesions. Patients in group 1 were transplanted with non-cultured melanocytes and keratinocytes. Patients in group 2 were treated using micro skin grafts after obtaining written informed consent. Ethical clearance was obtained from the Institute ethics committee, and the principles of the 1975 Declaration of Helsinki were followed.

Results: At the end of four months post-treatment, two patients (one in each group) did not follow up after removal of dressing at the first week. Results were analyzed at four months in 27 patients – 14 patients with 22 lesions in group 1 and 13 patients with 21 lesions in group 2. Repigmentation at 16 weeks post-surgery was evaluated. Excellent repigmentation (>90%) was seen in 45.45% of lesions in the non-cultured epidermal suspension (NCES) group as compared to 38.09% of lesions in the micro skin grafting (MSG) group, and this difference was not statistically significant ($p = 0.7597$). Repigmentation >75% was achieved in 54.54% of lesions in the non-cultured epidermal suspension (NCES) group compared to 38.09% of lesions in the micro skin grafting (MSG) group, and this difference was not statistically significant ($p = 0.3640$). Both the groups did not have any significant complications like scarring, milia, or any cobble stoning at the donor site. The recipient area had resistance to the spread of pigment at the margins of lesions. Six lesions in both the groups with excellent response, 3 in each group had achromic fissure or hypopigmented halos at the margins of lesions.

Conclusion: Both non-cultured epidermal suspension (NCES) and micro skin grafting (MSG) have been found to be safe and effective modalities with comparable efficacy in the surgical treatment of stable vitiligo. However, future trials on large sample sizes are warranted to validate our results.

Keywords: Surgical repigmentation, Stable vitiligo, Non-cultured epidermal suspension, Micro skin grafting

INTRODUCTION

Vitiligo, a common acquired hypopigmentory disorder resulting from melanocyte destruction, is a psychologically devastating condition, with lesions on exposed areas having a profound effect

on self-esteem and social interaction of patients.[1] Despite the availability of numerous medical and surgical modalities in vitiligo, the treatment remains a difficult task of vitiligo for the dermatologists as none of these treatments provide optimal response.

Surgical modalities in vitiligo are reserved for stable and refractory lesions. They have been classified mainly into tissue grafting and cellular grafting techniques. Each of these options has its pros and cons, as summarized in Table 1. Among the surgical options, non-cultured epidermal suspension (NCES), also known as non-cultured melanocyte keratinocyte suspension, and micro skin grafting (MSG) give promising results. Non-cultured epidermal suspension (NCES) is a simple, cost-effective method of cellular grafting that does not require cell culture, and the recipient area to be treated can be 5–10-times bigger than the donor area.[2,4] The main favorable factors of non-cultured epidermal suspension (NCES) are the lesser quantum of donor skin required to cover a big recipient area, less pain and discomfort during the procedure, and excellent color match. Micro skin grafting (MSG) is yet another type of smash grafting method which can be used to cover larger areas, in contrast to other methods of tissue grafting. The main favorable factors of micro skin grafting (MSG) are that it is a simple method, laboratory setup not required, and also micrografts are made to spread over larger areas as compared to other tissue grafting procedures.

Studies in the past have compared non-cultured epidermal suspension (NCES) and other procedures like suction blister grafting and transplantation of extracted outer hair root sheath suspension for the treatment of stable vitiligo.[5,6] Hence, we undertook this pilot study to compare the outcomes of non-cultured epidermal suspension (NCES) with that of micro skin grafting (MSG) in the treatment of stable vitiligo.

MATERIAL AND METHODS

Twenty-nine patients with clinically stable vitiligo lesions (defined as the occurrence of no new lesions and no increase in the size of preexisting lesions for the past six months) and age group between 12 and 70 years were enrolled in the study. Patients with body surface area > 30%, those with a history of a bleeding disorder, and keloidal tendencies were excluded from the study. The enrolled patients were randomized into two groups using simple randomization using computer-generated random numbers, and allocation concealment was done using opaque sealed envelopes. Group 1 comprised 15 patients with 23 stable vitiligo lesions, and group 2 comprised 14 patients with 22 stable vitiligo lesions. Patients in group 1 were transplanted with non-cultured melanocytes, and keratinocytes and patients in group 2 were treated using micro skin grafts after obtaining written informed consent. Ethical clearance was obtained from the Institute ethics committee, and the principles of the 1975 Declaration of Helsinki were followed.

Transplantation of non-cultured melanocyte keratinocyte suspension

Preparation of suspension

Under aseptic precautions, both donor and recipient sites were anesthetized by local infiltration of 1% lignocaine. The donor area, one-third to one-tenth the size of the recipient area, was selected on the lateral surface of the thigh. A thin split-thickness skin graft was harvested with the help of a sterilized shaving blade held in a Silverskin graft handle, and the donor area was dressed in sterile non-adherent vaseline gauze. The graft was washed immediately with normal saline and then transferred to a petri dish containing 5 ml of 0.25% trypsin and 0.02% EDTA (Himedia labs, Mumbai) and incubated overnight at 4°C. The next day, the petri dish was brought back to room temperature, 2 mL of the patient's serum was added to neutralize the action of trypsin. The solution was then pipetted out, and 5–10 mL of phosphate-buffered saline (PBS) (Himedia labs, Mumbai) was then added to the petri dish. The dermis was separated from the epidermis, and the dermal side of the epidermis was scraped with a curved jeweler's forceps to release cells from the basal layer of the epidermis into phosphate-buffered saline (PBS) solution. The dermal pieces and the remaining epidermal fragments were then removed, and the suspension thus obtained was

Table 1: Comparative features of tissue and cellular grafting techniques.

FEATURES [2,3,4]	TISSUE GRAFTING	CELLULAR GRAFTING
TYPES	Full-thickness punch grafting Split-thickness grafting Suction blister grafting Micro skin grafting (MSG) Ultra-thin epidermal grafting Flip top transplantation	Transplantation of: Cultured melanocytes Cultured epithelial grafts Non-cultured epidermal suspension (NCES) Non-cultured outer root sheath hair follicle suspension (NCORSHFS)
ADVANTAGES	Simple well standardized procedure	Can treat larger areas
DISADVANTAGES	More restricted to limited surface areas during one operative session	Complex techniques, require trained personnel and sophisticated equipment Increased cost of therapy

centrifuged at 89 g for 5 min. The supernatant was then discarded, and the pellet containing melanocytes and basal keratinocytes was resuspended in 0.5 to 1 mL of phosphate-buffered saline (PBS) depending on the size of the recipient area. Six to eight drops of methylcellulose were added to the suspension to make it denser. This suspension was loaded into a tuberculin syringe attached to an 18G needle.

Grafting procedure

The recipient site was moistened with normal saline, dermabraded using Maneckshaw manual dermabrader, and once pinpoint bleeding was seen, the suspension was uniformly spread over the area with the help of a tuberculin syringe. A surgical dressing composed of chlorhexidine gauze (Bactigras®: Smith & Nephew Healthcare Ltd. Hull, UK), collagen sheet, a sterile transparent occlusive dressing (Tegaderm O®: 3M Healthcare, St. Paul, MN, USA), and a sterile surgical pad was placed over the recipient area. Finally, the area was secured by bandaging with an elastic adhesive bandage, and the patient was advised to restrict movement at the site as much as possible.

Micro skin grafting

The lateral surface of the thigh was selected as the donor site as in the previous procedure. Under aseptic precautions, a thin split-thickness skin graft was taken from the donor area with the help of a sterilized shaving blade held in a Silverskin graft handle. The skin graft of thickness 0.15 – 0.3 mm was minced into small pieces of approximately 0.4–0.8 mm using scissors. The minced skin was then applied over the dermabraded recipient area uniformly, and the area was covered with multilayered dressings as in the previous procedure.

Post-operative advice and follow up

In both the groups, the same post-operative procedures were followed. The patient was discharged from the hospital and given oral antibiotics and anti-inflammatory agents for one week. Dressings were removed after one week, and the patient was advised to expose the recipient area to mid-day sun (11:00 am–2:00 pm), but only for a maximum of 30 minutes daily. All patients were followed up at 1, 2, 4, 8, 12, and 16 weeks respectively, to assess the extent of repigmentation. Color match was assessed in comparison to the surrounding normal skin. Clinical digital photographs were taken at each visit.

Efficacy outcomes

The primary measure of treatment efficacy was the percentage of repigmentation at 16 weeks after surgery. Repigmentation was graded as excellent (>90), very good (>75%–90%), good (>50%–75%), fair (>25%–50%) or poor (<25%). Adverse effects and any improvement in leukotrichia were also recorded.

Statistical analysis

Descriptive statistics were used, and an unpaired t-test was used for comparison of parametric data, and efficacy outcomes between the two groups were compared using Fisher's exact test. All statistical analysis was carried out at an 80% confidence interval, and $p < 0.05$ was considered significant.

RESULTS

At the end of 4 months post-treatment, two patients (one in each group) did not follow up after removal of dressing at the first week. Results were analyzed at four months in 27 patients—14 patients with 22 lesions in group 1 and 13 patients with 21 lesions in group 2.

The pretreatment characteristics of the patients in both groups were comparable, as shown in Table 2, and the frequency distribution based on the duration of stability is provided in Table 3.

Table 2: Patient and disease characteristics in both groups.

Parameters	NCES (group1) N = 15	MSG (group2) N = 14	P value
Number of lesions	23	22	
Type of vitiligo (G/S/F/M)	7/4/2/2	7/3/3/1	
Age (years) Mean ± SD	28.47 ± 11.86 (12 – 45 years)	26.93 ± 14.09 (14 – 65 years)	0.7523*
Gender (Female: Male)	1.1:1	2.5:1	0.5349$
Duration of disease (months) Mean ± SD	84 ± 73.2	66.86 ± 57.01	0.4899*
Duration of stable disease (months) Mean ± SD	42.07 ± 39.80	19.64 ± 14.47	0.0571*
Size of recipient area (cm^2) Mean ± SD	19.33 ± 17.11	14.3 ± 11.52	0.3651*
Size of donor area (cm^2) Mean ± SD	3.83 ± 1.98	4 ± 2.68	0.8498*

G- Generalized, S- Segmental, F- Focal, M- Mucosal
* unpaired t test;$ Fisher's exact test

Table 3: Duration of stable vitiligo in both the groups.

Duration of stable disease	NCES group (No. of patients)	MSG group (No. of patients)
6 months – 1 year	5	6
1 – 2 years	3	4
2 – 3 years	1	2
3 – 4 years	0	2
>4 years	6	0
Total	15	14

The recipient area ranged from 3.4 to 60 cm^2 in the non-cultured epidermal suspension (NCES) group and from 2.3 to 36 cm^2 in the micro skin grafting (MSG) group. The donor-recipient ratio varied from 1:2 to 1:10 in the non-cultured epidermal suspension (NCES) group and from 1:2 to 1:5 in the micro skin grafting (MSG) group. The lesions were located on both easy to treat (face, neck, chest, abdomen) and difficult to treat areas, including acral, lips, and over bony prominences.

Efficacy outcomes

Initial repigmentation was observed as early as two weeks in 6 of 22 lesions (27.2%) in the non-cultured epidermal suspension (NCES) group and 5 of 21 lesions (23.8%) in the micro skin grafting (MSG) group, and this difference was not statistically significant ($p = 0.7317$).

Repigmentation at 16 weeks post-surgery

Excellent repigmentation (>90%) was seen in 45.45% of lesions in the non-cultured epidermal suspension (NCES) group as compared to 38.09% of lesions in the micro skin grafting (MSG) group, and this difference was not statistically significant ($p = 0.7597$) (Figures 1 and 2). Repigmentation > 75% was achieved in 54.54% of lesions in the non-cultured epidermal suspension (NCES) group compared to 38.09% of lesions in the micro skin grafting (MSG) group, and this difference was not statistically significant ($p = 0.3640$). [Table 4].

The color of the repigmented area matched well with that of surrounding skin in 7 of 13 lesions (53.85%) in the non-cultured epidermal suspension (NCES) group and 9 of 13 lesions in the micro skin grafting (MSG) group (69.23%), and this difference was not statistically significant ($p = 0.6882$). The remaining six lesions in the non-cultured epidermal suspension (NCES) group and four lesions in the micro skin grafting (MSG) group had a lighter color match with the surrounding skin.

Response assessment based on site of vitiligo

Excellent repigmentation was seen in lesions located over the face and legs in both groups. Six lesions of lip vitiligo, 3 in each group, were treated, and all of them had poor response to treatment. Only one patient with an acral lesion over the right hand was treated in the micro skin grafting (MSG) group, which showed poor response to treatment.

Response assessment based on the stability of vitiligo

Patients with a duration of stable vitiligo of < 1 year, 5 in the non-cultured epidermal suspension (NCES) group and 5 in the micro skin grafting (MSG) group did not achieve any significant repigmentation. One patient with stable vitiligo of 6 months duration in the micro skin grafting (MSG) group achieved excellent repigmentation at three months, followed by the subsequent loss of pigmentation at four months with repigmentation of <75%. In patients with stable vitiligo of > 1-year duration, the repigmentation response > 75% was achieved in 7 of 10 patients in the non-cultured epidermal suspension (NCES) group (70%) and 5 of 9 patients in the micro skin grafting (MSG) group (55.5%).

Improvement in leukotrichia

Only one patient in the non-cultured epidermal suspension (NCES) group had complete repigmentation of leukotrichia at eight months post-treatment, while no improvement in leucotrichia was observed with micro skin grafting (MSG).

Complications

Both the groups did not have any significant complications like scarring, milia, or any cobble stoning at the donor site. The recipient area had resistance to the spread of pigment at the margins of lesions. Six lesions in both the groups with

Table 4: Comparison of repigmentation response in both the groups at 4 months follow up.

Response	Total		NCES Group		MSG Group	
	Number of lesions	Percentage (%)	Number of lesions	Percentage (%)	Number of lesions	Percentage (%)
Excellent	18	41.86	10	45.45	8	38.09
Very good	2	4.65	2	9.09	0	0
Good	3	6.98	1	4.54	2	9.52
Fair	3	6.98	-	0	3	14.28
Poor	17	39.53	9	40.9	8	38.09
Total	43	100	22	100	21	100

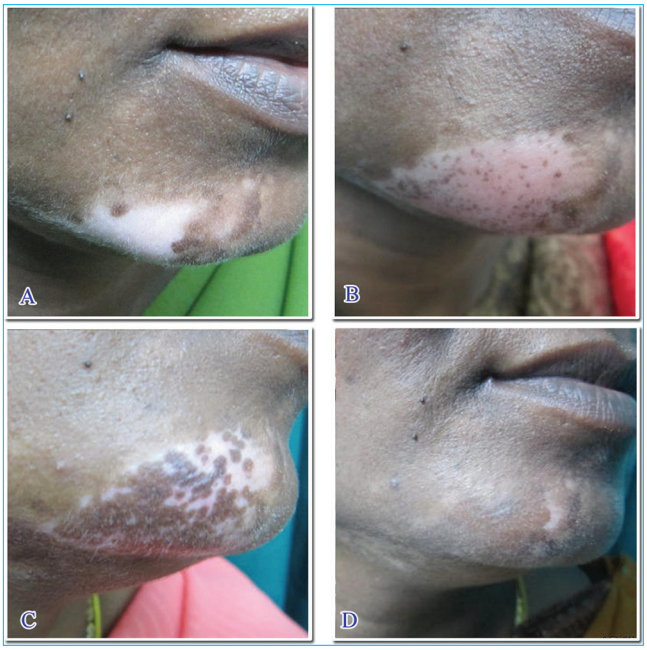

Figure 1: A patient in the non-cultured epidermal suspension (NCES) group with lesion of segmental vitiligo over the face showing almost 100% repigmentation. (A) Before surgery; (B) Lesion showing perifollicular repigmentation 2 weeks after surgery; (C) Lesion showing >50%repigmentation4 weeks after surgery; (D) 16 weeks after surgery

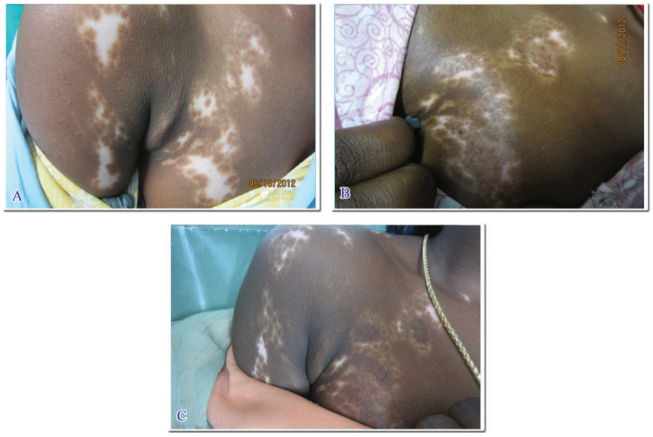

Figure 2: A patient in the micro skin grafting (MSG) group with lesions of segmental vitiligo over the right chest and arm showing >90%repigmentation. (A)Before surgery; (B) Lesions showing >75% repigmentation with diffuse pattern 4 weeks after surgery; (C) 16 weeks after surgery

excellent response, 3 in each group had achromic fissure or hypopigmented halos at the margins of lesions.

DISCUSSION

Current therapeutic modalities of vitiligo are mainly focused toward arresting the progression of the disease and having repigmentation by means of surgical and non-surgical methods. Despite many medical treatment options for vitiligo, majority of patients do not respond with a satisfactory degree of repigmentation. Surgical techniques are mainly developed for patient's refractory to medical treatments.

Non-cultured epidermal suspension (NCES) in stable vitiligo was first noted by Gauthier and Surleve-Bazeille[7] in 1992. In the original technique described, a donor tissue taken from the scalp by superficial shaving was treated with 0.25% trypsin for 18 hours, and the cellular suspension was made. This cellular suspension was injected into blisters induced by liquid nitrogen at the recipient area. Several years later, in 1998, Olsson and Juhlin[8] reported a modified but improved method wherein the donor skin obtained from the gluteal region was trypsinized in 50 minutes, and the cellular suspension obtained was directly put on to a dermabraded vitiligo lesion. Van Geel et al.[9] modified these two methods in 2001 by introducing hyaluronic acid as a biodegradable barrier to increase the thickness of the suspension. Since then, several studies have been done using this technique with variable results.

In our study, we had combined the procedures described by Van Geel et al.[9] for obtaining the cellular suspension and that of Holla et al.,[10] for making the cellular suspension using phosphate-buffered saline (PBS) instead of melanocyte media. We did not use any specific trypsin inhibitor, and the patient's own serum was used to neutralize the action of trypsin. Methylcellulose drops were used instead of hyaluronic acid to increase the thickness of the suspension. All these steps reduced the cost of the procedure significantly as both melanocyte media and specific trypsin inhibitors are expensive. The cost-effectiveness was further improved by the addition of methylcellulose as the viscous agent.

Micro skin grafting (MSG) provided promising results in large granulating wounds and extensive burns in studies carried out by Nanchachal et al.[11] and Zhang et al.[12] Hence, micro skin grafting (MSG) was first time effectively tried to treat stable vitiligo by Gupta[13] based on these observations. Tsukamoto et al.[14] also described excellent repigmentation in patients with facial stable segmental vitiligo treated with micro skin grafting (MSG) followed by PUVA therapy. In our study, we spread the minced grafts by direct spread method as described by Gupta.[13]

Both non-cultured epidermal suspension (NCES) and NCORSHFS have been found to be safe and effective modality with comparable results in the surgical treatment of stable vitiligo.[5,6] In our study, at the end of 16 weeks, repigmentation was more visible in the non-cultured epidermal suspension (NCES) group than the micro skin grafting (MSG) group, though not statistically significant.

Our observation regarding the pattern of repigmentation was as per the findings of Sahni et al.,[15] who noted perifollicular repigmentation as the most common pattern in vitiligo lesions, followed by diffuse repigmentation in non-cultured epidermal suspension (NCES). Irrespective of method, elbows and fingers are the most difficult areas to repigment.[16] We agree with Mulekar[17] that stability in vitiligo of 6 months is too short a period, and disease may still be active. For leukotrichia, non-cultured epidermal suspension (NCES) and micro skin grafting (MSG) may not be the suitable modality of surgical treatment.[18]

Complications other than the halo phenomenon were not observed in our study. According to Sobhy et al.,[19] the lack of pigmentation at the periphery of the transplanted skin may be due to some melanocyte destroying factors or due to influx of keratinocytes from the border. This influx of keratinocytes at the margins appeared as a white rim. This can be taken care of by increasing dermabrasion beyond the border of the lesion. Olsson and Juhlin[16] found that the white edge can be reduced usually in patients treated with the epithelial sheets than patients treated using free cells in suspension.

Limitations of the study

Lack of randomization, controls, blinding and objective measures, and small sample size, which led to lack of statistical significance for many measures, were significant limitations of our study. Moreover, it was a pilot trial with a small number of patients.

CONCLUSION

Both non-cultured epidermal suspension (NCES) and micro skin grafting (MSG) have been found to be safe and effective techniques with comparable efficacy in the surgical treatment of stable vitiligo. However, future trials on large sample sizes are warranted to validate our results.

Declaration of patient consent

Institutional Review Board (IRB) permission obtained for the study.

Financial support and sponsorship

Nil.

Conflicts of interest

The authors Devinder Mohan Thappa is the Editor-In-Chief. Laxmisha Chandrashekar and, Malathi M are on the Editorial Board of this journal. They have no conflict of interests.

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How to cite this article: Manickam N, Thappa DM, Chandrashekar L, Friji MT, Malathi M. Comparison of micro skin grafting and transplantation of non-cultured melanocyte keratinocyte suspension for the treatment of stable vitiligo: A pilot study. Cosmoderma 2021;1:2.