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A Study of Autologous Non-Cultured Epidermal Suspension (NCES) Transplant in Patients with Stable Vitiligo without the Use of NB-UVB

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ABSTRACT

Introduction: *Vitiligo is a common form of localized depigmentation and an important public health problem which affects around one percent of the global population and about two percent of the population in India. The present study aimed to document the results and side effects of non-cultured melanocyte transplant in patients with stable vitiligo without post-procedure use of NB UVB.*

Methods: *The present study was a prospective interventional study conducted among patients with stable vitiligo who were attending the outpatient department (OPD) of dermatology of a tertiary center*

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of Delhi. Patients with stable vitiligo aged >10 years who gave their consent for non-cultured epidermal suspension (NCES) were included in the study. Autologous skin graft of size 0.2 mm was taken from the donor site and a melanocyte cell suspension was prepared using standardized procedure, which was later transplanted to the recipient area. All patients were asked to come for follow-up at the OPD after seven days, 15 days, one month, followed by every month till six months post-transplantation, and repigmentation was assessed visually by both graph paper and photography. Data was analyzed using SPSS vs. 21.

Results: *In our study, the majority of patients had vitiligo vulgaris (27 subjects with 47 patches). A total number of 59 patches were transplanted, of which 38 (64.4%) achieved excellent repigmentation, 28.8% good repigmentation, 3.4% fair repigmentation and 3.39% poor repigmentation. A total of 1302.5 cm² of the depigmented area was operated by NCES and the repigmentation of 874.5 cm² (67.14%) was achieved by six months.*

Conclusion: *The present study found that the majority of patients (93%) with vitiligo achieved excellent or good repigmentation after NCES and the rate of complication was very low.*

Keywords: vitiligo, melanocyte transplant, epidermal suspension, autologous transplant.

INTRODUCTION

Vitiligo is a chronic depigmentation disorder of the skin or mucosa which represents a growing public health problem worldwide. It is a multifactorial polygenic disorder associated with complex pathogenesis, usually asymptomatic, caused by a loss of functional melanocytes and often associated with other autoimmune diseases (1, 2). Vitiligo affects approximately 1% of the world population of all skin types, usually before the age of 20 (3), with a female to male ratio of 1.4:1 (4). The incidence of vitiligo in India is significantly high, ranging from 3% to 4%, with a peak of up to 8.8% being reported in the states of Rajasthan and Gujarat (5).

Vitiligo can be clinically classified as (a) non-segmental or generalized vitiligo that includes acrofacial, vulgaris, universalis and mixed forms; and (b) localized vitiligo, which can affect either one segment or two or multiple segments and includes focal, segmental and mucosal forms. There are also mixed and undetermined forms of vitiligo.

Repigmentation is seen less often with the use of conservative treatment option available at the present time. Surgical treatment can be a choice and it is usually recommended for patients with stable vitiligo (with no change in the number of lesions or their morphology for a period of one year) for which other treatment modalities had no significant results.

Surgical treatments include suction blister grafts, punch grafts, minigrafts, split thickness skin grafts and cellular grafts (6, 7). There are two types of cellular grating, including cultured and non-cultured melanocyte transfer. Cultured melanocyte transplant is a complex procedure requiring well equipped laboratories and skilled technicians, which is time consuming. Cultured melanocyte transplant however shows that melanocytes count can be increased by up to 100 times.

Non-cultured epidermal suspension is a new simpler cellular grafting technique which can be done with the help of basic laboratory settings; it is less time consuming, less tedious and more area can be covered in a single sitting. Larger recipient areas can be covered by graft taken from a comparatively smaller donor site compared to the older techniques (donor:recipient 1:10). However, they carry the risk of complications such as scarring, hyperpigmentation and color mismatch.

This study aimed to observe the results and side effects of non-cultured melanocyte transplant in patients with stable vitiligo without post-procedure use of narrowband ultraviolet B phototherapy (NB UVB). ❑

MATERIALS AND METHODS

Study design: prospective interventional study.
Study setting: OPD of Dermatology, Venereology and Leprology of a tertiary health center, Delhi, India.

Study duration: one year, between August 2017 and August 2018.

Study population: patients with stable vitiligo attending the OPD.

Inclusion criteria: patients with stable vitiligo (*as per* IADVL taskforce definition of stability) (8); patients consenting for NCES; patients aged 10 years or above; patients with an area of each target patch of at least 5 cm^2.

Exclusion criteria: involvement of more than 30% of the body surface area; patients with a history of koebnerization and/or keloid development at the site of injury; pregnant and lactating women; patients with immune-compromised status; patients with bleeding disorders; patients who were taking steroids or any other immunosuppressant for vitiligo during the last one month before surgery.

Methodology

All patients with stable vitiligo coming to the OPD of Dermatology between August 2017 and January 2018, who met the inclusion criteria, were selected for the present study after taking their written informed consent. Details regarding vitiligo, personal and family history, and treatment history were collected using a semi-structured questionnaire. Vitiligo activity was calculated using VIDA scale, local examination of the vitiligo site was done, target patches of vitiligo were selected and their area was measured using graph paper in square centimeters (Figure 1) and recorded; also, a high-resolution picture of target patches for analysis of repigmentation was taken. Photography of the target site, donor site and repigmentation site. Routine lab tests were done before taking the skin graft.

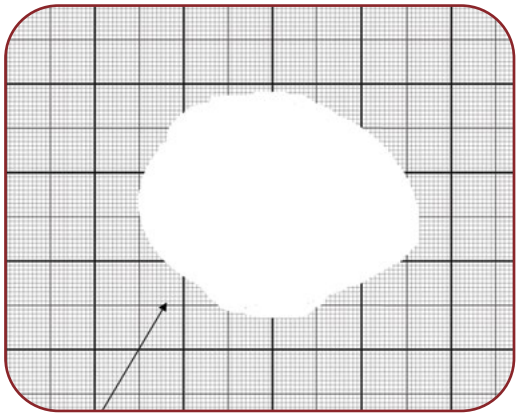


FIGURE 1. Area of the lesion – graph paper was used to determine the area of target patch

The following formula was used:

$$\text{No. of large square} + \frac{\text{No. of medium square}}{4} + \frac{\text{No. of small square}}{10} = \text{area in } cm^2$$

Transplant procedure

1. Donor site

A donor area of $1/10^{th}$ of the recipient area was marked on the lateral aspect of the thigh. The area was cleaned using 10% povidone iodine solution and anesthetized with 1% lignocaine with adrenaline. The skin was stretched and a thin split thickness graft of 0.2 mm was taken with a silvers knife. The superficial wound was covered with sterile chlorhexidine dressing, followed by elastic and adhesive compression band (Figure 2a).

2. Cell separation procedure

The skin graft received from donor site was processed with 6 mL of 0.25% trypsin-ethylenediaminetetraacetic acid (EDTA) in a Petri dish. Then, it was completely submersed in the solution with the epidermal side facing up. Afterwards, the mixture in the Petri dish was incubated at 37 °C for 50 min. After incubation, the graft was transferred into a Petri dish containing 8 mL of melanocyte nourishment medium which helped in waning off the action of trypsin by acting as a diluting agent; therefore, no trypsin inhibitor or phosphate buffer saline was needed.

The epidermis and dermis were slowly differentiated by using Jewelers forceps. Scrapping of the epidermal surface was done to avoid remains of any pigment on their surface. The content of the Petri dish was centrifuged for six minutes at 3000 rpm. Then, the cell pellets settled at the bottom after centrifugation, consisting of cells from stratum basale and lower half of the stratum spinosum that were rich in melanocytes, were collected and the supernatant part was discarded. The resulting pellets were mixed with 0.8 mL of nutrient medium and slowly put into a 1 mL syringe after taking proper aseptic precautions (Figure 2b-2g).

3. Recipient site (vitiliginous area)

The vitiliginous areas were marked and cleaned with 10% povidone iodine and anesthetized with 1% lignocaine without adrenaline. The area was resurfaced down to the papillary dermis (till pinpoint bleeding) with a diamond fraise wheel. Application of cell suspension over the denuded

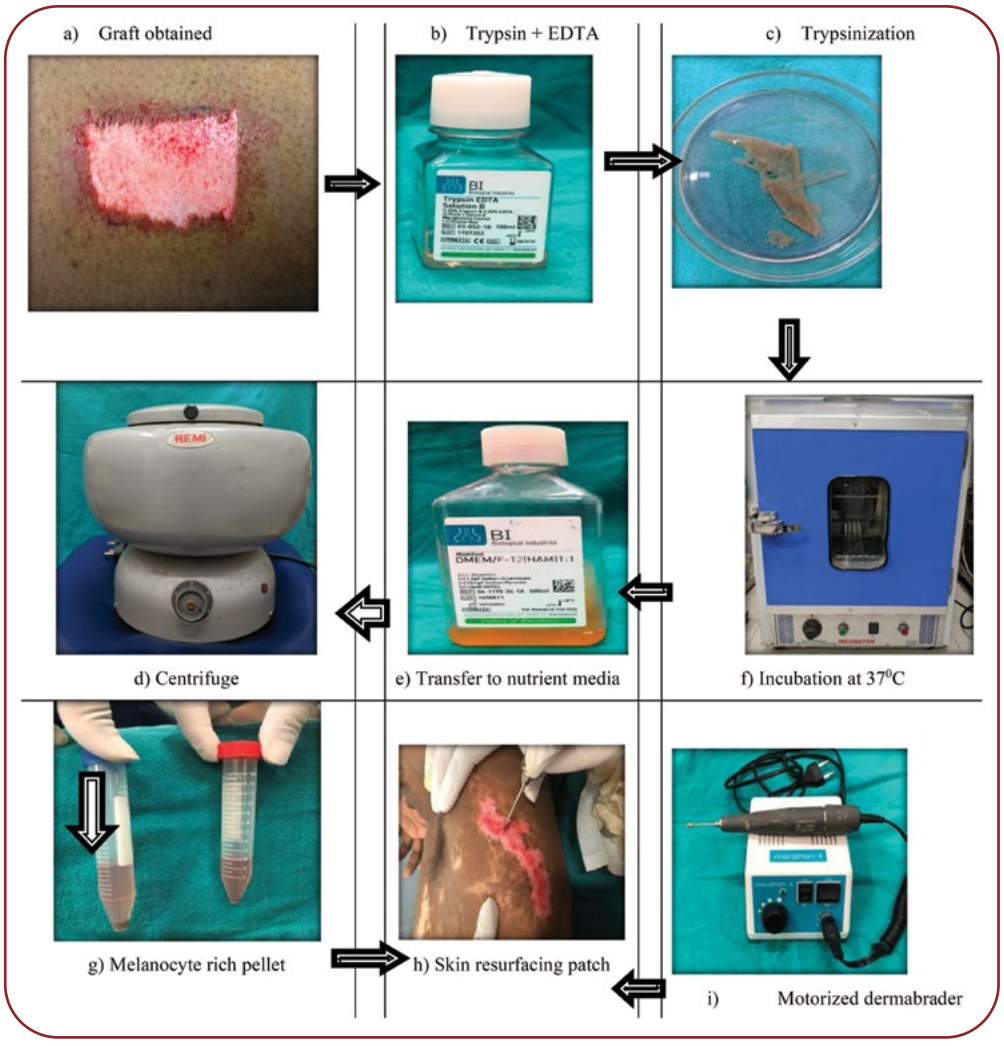


FIGURE 2. Steps of non-cultured epidermal suspension (NCES) transplant

recipient area was done uniformly and gently using a spatula. The area was covered with sterile gauze pieces and held in place by transparent film dressing or elastic adhesive bandage to secure its location. Patients were advised to remain supine for the next 30 minutes (Figure 2h).

4. Post-operative care and follow-up

Appropriate medical management using suitable medicines for five days during the post-transplant period was done. Dressing was kept for seven days and then it was removed from both the donor and recipient areas. All patients were asked to come for follow-up at the OPD after seven days, 15 days, one month, then every month till six months post-transplantation, and repigmentation was assessed visually by both graph paper and photography. Patients were also assessed by an independent observer.

5. Evaluation of repigmentation

For more objective assessment, the repigmentation pattern was assessed by tracing the lesion, repigmented areas were marked on translucent paper and then copied to graph paper. Percentage of repigmentation was calculated by dividing the area of repigmentation by the area of depigmentation and multiplying by 100. Also, pictures for computerized analysis of the extent of repigmentation were taken with the help of software ImageJ. The repigmentation pattern was noted as 'diffuse', 'perifollicular' or 'marginal'. The donor area was examined for scarring and koebnerization and further tabulated. Patient satisfaction forms were given to patients to

report any adverse events felt and satisfaction with the procedure.

Repigmentation was assessed at each follow-up visit and scores were given as follows:

- 0-25%: poor repigmentation
- 25-50%: fair repigmentation
- 50-75%: good repigmentation
- 75-100%: excellent repigmentation.

Statistical analysis

Data was collected using a semi-structured questionnaire and it was entered in Microsoft spreadsheet and analyzed after data cleaning by using SPSS statistical software version 21.0. Continuous data were presented as mean ± SD and median (IQR) and categorical data in the form of frequency and percentage (%). Appropriate parametric and non-parametric tests were used wherever applicable after testing distribution of data whether normally distributed or not by using Kolmogorov-Smirnov test.

Ethical issues

The study protocol was presented to the institutional ethical board of North Delhi Municipal Cooperation Medical College (N.D.M.C) and Hindu Rao Hospital, Delhi, India, and approval was sought before the start of the study. Written informed consent was taken from participants and parents of minor patients. Confidentiality of data was maintained. For subjects with complications, appropriate management was done as per guideline.

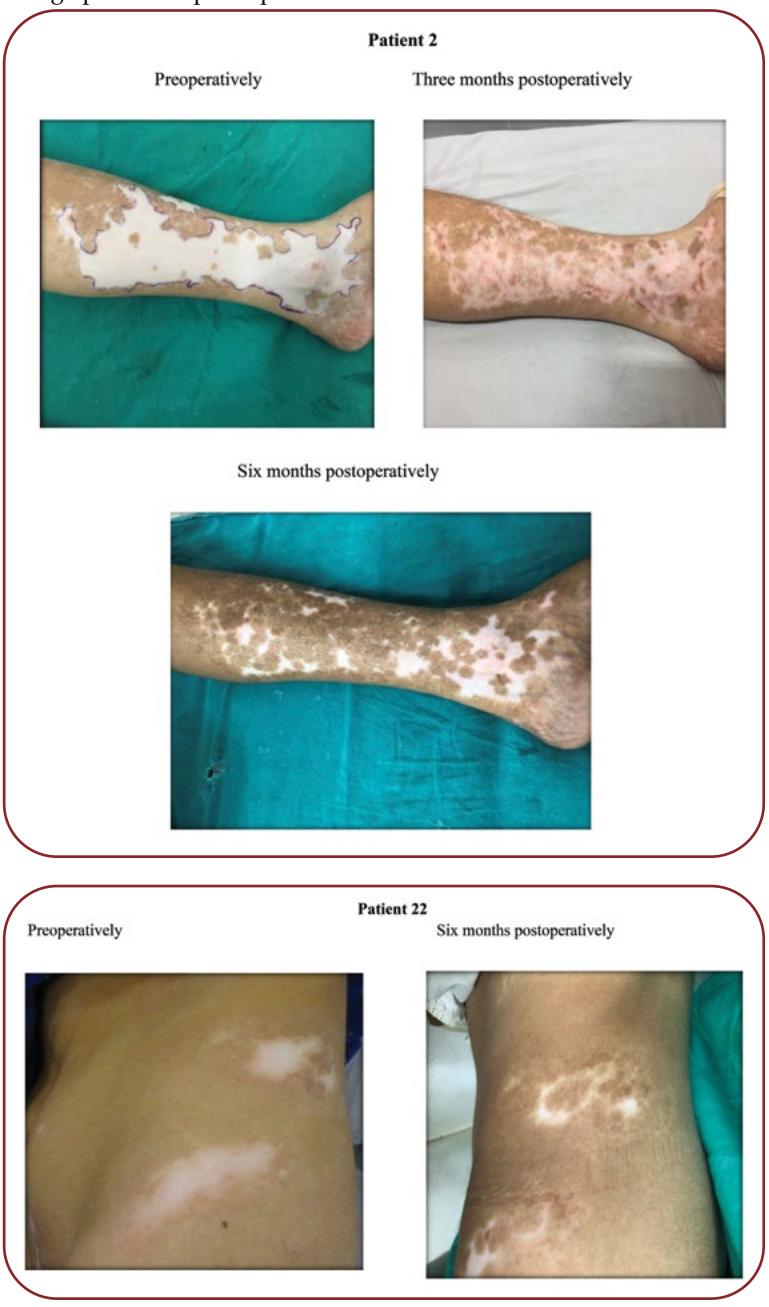
RESULTS

In the present study, 39 patients were included between August 2017 – January 2018 and they were followed for the next six months till August 2018. One patient was lost to follow-up and was therefore excluded from analysis. A total of 1302.5 cm2 of depigmented area was operated by NCES and the repigmentation of 874.5 cm^2 (67.14%) was achieved by six months. Patients lost to follow-up after surgery were excluded from the study results. Around 38 out of 59 patches (64.41%) achieved excellent repigmentation (>75%), 17/59 (28.81%) patches good repigmentation (>51-75%), 2/59 (3.39%) fair repigmentation (31-50%) and 2/59 (3.39%) patches poor repigmentation (<30%) (Table 1, Supplementary Figure 1). ❑

TABLE 1. Distribution of transplants patches according to repigmentation score

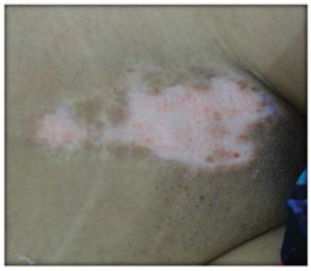
Repigmentation score	Frequency	Percentage
Excellent (>75%)	38	64.41%
Good (>51-75%)	17	28.81%
Fair (31-50%)	2	3.39%
Poor (<30%)	2	3.39%
Total	59	100%

SUPPLEMENTARY FIGURE 1. Pre- and post-operative changes in vitiligo patches in participants

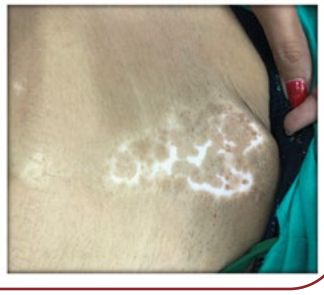


Patient 27

Intraoperatively

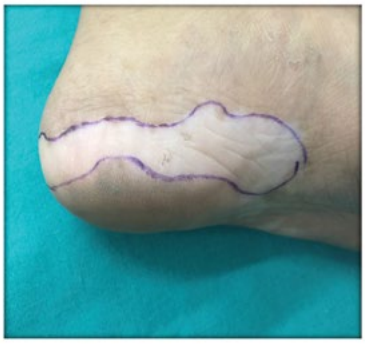


Six months postoperatively

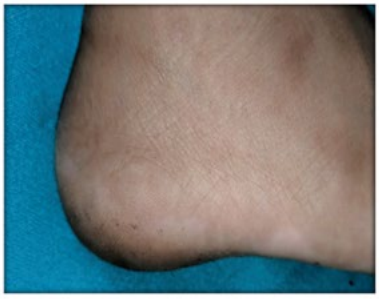


Patient 1

Preoperatively

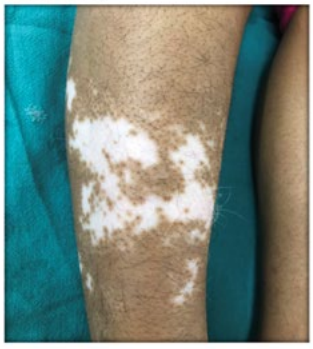


Six months postoperatively

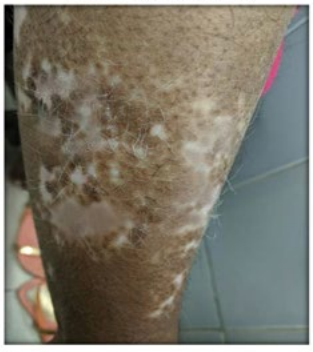


Patient 29

Preoperatively

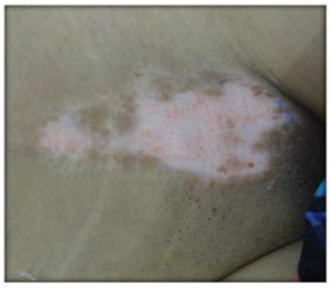


Six months postoperatively

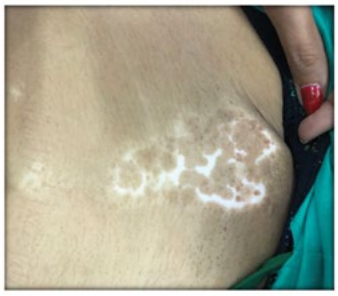


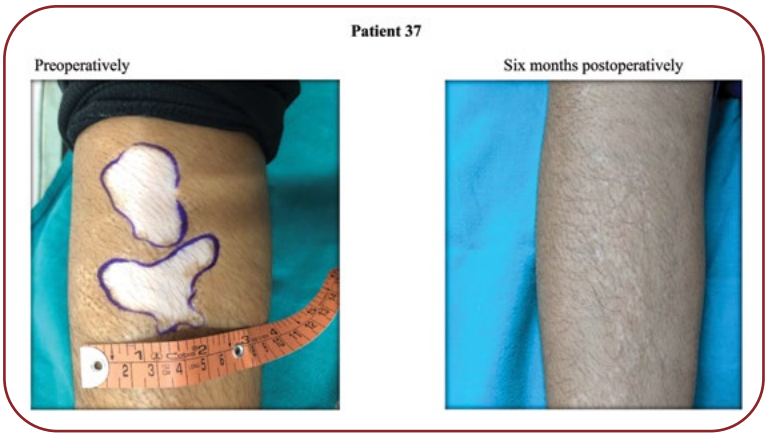
Patient 27

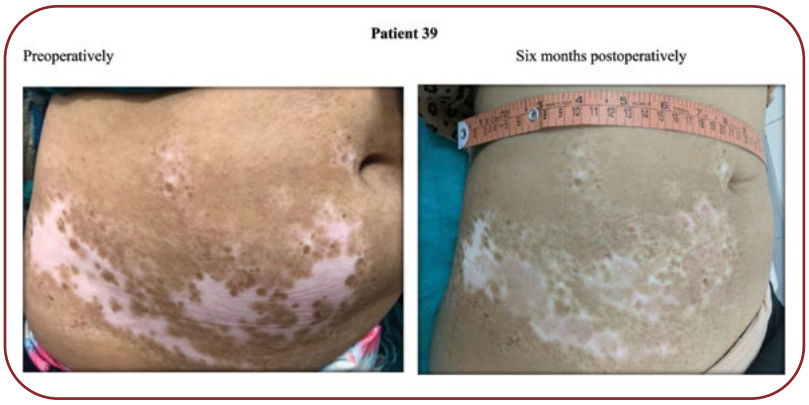
Intraoperatively



Six months postoperatively







Pattern of pigmentation

The majority of patches – 54 (88.52%) – showed diffuse repigmentation and only six (9.83%) showed perifollicular repigmentation. One (1.85%) patch showed no sign of repigmentation. At the end of two months, two patients who had initial perifollicular repigmentation were lost to follow-up (Table 2).

TABLE 2. Distribution of patches according to the pattern of repigmentation

Pattern of repigmentation	Frequency	Percentage
Diffuse	54	88.52%
Perifollicular	6	9.83%
Marginal	0	0
No pigmentation	1	1.85%

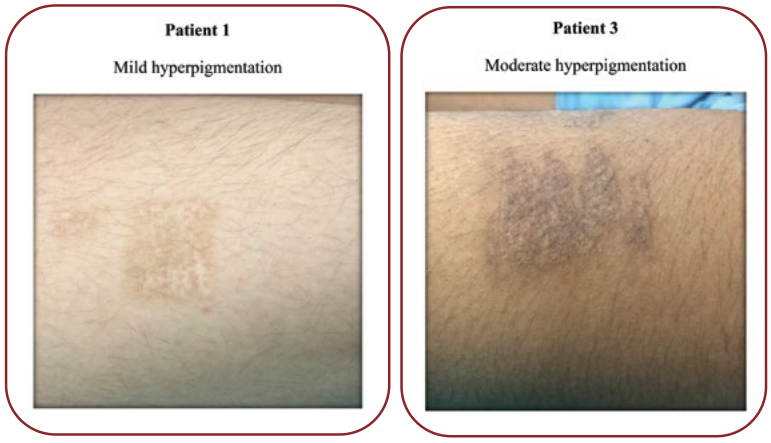
Complications

Three out of 38 patients (7.98%) had developed hyperpigmentation at the donor site. No scarring, infection or koebnerization was seen. One patient who was lost to follow-up after the first post-procedure visit had no infection and the donor site was in healing phase (Table 3, Supplementary Figure 2).

TABLE 3. Distribution of patches according to complication

Site	Complication	Frequency	Percentage
Donor site	No	35	92.11%
	Yes	3	7.98%
Recipient site	No	61	93.85%
	Yes	0	0
	Lost for follow-up	4	6.15%

SUPPLEMENTARY FIGURE 2. Complications at the donor site



DISCUSSION

In the plethora of various treatment modalities for vitiligo, a significant number of patients fail to respond to conservative treatment or get a satisfactory degree of repigmentation. Autologous NCES is a well-established surgical modality for patients with stable vitiligo. Several techniques to perform NCES have been described. However, the techniques of vitiligo surgery are in a constant state of evolution, with efforts being made to develop simpler methods to obtain long-standing and uniform repigmentation with minimal side effects.

In our study, the graft was obtained from the lateral aspect of the thigh in a ratio of 1:10 (donor to recipient) with the use of silvers knife. In the original procedure described by Gauthier and Surleve, the graft was obtained from the occipital region of the scalp by superficial shaving with the help of a razor blade (9), whereas in 1998 Olsson and Juhlin modified the technique by obtaining the graft from the gluteal region using a Goulian biopsy knife (10). In studies conducted by Mulekar (11), Sobhy *et al* (12) and Zawahry *et al* (13), the graft was obtained from the gluteal region. In a study by Pandya *et al* (14), the graft was obtained by the silvers knife from the thighs, buttocks or waist. Verma *et al* (15) and Singh C *et al* (16) obtained graft from the lateral aspect of the thigh, which was in concordance with our study.

The majority of patients in the current study (50%) were aged between 21-30 years, with female preponderance of 64.10%, which was in accordance with findings reported by Zawahry *et al* (13), Pandya *et al* (14), and Verma *et al* (15), with their study participants being aged between 20-30 years, but in contrast to studies performed by Olsson and Juhlin *et al*, with the majority of their patients belonging to the age group of 10-30 years, with female predominance of 65.15% (10).

Regarding the type of vitiligo, the majority of our study participants [69.23% (27)] had vitiligo vulgaris, followed by focal vitiligo [20.51% (8)]. In Mulekar's study on 184 patients, 67% of subjects had vitiligo vulgaris and 10.3% focal vitiligo (17), while Zawahry *et al* studied 25 patients, of which 21 had observed generalized vitiligo and two, focal vitiligo (13). In Pandya *et al*'s study, 92.6% of all patients had vitiligo vulgaris)14). In Verma *et al*'s study, 11 patients (64.7%) had vitiligo vulgaris, three subjects (15.8%) focal vitiligo and one patient (5.3%) acrofacial vitiligo (15).

In our study, the duration of stability ranged from one year to 10 years (3.03 ± 2.03) as follows: between 1-3 years in 27 (69.33%) patients and between 4-6 years in nine (23.08%) subjects. There are conflicting reports on the relationship between stability and repigmentation. The stability period ranged between 11-92 months in a study conducted by Van Geel *et al* (18) and between 2-4 (2.25±0.55) years in a study performed by Sobhy *et al* (12). Olsson and Juhlin and Van Geel *et al* found a positive correlation between stability and repigmentation, while Sobhy *et al* found that the duration of stability was not statistically significant. Our study was in accordance with Sobhy *et al*, showing no correlation between the duration of stability and repigmentation.

A total of 1302.5 cm^2 of depigmented area was operated by NCES, out of which repigmentation of 874.5 cm^2 (67.14%) was achieved by six months; 33/65 (50.77%) vitiligo patches were on the lower extremity, 11/65 (16.92%) on the abdomen, 6/65 (9.23%) on the upper extremities and back, respectively, 2/65 (3.08%) on the pubis, one each (1.54%) on the head, face and gluteal region in a non-segmental pattern.

In our study, the majority of patients had vitiligo vulgaris (27 subjects with 47 patches). Results were excellent in 32/47 (68.1%) patients, good in 14/47 (29.8%) subjects and fair in 1/47 (2.1%) patients. Out of seven patients with focal vitiligo, four (57.1%) subjects achieved excellent repigmentation and the remaining three (42.9%) good repigmentation. None of the patients with vitiligo vulgaris or focal vitiligo showed poor repigmentation.

In the study performed by Van Geel *et al*, 33 patients had vitiligo vulgaris and five mixed vitiligo. They reported >75% of repigmentation in 18 (55%) subjects and two (40%) patients, respectively (18). In our study, the majority of the patients [32/47 (68.1%)] had vitiligo vulgaris with excellent repigmentation.

Sobhy *et al* (12) concluded that the site of lesion did not have a significant effect on the results, but the mean percentage of repigmentation was greater in patients with lesions over the face (87.75±13.22%), back (86.65±12.53%) and chest (83.50±14.48%), which was in con-

trast to the findings of Pandya *et al* (14), who reported that the most common sites included the feet (45.1%), legs (29.4%), hands (9.8%), knees (3.9%) and face (3.9%). Pandya *et al* (14) also reported favourable results for the legs, feet, face and forearms showed, and unfavourable results for the elbows and the acral areas of the hand; this was in accordance with our study, but it could also be due to the higher number of patientswith depigmented lesions over the lower extremities.

Pandya *et al* (14) found a positive correlation with light exposed areas, whereas Mulekar *et al* (19) found poor response in the sun exposed areas and concluded that sun maybe a traumatic factor. Sobhy *et al* (12) reported better prognosis in exposed areas (12). The differences observed between various studies may be due to the variation in intensity of sun radiation in the study area or to person-to-person variation in melanocytic response to ultraviolet stimulation. In our study, there was no difference in repigmentation in sun exposed or sun protected area.

Various complications of NCES include hyperpigmentation, scaring, infections, milia formation, koebnerization, achromic fissures and new depigmented patches. In our study, the only complication was mild to moderate hyperpigmentation in three (7.69%) of 39 patients over the donor site. In a study by Van Geel *et al*, color mismatch was observed in 64% of patients, with 36% of those having hyperpigmentation and 28% hypopigmentation (18). The degree of colour mismatch was mild (54%) to moderate (34%), 12% of patients had severe colour mismatch. Pandya *et al* (14) found infection at the donor site in two (7.4%) patients, infection at the recipient site in three (11.1%) subjects and koebnerization at the donor site in one patient. In a study by Verma *et al*, new depigmented macules developed in one patient and infection of the donor site in another subject (15). No infection or koebnerization was seen in our study.

The present study had few limitations. The follow-up had a duration of six months, which was insufficient to evaluate delayed complications. Patients with more than 30% of the body surface area could not be operated. Sample size was not calculated using standardized formula. ❑

CONCLUSION

In the current study, we found a high level repigmentation area (67%) after NCES by six months. The majority of patches (64.41%) showed excellent repigmentation and 28.81% good repigmentation. Non-cultured epidermal suspension was associated with a very low rate of complications at the donor site. A randomized multicentric control study comparing different surgical modalities with long term follow-up is required to validate the findings of the present study. ❑

Conflicts of interest: none declared.
Financial support: none declared.

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