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Successful Repigmentation of Recalcitrant Stable Vitiligo with Autologous Non-cultured Epidermal Cell Suspension Technique

Savita Chaudhary, Gopal Prasad Poddar, Moin Ahmad Siddiqui, Ratnika, Ketki, Reyan Abdul Jamil

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ABSTRACT

Introduction: Vitiligo is a chronic condition characterized by loss of skin pigment, often unresponsive to medical treatment when the disease remains inactive. The Autologous non-cultured epidermal cell suspension (NCES) technique has proven to be an effective surgical option for such refractory cases.

Methods: In this study, 10 patient with a single, stable vitiligo patch, showing no response to conventional treatment, underwent NCES. Epidermal cells were isolated from a thin skin graft taken from pigmented skin. After processing the cells with trypsin and centrifugation, the cell suspension was applied to the prepared recipient area. Postoperative care included topical treatments and phototherapy. Clinical assessment and photographic documentation were performed during follow-up visits

Results: The patient presented with gradal repigmentation, with significant improvement by 8 weeks and excellent repigmentation (> 75%) by 12 weeks. The procedure was well tolerated, with only mild, transient adverse effects such as burning sensation and post-inflammatory hyperpigmentation.

Conclusion: NCES is a safe, effective, and cosmetically satisfactory surgical intervention for non-progressive vitiligo resistant to medical treatment.

KEYWORDS: *Vitiligo, Non-cultured epidermal cell suspension, Surgical treatment, Repigmentation*

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INTRODUCTION

Vitiligo is a common skin condition these days that causes the skin to lose its colour. It affects 0.5% to 1% of persons of all races and genders around the world.[1] Currently, to treat vitiligo, some usual methods employed are phototherapy techniques like ultraviolet radiation, narrowband ultraviolet B (NB-UVB), lasers, topical steroids, topical immune-modulators, and topical vitamin D. But these methods are time-consuming and sometimes don't give enough relief, while surgery might work better for stable vitiligo. The main purpose of these operations is to move melanocytes from healthy donor skin to the area that has lost its colour. The doctor said that tissue and cellular grafting were two of the many surgical techniques that could help the patient regain their skin colour. Several kinds of graftings are available such as full complete thickness punch grafts, very soft and thin dermo-epidermal grafts, and suction blister epidermal grafting. Many problems and constraints make tissue and cell grafting tricky to employ. Tissue grafting simply uses the surface area of the local tissue, and the therapy only happens on the top tissue area. For cellular grafting, it is a mix of pure melanocytes and keratinocytes that have been made.

Non-cultured epidermal cell suspension (NCES) is a very common method used for grafting due to the least amount of instruments and space required in the lab. It took less time and covered more ground in one session than cellular therapies. The graft came from a smaller donor site than before (donor:recipient ; 1:10).[2]

Department of Dermatology , Era's Lucknow Medical College and Hospital, Era University, Lucknow, Uttar Pradesh, India-226003

Corresponding Author: Gopal Prasad Poddar

Email: poddargopal19@gmail.com

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MATERIAL AND METHODS

This study was conducted in 10 confirmed patients of stable vitiligo. who attended the outpatient department of Dermatology at Era's Lucknow Medical College and Hospital, Lucknow. Individuals presenting with a solitary, stable vitiligo patch that showed no response to any medical intervention were incorporated into our study. Individuals under 12 years of age and over 50 years, those with unstable vitiligo patches, a history of Koebner's phenomenon, a tendency for keloidal and hypertrophic scars, bleeding disorders, immunocompromised conditions, active infections at the treatment site, those who declined consent, and patients with unrealistic expectations were not included in the study. A comprehensive history was obtained, and a thorough cutaneous and systemic

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examination was conducted prior to the procedure. Both routine and specialized investigations were conducted as necessary. Informed consent concerning the procedure and its outcomes was obtained from each patient. Approval for ethical considerations was secured. A NCES stable patch techniques included.

PROCEDURE

1. The lateral area of thighs, buttocks, and waist was selected as the donor site.
2. Hairs were shaved off from the recipient and donor sites, followed by application of povidone-iodine and infiltration of 2% lidocaine.
3. A very thin split-thickness dermal tissue graft was removed with a razor blade and put into a cell-culture dish with a trypsin-EDTA solution.
4. The cell-culture dish containing skin graft, along with trypsin-EDTA solution, was placed in an incubator at a temperature of 37°C for 45 minutes. Furthermore, anti-trypsinization was done using trypsin-neutralizing solution.
5. The epidermal cells were carefully separated from the dermal parts and were centrifuged for a time period of 6 minutes at 37°C at 3000 rpm.
6. Both motor and manual dermabrasion were done at recipient site until an endpoint with tiny pinpoint bleeding was reached.
7. Dermabrasion was done 2 mm outside of the lesion's edges to stop the halo effect.
8. After centrifugation, the pellets were collected and transferred to recipient site.
9. The suspension was evenly applied over the recipient area and was dressed with bovine collagen.
10. To keep the gauze from sticking to the graft site, antibiotic-soaked gauze was used to make a three-layered dressing (with a collagen sheet) at both the donor and recipient sites.
11. Antibiotics and analgesics were prescribed for a period of 7 days post procedure.
12. The patient was instructed to immobilize the recipient site for at least 7 days at least for 7 days.
13. The bandage and dressing were changed daily.
14. The dressing was removed from the receiver's site after 7 days, and antibiotic ointment was recommended for a minimum of 2 weeks.
15. Systemic and topical PUVA therapy was given.

Patients were followed up at day 7, 4th week, 8th week and 12th week post procedure. Post-operative assessment was done by recording subjective response from the patient, and clinical assessment was done along with the digital photography of area, taken before and after procedure at every follow-up.

To examine subjective repigmentation improvement over patch, patients were asked:

Table 1: To examine subjective repigmentation improvement over patch, patients were asked:

Improvement mode	Recovery %
Mild mode	0–25%
Moderate mode	26–50%
Marked mode	51–75%
Complete mode	76–100%

Table 2: Extent of pigmentation was assessed clinically following post procedure as follows:

Improvement grade	Repigmentation %
Excellent grade	>75%
Very Good grade	51-75%
Good grade	26-50%
Poor	<25%

RESULTS

The total of 10 patients, in which 6 (60%) were females, and 4 (40%) were males, with a mean age of patients was 26.2 ± 4.6 years.

The finding showed that skin repigmentation was excellent in 7 (70.0%) patients with ≥ 75% repigmentation of the treated area and good in 3 (30.0%) of patients with 51-75% of repigmentation. Few complications like pruritus, burning, and post-inflammatory hyperpigmentation were seen after procedure but, all of these complications were mild and self-limiting, itching and redness were not observed in any patient, both sites treatment as well as donor sites.

Table 3: Sociodemographic and clinical diagnostic parameters of patients

Sample size of patients N=10	
Mean ± age	26.2 ± 4.6 years
Sex Males	4 (40%)
Females	6 (60%)
Family history positive	2 (20%)
Negative	8 (80%)
Mean duration of disease	4.05 ± 1.03 years
Mean duration of stability	2.32 ± 0.9 years
Involved sites	
Legs	3 (30%)
Upper back	3 (30%)
Feet	2 (20%)
Lower back	2 (20%)
Subjective assessment	
Complete improvement (76-100%)	8 (80%)
Marked improvement (51-75%)	2 (20%)
Moderate improvement (26-50%)	0
Mild improvement (0-25%)	0
Extent of repigmentation	
Excellent (>75%)	7 (70%)
Very good (51- 75%)	3 (30%)
Good (26- 50%)	0
Poor (0- 25%)	0

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DISCUSSION

Billingham and Medawar performed the first investigation and execution of non-cultured upper epidermal cellular grafting utilizing piebald guinea pig skin.[3] This new procedure has a big effect, and it's easier to observe in Fitzpatrick skin types IV, V, and VI because of the clear difference. Non-cultured epidermal suspension is the predominant and preferred intervention for both segmental and stable non-segmental vitiligo, and the study also shows that they do not respond to pharmacological therapies. To evaluate stability, Prasad and Gupta *et al.*[5] proposed a definition marked by the lack of disease progression in their previous study. This method is superior to the cell culture method because it is simpler, less expensive, and doesn't need high-tech facilities. In this study, we applied the methodology of non-cultured epidermal cell suspension, the sample size of our study was 10 and the patients had a solitary stable resistant patch of vitiligo. In this study, patients range from ages of 21 and 35, with an average mean age of 26.2 ± 4.80 years. Six of the ten patients (60%) were women, and four (40%) were men. Our study also

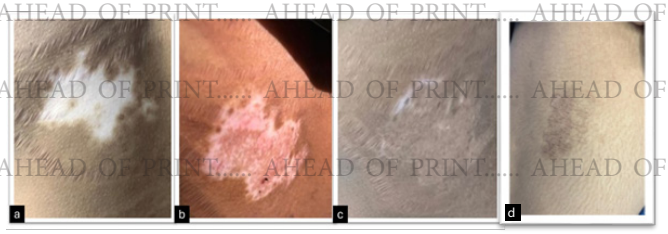

Figure 2: Single stable vitiligo patch over right lower abdomen at baseline (a), repigmentation at 4 weeks (b), repigmentation at 8 weeks (c), post inflammatory hyperpigmentation at donor site (d)

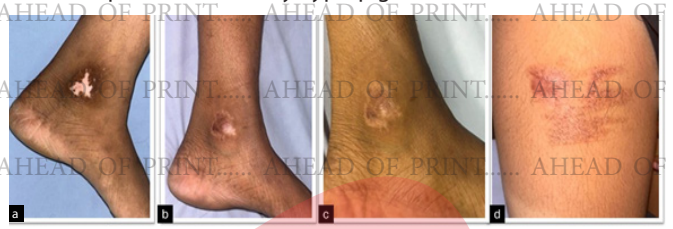

Figure 3: (a) Single stable vitiligo patch over left medial malleolus at baseline, (b) repigmentation at 4 weeks, (c) repigmentation at 8 weeks, (d) post-inflammatory hyperpigmentation at donor site.

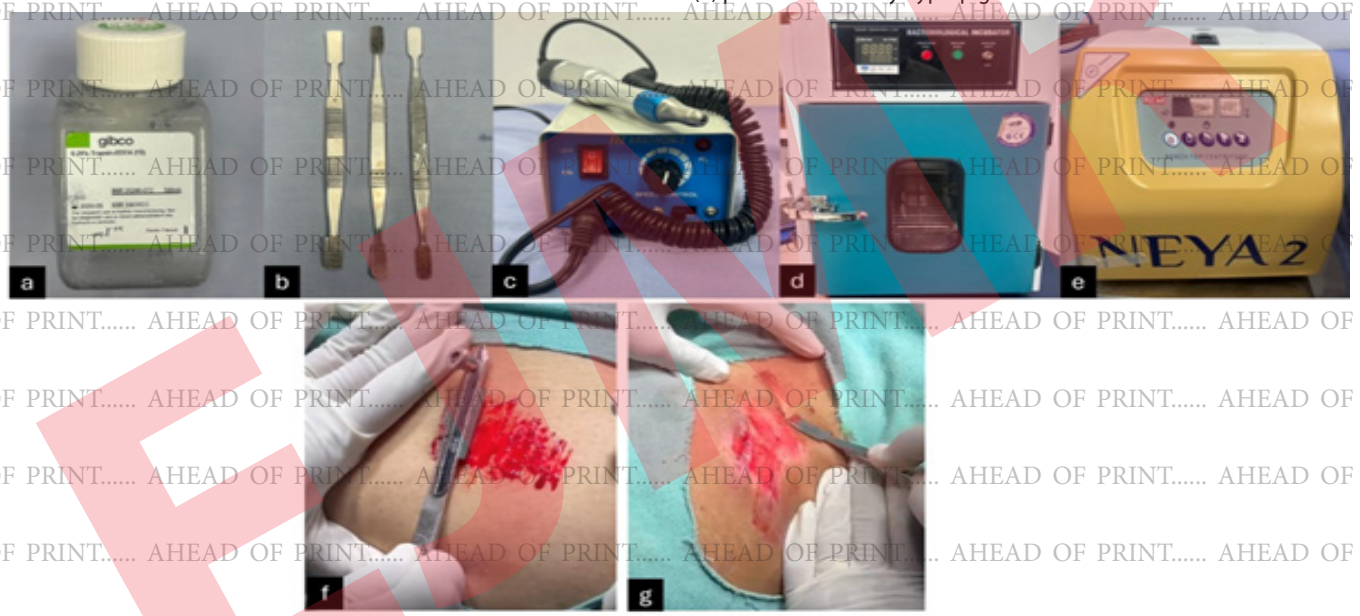

Figure 1: Trypsin EDTA solution (a), manual dermabrader(b), motor dermabrader(c), incubator(d), centrifuge machine(e), taking graft from donor site with help of humby's knife(f), preparing the recipient site using manual dermabrader(g)

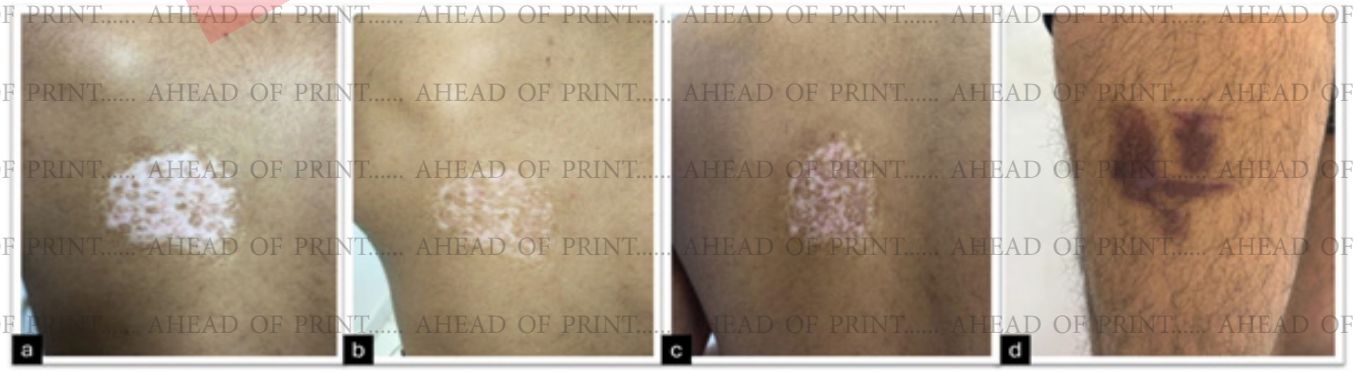

Figure 4: Single stable vitiligo patch over left upper back at baseline (a), repigmentation at 4 weeks (b), repigmentation at 8 weeks (c), (d) post-inflammatory hyperpigmentation at donor site (d)

aligns with previously reported studies done by Zawahry *et al*.,[6] Pandya *et al*.,[7] and Verma *et al*.[8]

After collecting family histories from all patients, only 2 (20%) demonstrated a positive familial history in our study, consistent with the results of Mahajan *et al*.[9] We found that the time duration of problem ranged from 1.5 to 6 years, with an average duration of disease of 4.05 ± 1.03 years. Whereas, the duration of stability varied from approximately 1.2 to 4 years, the average duration of stability was 2.32 ± 0.9 years. The study done by Sobhy *et al*.[10] and our findings match and confirm these conditions.

The findings show that legs and upper back were the most prevalent regions where patients had problems. Three out of ten patients had problems in there leg. Pandya *et al*.[7] also reported that 29.4% of the people in their study had issues with their legs.

After 12 weeks, 7 (70%) of the cases showed excellent repigmentation (>75%), and 3 (30%) of the cases showed good repigmentation (75–90%). Kaur *et al*.[11] and Chandela *et al*.[2] demonstrated that 65% and 64.41% of their patients exhibited excellent repigmentation.

In line with previous studies by Chandela *et al*.[2], Kaur et al.[11], Pandya et al.[7], our patients also had some complaints, such as itching, burning sensation and post-inflammatory hyperpigmentation over the area after the overall surgical procedure, but after some time, all the results gradually improved.

CONCLUSION

Our studies on NCES in the treatment of vitiligo present mixed results ranging from excellent to poor. Expertise in the procedure is critical to getting a favourable therapeutic outcome. The skilled use of equipment, the manner of collecting material from donor sites, recipient site preparation, and proper care following the procedure can impact the treatment result. Although our study has some limitations, study was conducted with small sample size and there was no other comparison group. Hence, more studies with larger sample size are needed.

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Orcid ID:

Savita Chaudhary - https://orcid.org/0009-0006-4692-4315

Moin Ahmad Siddiqui - https://orcid.org/0000-0002-1927-1455

Ratnika - https://orcid.org/0009-0001-4698-4741

Gopal Prasad Poddar - https://orcid.org/0009-0000-6845-3792

Ketki - https://orcid.org/0009-0008-3218-0786

Reyan Abdul Jamil - https://orcid.org/0009-0001-8735-7808