Original Research Article

A study of autologous epidermal non-cultured cell suspension in stable vitiligo patients

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Abstract

Background: To study the extent of repigmentation after autologous epidermal non-cultured cell suspension in stable vitiligo patients. **Patients and Methods:** A prospective, longitudinal study was conducted to study the extent of repigmentation after autologous epidermal non-cultured cell suspension in 30 stable vitiligo patients. **Results**: By subjective assessment, 10% (3) patients showed poor response, 30% (9) showed good response and 60% (18) patients showed excellent response after follow-up at 6 months with good patient satisfaction. By objective assessment 10% (3) patients showed poor response, 13.3% (4) showed fair response and 23.3% (7) patients showed good response, 53.3% (16) patients showed excellent response after follow-up at 6 months. Extent of repigmentation was evaluated as - 10% (3) showed poor response, 10% (3) showed fair response and 20% (6) patients showed good response, 60% (18) patients showed excellent response after follow-up at 6 months. **Conclusion:** Autologous non-cultured epidermal cell suspension in stable vitiligo is a safe, effective, simple method, with superior quality of repigmentation.
Keywords: Vitiligo, vitiligo surgery, grafting, dermabrasion, repigmentation, non-cultured melanocytes.

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INTRODUCTION

Vitiligo is an autoimmune disorder characterized by asymptomatic, localized and/or generalized depigmentation of the skin and/ or mucous membranes in the form of typical chalky-white or milky macule(s)1 with incidence ranges from 0.1 to > 8.8% across India,[1,2] most of the studies showing female preponderance.1 Many treatment modalities are currently used for vitiligo like topical, systemic, phototherapy, lasers and surgical methods. Recent surgical advances include autologous non-cultured epidermal cell suspensions and cultured melanocyte suspensions.[3] A study was undertaken to evaluate the extent of repigmentation after autologous epidermal non-cultured cell suspension in stable vitiligo patients. A complete history with precipitating factors, previous medical treatment, any associated diseases, was obtained followed by a complete physical examination. Routine blood investigations were done and tests for thyroid function done where required. Preoperative photographs of donor and recipient area were taken. Postoperative pigmentation was evaluated using scoring system and digital photography.

PATIENTS AND METHODS

Patients

Study population:

The study is conducted on a study population of 30 patients presenting with stable vitiligo attending OPD in Department of DVL, Santhiram Medical College and General Hospital, Nandyal.

Inclusion criteria:

1) Stable vitiligo not responding to medical treatment, (stability as defined by IADVL taskforce)

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Exclusion criteria:

1. Age < 12 years, > 50 years
2. Unstable vitiligo
3. Koebner's phenomenon
4. Keloidal and hypertrophic scar tendency
5. Patients with platelet disorders, anemia or bleeding disorders.
6. Immunocompromised patients like HIV, Hepatitis B or C positive
7. Patients who have active skin disease/infection at treatment area.
8. Un-cooperative patients or patients unable to understand the protocol or give informed consent

Methods

1. Ethical clearance from the instituitional ethics committee
2. Informed written consent
3. Pre-structured proforma
4. Preoperatively, patients will be assessed and,
5. Investigated with routine investigations and special investigations when required.

The data has been collected and analysed in an excel sheet. Proportions are calculated for the data.

RESULTS

Criteria for evolution	Score				Factor	Total
	0	1	2	3		
A.Extent of pigmentation	≤50%	51%-70%	71%-90	>90	X5	
B. Color match	Poor	Good	Excellent	-	X3	
C. Complications (Recipient area)**	Minimal cobblestone/variegated appearance	Moderate to marked cobblestone/variegated appearance milia	Thick graft margins, wrinkles over graft ,type patch appearance	Keloid hypertrophic scar	X-3	
D. Complications (Donor area)**	Minimal hypo/hyperpigmentation, minimal scarring	Moderate hype/hyper pigmentation, moderate scarring	Gross hype/hyperpigmentation, unsightly scarring	Keloid depigmentation	x-2	
Result: 17-21-Excellent; 12-16-Good;7-11-Fair; 6 or less-Poor					**(A+B+C+D)**	

*Excellent color match-Minimal hypo or hyperpigmentation of grafted area not requiring camouflage.
*Good color match-Moderate hypo or hyperpigmentation amenable to light camouflage.
*Poor color match-Gross hypo or hyperpigmentation difficult to cover up with ordinary make up.
**In presence of complications belonging to more than one score, only the highest score should be multiplied the factor

On removal of dressing on the recipient area, crusted scabs were seen partially attached to dermabraded area, along with erythematous achromic areas. Repigmentation usually started by 2- 3 weeks and was complete within 6 months in most of the patients. Earliest onset of pigmentation was observed within 10 days with pin-point pigmentation. In some patients with perifollicular type of repigmentation, initially islands of pigmentation was seen which later coalesced to form uniform repigmentation. Whereas in other patients, diffuse pigmentation was seen in central part of vitiliginous area, then gradually spread to pigment surrounding margins. Repigmentation was rapid and maximum in initial 3-4 months, then slowed in the following months. Repigmentation in donor area was of perifollicular type in all patients and was completed within 1- 6 months.

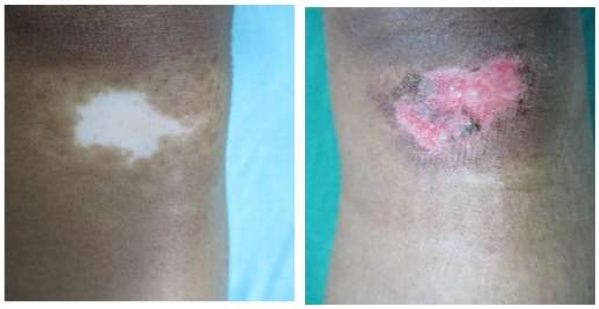

Photo 1: Before procedure **Photo1b:** After dressing removal

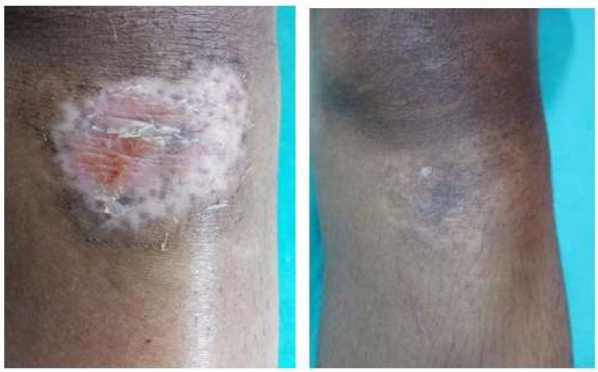

Photo 1c: After 10 days of procedur **Photo 1d:** After 6 months

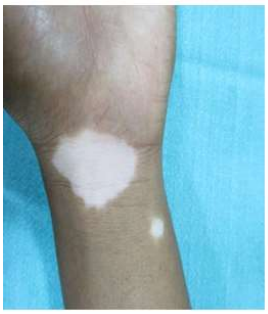
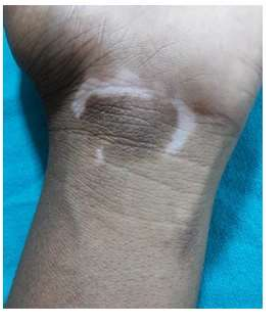
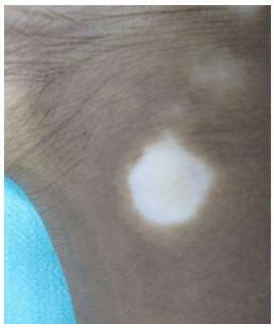
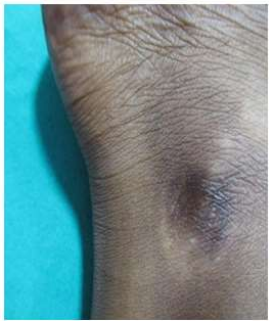

Photo 2a: Before procedure **Photo 2b:** After 6 months **Photo 3a:** Before procedure **Photo 3b**: After 6 months


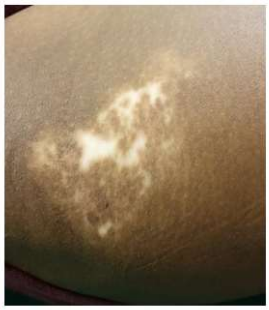
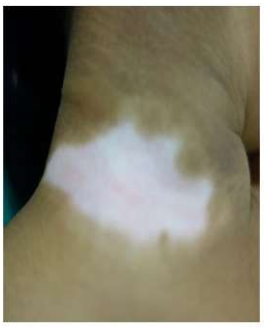
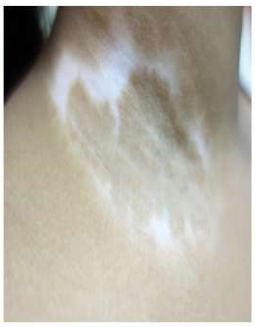

Photo 4a: Before procedure **Photo 4b:** After 6 months **Photo 5a :** Before procedure **Photo 5b:** After 6 months

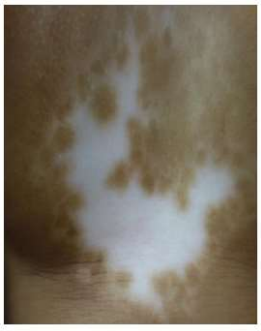


Photo 6a: Before procedure **Photo 6b:** After 6 months

DISCUSSION

This procedure is also known by other names like non-culture melanocyte transfer, basal cell suspension technique, non-culture cellular transplant.7 The principle is seeding of melanocytes i.e. introduction of melanocytes from normal skin into a region of depigmented skin 8. The basic principle is to separate the basal cells and the melanocytes by trypsinization, and prepare a suspension which is applied on the dermabraded lesional skin 9. During the initial 7 days of healing, melanocytes and keratinocytes present in the grafted material, multiply and repigment the depigmented area. Further pigmentation can be accelerated by topical psoralen therapy 9, NBUVB and PUVA. However, some authorities use phototherapy only if there is delay in onset of repigmentation 6,10. Pigmentation in the treated areas gradually increases in size due to melanocyte proliferation and migration under the influence of cytokines secreted by surrounding keratinocytes. Hence, melanocytes taken from a small donor can pigment a much larger recipient area 9.A total of 30 patients were included in this prospective longitudinal study and followed up at 1 month, 3 months and 6 months. All patients completed the study.

Table 1: Age

STUDY	AGE GROUP between	PROPORTION
PRESENT STUDY 20 patients	12 – 50 years Youngest -12 years Oldest - 50 years.	Highest proportion - 40 % (8) patients in group 21-30 years
Sobhy [11] *et al*	13 – 33 years	Highest proportion - 60% in age group <15 years

Table 2: Sex

STUDY	MALES	FEMALES
Present study	33.3% (10) patients	66.7% (20) patients
Sobhy [11] *et al*	7 patients	13 patients
Rajesh Verma [12] *et al*	52.6% (10)	47.4% (9)

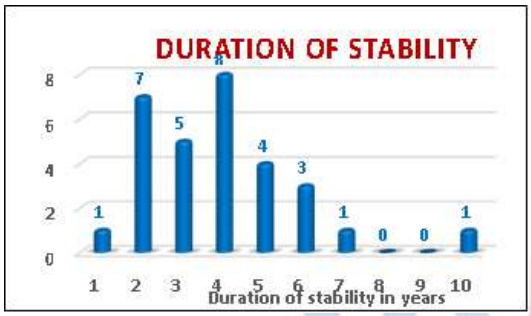

Figure 1: Duration of stability

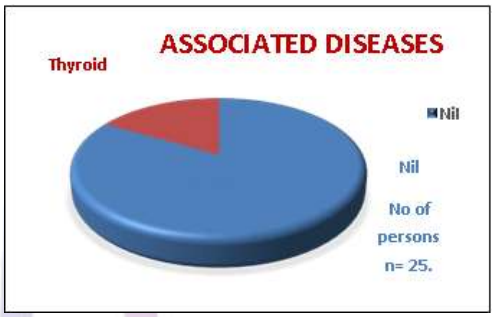

Figure 2: Associated Diseases

Sobhy13 *et al* found that the most important variable is the stability of the disease (2 years or more) rather than the duration of the disease. But, in the present study, patients with stability less than 2 years were also included according to IADVL task force guidelines regarding the stability.

In this study, association with only thyroid disease has been noted, 83.3% (25) patients had normal thyroid function tests, whereas 16.7% (5) patients had abnormal thyroid function tests. All 5 patients with abnormal thyroid function tests were females.

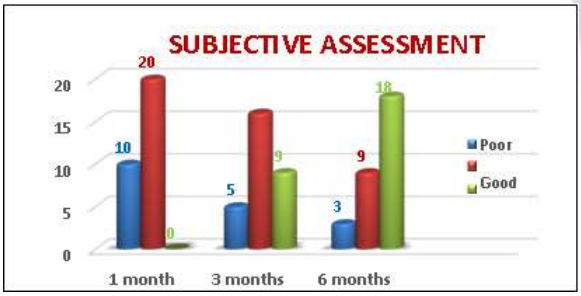

Figure 3: Subjective Assessment **Figure 4:** Objective assessment

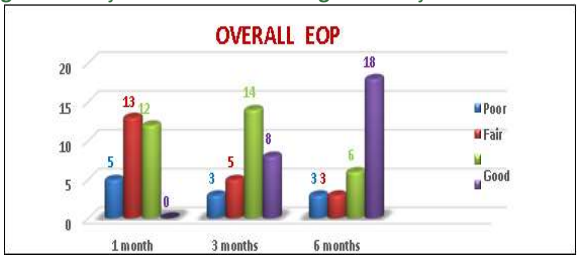

Figure 5: Overall EOP

After follow-up at 6 months, 10% (3) showed poor response, 30% (9) showed good response and60% (18) patients showed excellent response.

After follow-up at 6 months, 10% (3) showed poor response, 13.3% (4) showed fair response and% (7) patients showed good response, 53.3% (16) patients showed excellent response.Gauthier14 in study has reported incomplete repigmentation in 20% of patients, probably due to type of vitiligo.
After follow-up at 6 months 10% (3) showed poor response, 10% (3) showed fair response and20% (6) patients showed good response, 60% (18) patients showed excellent response.

Table 4:

	COMPARATIVE REPIGMENTATION IN OTHER STUDIES (IN %)			
STUDY	EXCELLENT	GOOD	FAIR	POOR
PRESENT STUDY	60	20	10	10
Munish paul[6]	65	18	8	8
Sobhy[11] *et al*	35	55	10	NIL
RajeshVerma[12] *et al*	NIL	62	20	18
Pandya[15] *et al*	52.2	–	–	–

AGE AT PRESENTATION VERSUS EXTENT OF PIGMENTATION AT 6 MONTHS

At the end of 6 months, excellent repigmentation was seen in 100% (5) patients in age group 11- 20 years and repigmentation observed was better and occurred earlier in younger patients than in older patients.Sobhy11 *et al*, also reported earlier repigmentation and better prognosis in age group <15 years than in older patients, probably due to younger cell that grows and multiplies rapidly.Czajkowski R9, also has found that the time of proliferation of melanocytes in invitro culture conditions depends on the age of the patient and, younger the patient, faster is the melanocytes proliferation.

ADVERSE EFFECTS IN DONOR AND RECIPIENT AREA

Though initially some patients developed hyperpigmentation and mild textural abnormality at donor site, after few months, they gradually decreased to match with surrounding area. Similarly, hyperpigmentation at recipient area too gradually decreased and they were not a significant causeof distress to the patients. Further, in this study, none of the patients had developed donor site infection, hypopigmentation and scarring.

STUDY	DONOR AREA	RECIPIENT AREA
PRESENT STUDY	Hyperpigmentation – 20% (6) patients Infection/hypopigmentation scarring - NONE	Hyperpigmentation – 33.3% (10) patients
Van geel *et al*	Minor textural changes - 65%	Color mismatch - 80.4% (54)
Gauthier[14]	No hypopigmentation, scars or keloid	Imperfect color matching - 20%
Munish paul[6]	Hyperpigmentation - 5	Hyperpigmentation - 12% (6) patients Hypopigmentation - 8% (4) patients Hypopigmented border -12 patients
Pandya[15] *et al*,	Infection - 7.4% Koebner's response - 1 patient	–
Sobhy[11] *et al*	–	Hyperpigmentation - 1 patient Hypopigmentation - 1 patient

CONCLUSION

In the present study, by subjective assessment, 10% (3) patients showed poor response, 30% (9) showed good response and 60% (18) patients showed excellent response after follow-up at 6 months with good patient satisfaction. By objective assessment 10% (3) patients showed poor response, 13.3% (4) showed fair response and 23.3% (7) patients showed good response, 53.3% (16) patients showed excellent response after follow-up at 6 months. Extent of repigmentation was evaluated as - 10% (3) showed poor response, 10% (3) showed fair response and 20% (6) patients showed good response, 60% (18) patients showed excellent response after follow-up at 6 months. Repigmentation was uniform, matched

well with surrounding skin and usually completed within 6 months. Cosmetic acceptability is also superior as there is no cobble-stoning or scarring seen in other methods, giving good patient satisfaction. The study confirms that repigmentation by autologous non-cultured epidermal cell suspension in stable vitiligo is a safe, effective, and simple method, with superior quality of repigmentation, very few adverse effects and high level of patient satisfaction with possibility to repigment vitiliginous skin manifold larger than the donor skin.

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