Original Research Paper

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ASSESSING THE EFFICACY AND STABILITY OF FOLLICULAR CELL SUSPENSION IN THE REPIGMENTATION OF STABLE VITILIGO

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ABSTRACT

INTRODUCTION: Vitiligo is a common depigmenting skin disease with significant psychological & social stigma, profoundly affecting the quality of life especially when located in exposed areas of the body. Although various treatment modalities are there for vitiligo, none of them are curative. Hair follicle melanocytes are an attractive source of donor cells over epidermal melanocytes with features like remarkable capacity to synthesize melanin and are a rich source of 3 different stem cells–. Surgical modalities are, thus, an excellent treatment option for patients with focal stable vitiligo. Among which follicular cell suspension is a novel, minimally invasive, safe and effective surgical modality in focal stable vitiligo. **AIM:** To determine the efficacy and stability of follicular cell suspension in the repigmentation of stable vitiligo **METHODOLOGY:** After written consent and routine investigations, 43 patients (29 females, 12 males, 2 were lost to follow-up) with focal stable vitiligo attending the Dermatology OPD, were included in the study. Anagen hairs from the occipital area were selected. Under field block anesthesia, 1mm manual punch was done in the scalp, the extracted hair follicles washed, incubated for 3 cycles. The cell suspensions were then filtered to form a final cell pellet. The recipient area was cleaned, dermabraded, and drops of cell suspension was spread over the surface and collagen dressing was done7. Postoperative care given and dressing was removed after 7 days. Monthly visits with photographs were done for 1 year. **RESULTS:** Response was graded as Grade 4- Excellent (90-100% repigmentation), Grade 3- Good (75-90% repigmentation), Grade 2- Fair (50-74%) and Grade 1- Poor (<50%). This study showed excellent repigmentation in 63.4% of patients followed by good repigmentation in 26.8% of patients. Types of repigmentation seen in our study were as follows diffuse (73.2%), dotted (4.8%), marginal (17.1%), perifollicular (4.9%). The repigmentation was almost similar to skin colour in 73.1% of patients. Erythema (7.3%) and hyperpigmentation (4.9%) were the adverse effects seen in recipient site. **CONCLUSION:** Our study has demonstrated that non-cultured follicular cell suspension, a novel, minimally invasive technique with minimal side-effects is successful in treating stable vitiligo patients.

KEYWORDS

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INTRODUCTION

Vitiligo is caused by the autoimmune destruction of melanocytes that presents with depigmentation of skin and mucous membrane characterized by milky white-coloured macules and patches with various patterns of distribution . It runs an unpredictable course and duration with asymptomatic chronic cosmetic disfigurement causing significant social stigma, with serious implications for mental health . The pathogenesis is multifactorial, including genetic predisposition, autoimmunity, and environmental factors .

Amongst the various surgical treatment modalities, replenishment of melanocytes selectively within the vitiliginous macules by autologous melanocyte transplantation, by either tissue graft or cellular graft is promising . Transplantation of autologous non-cultured extracted hair follicle outer root sheath cell is a novel surgical method for the treatment of vitiligo Surgical modalities work best in stable and segmental vitiligo. In segmental vitiligo, the causative factor usually disappears, thus leaving well-defined depigmented lesions. There's no consensus regarding the minimum period of stability. It is usually considered as stable if there is no progression of old lesions and/or development of new lesions during the past one year.

AIM:

The aim of the study is to determine the efficacy of follicular cell suspension in the repigmentation of stable vitiligo.

OBJECTIVES:

To assess the efficacy and safety of follicular cell suspension in the repigmentation of stable vitiligo by performing objective evaluation based on clinical improvement & photographic evidence.

To observe any untoward events or side effects.

METHODS AND MATERIALS:

PLACE OF STUDY:
Department of Dermatology, Venereology and Leprosy Government Stanley Medical College &Hospital, Chennai.

TYPE OF STUDY: PRE & POST STUDY DESIGN.
TARGET POPULATION: Patients attending vitiligo clinic in dermatology outpatient department.

STUDY POPULATION:
Patients with focal stable vitiligo.

Duration : 1 year
SAMPLE SIZE:
Sample size calculated based on the formula:

n= 4pq/d2
Sample size= 43 patients
Calculation was done at 95% confidence interval at power 80%.

SAMPLING METHOD:
Convenient sampling

PRE PROCEDURE WORK UP:
Complete blood count, Renal function test, Liver functions test, Bleeding time, clotting time, blood glucose, Thyroid function tests - TSH, T3,T4 levels, Screeing for HIV, RPR, Hepatitis-B and C, latent tuberculosis.

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Table 1: Inclusion & Exclusion Criteria

PROCEDURE :

43 patients clinically diagnosed as focal stable vitiligo who fulfilled the inclusion criteria were included in the study. Patients were explained thoroughly about procedure, treatment response, post-operative follow up, adverse events in regional language and got informed written consent from patients.

PROCEDURE PROPER (FOLLICULAR CELL SUSPENSION):

INCLUSION CRITERIA:	EXCLUSION CRITERIA:
Age - 18 years and above	Patients with a history of bleeding disorders, keloidal tendency, patients with unrealistic expectations
Patient willing for procedure	Patients with a history of recurrent herpes labialis, HIV and HBsAg, hepatic or renal disease, epilepsy, or any major medical illness, pregnancy
Patients clinically diagnosed as stable vitiligo	Patients with active infection at the local site
Patient willing for regular follow up	Patients with a history of psoriasis or lichen planus because of risk of Koebner phenomenon(10)
Patients who did not take treatment for the past 1 month	Patients on anticoagulant medications (aspirin, warfarin, and heparin)

- After trimming to 2mm, actively growing anagen hairs, which are usually more pigmented due to increased melanin content, were selected from the occipital area of the scalp(11)
- Field block anesthesia with 2% lignocaine was given, after which approximately 20 hair follicles were obtained with the help of 1 mm manual punch with care to avoid transection of hair-follicle. Then, the hair-follicular unit was gently pulled out using a hair-follicle holding forceps(12)
- The donor area was dressed using 2% mupirocin ointment and sterile cotton pads.
- In the laboratory under all sterile conditions, the extracted hair-follicles were washed with phosphate buffered saline (PBS), containing suitable antibiotics and antimycotics such as penicillin, streptomycin, and amphotericin B, for about 3 times(13)
- The hair-follicles were then incubated with 0.25% trypsin – 0.05% ethylene diamine tetra acetic acid (EDTA) at 37°C for 90 min to prepare the single cell suspension.
- Within 15-20 min of incubation, the cells start loosening from each other. The hair-follicles were subsequently placed in another tube of trypsin and EDTA. Trypsin inhibitor was added in the former tube to cease the reaction and prevent the digestion of separated cells by trypsin(14)
- At the end of three such cycles, a thin keratinous shaft will be left behind
- The cell suspensions of all the tubes were added in a single tube and then filtered through a 70 m cell strainer to prepare a single cell suspension
- The final cell pellet was obtained by centrifuging the combined cell suspension at 1000 rpm for 5 min, which was again suspended in the PBS
- The total amount of time for the laboratory procedure takes about 2-3 hours. (15)

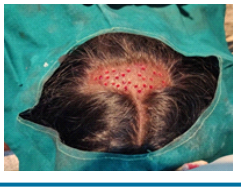

Fig1: Donor site

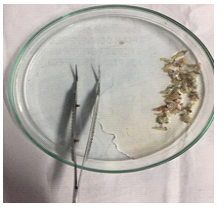

Fig 2: Hair follicles being washed with PBS (phosphate buffered saline) with antibiotics

Fig 3: Bacteriological incubator - hair follicles were incubated in 0.25% trypsin -0.05% EDTA

Transplantation:

- The recipient site was cleaned with betadine-surgical spirit and draped. Under local anaesthesia, dermabrasion was done until tiny bleeding points appear beyond which dermabrasion should not be carried, else scaring will ensue.
- Dermabrasion was done about 5 mm beyond the margin of vitiligo patch to avoid the halo phenomena
- Using an 18 G needle attached to a tuberculin syringe or a pipette, few small drops of suspension are placed over the denuded surface. This was then spread evenly to cover all the dermabraded area. A meshed collagen sheet was placed over the suspension and was covered with sterile Vaseline /chlorhexidine gauze, over which surgical pad was put, and the dressing stabilized by a transparent dressing film and an elastic bandage. The patients were observed for 1 hour after procedure.(16)

POSTOPERATIVE CARE:

- The patients were advised to keep the dressing dry and minimize local manipulation
- Systemic antibiotics were prescribed for a minimum period of 7 days
- The dressing was removed at 7 days of follow-up
- Patient were followed up monthly during the 1-year study period and at each follow up visit repigmentation was assessed and serial photographs were taken.

FOLLOW UP AND EVALUATION OF TREATMENT RESPONSE:

Table 2: Grading of treatment response

GRADING OF RESPONSE	% OF REPIGMENTATION
Grade 1 – POOR	< 50 %
Grade 2 – FAIR	50 – 74%
Grade 3 – GOOD	75 – 89%
Grade 4 – EXCELLENT	– 90 100%

- The repigmentation pattern was noted as “diffuse,” “marginal”, “perifollicular,” or “dotted”. A note was also made on the color matching of the repigmented skin as “lighter than,” “similar,” or “darker than” normal skin or mixed variant.
- The clinical outcome was documented every month by standardized photographs for up to 6 months. The repigmentation was assessed subjectively by comparing pretreatment and post treatment photographs.
- The standard Dermatological Quality of Life Index questionnaire was used to assess improvement in quality of life.

STATISTICAL ANALYSIS:

- The collected data were analyzed with IBM.SPSS statistics software 23.0 Version.
- To describe about the data descriptive statistics frequency analysis, percentage analysis was used for categorical variables and the mean & S.D were used for continuous variables.
- The paired sample t-test was used to find the significant difference between the bivariate samples in paired groups. For the multivariate analysis for repeated measures the Friedman test was used followed by Wilcoxon signed rank test. Chi-Square test was used to find the significance in categorical data
- In all the above statistical tools the probability value 0.05 is considered as significant level.

VOLUME - 7 | ISSUE - 3 | July - 2023 | ISSN (O) : 2663-2888 | ISSN (P) : 2663-287X

OBSERVATION AND RESULTS

TABLE 3: DEMOGRAPHIC PROFILE

PATIENT DETAILS	VARIABLES	Frequency	Percent
AGE	18 - 20 yrs	10	24.4
	21 - 25 yrs	15	36.6
	26 - 30 yrs	9	22.0
	31 - 35 yrs	4	9.8
	>35 yrs	3	7.3
	Total	41	100.0
SEX	Female	29	70.7
	Male	12	29.3
TYPE OF REPIGMENTATION	Diffuse	30	73.2
	Dotted	2	4.8
	Marginal	7	17.1
	Perifollicular	2	4.9
COLOUR MATCHING OF REPIGMENTED SKIN	Darker	2	4.9
	Lighter	1	2.4
	Mixed	8	19.5
	Similar	30	73.1
SIDE EFFECTS- DONOR SITE	Nil	41	100.0
SIDE EFFECTS- RECIPIENT SITE	Erythema	3	7.3
	Hyperpigmentation	2	4.9
	Nil	36	87.8
TOTAL	41	100.0	

TABLE 4: RESULTS – GRADE OF REPIGMENTATION - FOLLOW-UP OF STUDY POPULATION (% of patients)

	PERCENTAGE OF PATIENTS AT EVERY MONTH ON FOLLOW UP					
GRADE OF REPIGMENTATION	1ST MONTH	2ND MONTH	3RD MONTH	4TH MONTH	5TH MONTH	6TH MONTH
Grade 1 – Poor (<50%)	100	85.4	41.5	4.9	4.9	4.9
Grade 2 – Fair (50-74%)		14.6	51.2	56.1	24.4	4.9
Grade 3- Good (75-89%)			7.3	31.7	48.8	26.8
Grade 4 – Excellent (90-100%)				7.3	22.0	63.4

TABLE 5: STATISTICAL ANALYSIS - GRADE OF REPIGMENTATION (FOLLOW UP – 6 MONTHS)

Followup	Mean	S.D	N	2 – value	P-value
1st month	1.00	0.00	41	187.741	0.0005 **
2nd month	1.15	0.36	41		
3rd month	1.66	0.62	41		
4th month	2.41	0.71	41		
5th month	2.88	0.81	41		
6th month	3.49	0.81	41		

** Highly Significant at P < 0.01 level- Follow up comparison by FRIEDMAN TEST

TABLE 6: STATISTICAL ANALYSIS – DERMATOLOGICAL LIFE QUALITY INDEX (DLQI) – BEFORE AND AFTER TREATMENT

DLQI COMPARISON BEFORE & AFTER TREATMENT – PAIRED t TEST						
DLQI	VARIABLE	Mean	S. D.	N	t – value	p-value
	BEFORE TREATMENT	14.07	4.42	41	14.948	0.0005 **
	AFTER TREATMENT	4.00	4.63	41		

**Highly significant at p<0.01 level

CLINICAL PHOTOGRAPHS - PRE & POST PROCEDURE PHOTOGRAPHS

Figure 4: Serial photographs of patient pre procedure, post procedure and at every 2nd month – a) Excellent repigmentation seen in Patient 1, b) excellent repigmentation seen in Patient 2, c) good repigmentation seen in Patient 3

DISCUSSION:

In our study, there is a preponderance of female patients, young adults (18- 25 years) and college students, which signifies their cosmetic concern and that vitiligo still remains a social stigma.

Most the patients had diffuse + marginal (90.4%) type of repigmentation followed by dotted and peri follicular type of repigmentation. The repigmentation was almost similar to skin color in most of the patients (73.1%), and few had mixed variety and rarely patients had repigmentation with darker and lighter to the skin color.

This study showed excellent repigmentation in 63.4% of patients followed by good repigmentation in 26.8% of patients with no visible residual scarring of the donor area and good color match of the recipient. Only few patients had minimal side-effects even in the recipient area. If the skin texture was not altered the efficacy was found to be superior to the lesion with the altered skin.

The mean quality of life of the patients improved from 14.07 to 4.00. i.e., the disease had a very large effect on patient's life before treatment which has improved to having a small effect on patient's life after treatment at 6 months of follow-up.

CONCLUSION

- Follicular cell suspension was found to be more effective, safe, with fewer side effects in the stable vitiligo if the lesion has not been treated with topical treatment chronically prior to the procedure.
- This novel, minimally invasive, scarless technique helpful in improving the Quality of Life (QoL) in patients with stable vitiligo

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REFERENCES:

1. Sawatkar. Follicular cell suspension: A new surgical modality for the treatment of vitiligo [Internet]. [cited 2021 Oct 30]. Available from: https://www.pigmentinternational.com/article.asp?issn=2349-5847;year=2015;volume=2;issue=1;spage=4;epage=8;aulast=Sawatkar
2. Takeo M, Lee W, Ito M. Wound Healing and Skin Regeneration. Cold Spring Harb Perspect Med. 2015 Jan;5(1):a023267.
3. Legué E, Sequeira I, Nicolas J. Hair follicle renewal: authentic morphogenesis that depends on a complex progression of stem cell lineages. Development. 2010;
4. Vitiligo and Other Disorders of Hypopigmentation | Plastic Surgery Key [Internet]. [cited 2021 Oct 30]. Available from: https://plasticsurgerykey.com/vitiligo-and-other-disorders-of-hypopigmentation-2/
5. Sangma LN, Nath J, Bhagabati D. Quality of life and psychological morbidity in vitiligo patients: a study in a teaching hospital from north-East India. Indian J Dermatol. 2015 Apr;60(2):142–6.
6. The pathogenesis of vitiligo - PubMed [Internet]. [cited 2021 Oct 30]. Available from: https://pubmed.ncbi.nlm.nih.gov/23673299/
7. Falabella R. Surgical treatment of vitiligo: why, when and how. J Eur Acad Dermatol Venereol. 2003;17(5):518–20.
8. Savant SS. Surgical therapy of vitiligo: current status. Indian J Dermatol Venereol Leprol.

2005 Oct;71(5):307–10.
9. Shah AN, Marfatia RK, Saikia SS. A Study of Noncultured Extracted Hair Follicle Outer Root Sheath Cell Suspension for Transplantation in Vitiligo. Int J Trichology. 2016 Apr 1;8(2):67–72.
10. Njoo MD, Das PK, Bos JD, Westerhof W. Association of the Köbner phenomenon with disease activity and therapeutic responsiveness in vitiligo vulgaris. Arch Dermatol. 1999 Apr 1;135(4):407–13.
11. The hair follicle melanocytes in vitiligo in relation to disease duration - Anbar - 2009 - Journal of the European Academy of Dermatology and Venereology - Wiley Online Library [Internet]. [cited 2021 Oct 30]. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/j.1468-3083.2009.03233.x
12. Gho C g., Braun JE f., Tilli C m. l. j., Neumann H a. m., Ramaekers F c. s. Human follicular stem cells: their presence in plucked hair and follicular cell culture. Br J Dermatol. 2004;150(5):860–8.
13. Clinical and treatment characteristics determining therapeutic outcome in patients undergoing autologous non-cultured outer root sheath hair follicle cell suspension for treatment of stable vitiligo - Vinay - 2015 - Journal of the European Academy of Dermatology and Venereology - Wiley Online Library [Internet]. [cited 2021 Oct 30]. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/jdv.12426
14. Shah AN, Marfatia RK, Saikia SS. A Study of Noncultured Extracted Hair Follicle Outer Root Sheath Cell Suspension for Transplantation in Vitiligo. Int J Trichology. 2016 Apr 1;8(2):67–72.
15. Noncultured extracted hair follicle outer root sheath cell suspension for transplantation in vitiligo - PubMed [Internet]. [cited 2021 Oct 30]. Available from: https://pubmed.ncbi.nlm.nih.gov/21275943/
16. Experimental study and clinical observations of autologous hair follicle cell transplants to treat stable vitiligo - Indian Journal of Dermatology, Venereology and Leprology [Internet]. [cited 2021 Oct 30]. Available from: https://ijdvl.com/experimental-study-and-clinical-observations-of-autologous-hair-follicle-cell-transplants-to-treat-stable-vitiligo/
17. Silpa-Archa N, Griffith JL, Huggins RH, Henderson MD, Kerr HA, Jacobsen G, et al. Long-term follow-up of patients undergoing autologous noncultured melanocyte-keratinocyte transplantation for vitiligo and other leukodermas. J Am Acad Dermatol. 2017 Aug 1;77(2):318–27.