VOLUME - 10, ISSUE - 05, MAY- 2021 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Dermatology

STUDY OF THE EFFICACY OF NON CULTURED MELANOCYTE TRANSFER IN PATIENTS WITH STABLE VITILIGO

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ABSTRACT

BACKGROUND: Vitiligo is an acquired, hypomelanotic autoimmune inflammatory skin disorder which poses great therapeutic challenges. Treatment with transfer of non-cultured melanocytes has been found to be useful, thus proving the efficacy of the technique as a treatment option in patients with stable vitiligo who have been resistant to other treatment options.

METHODS: This prospective study was done to find out the efficicacy of transfer of non cultured melanocytes in vitiligo patients with stability of 6 months or more after obtaining a written informed consent over a period of 23 months. This method involves harvesting of epidermal grafts from which melanocytes (along with keratinocytes) are separated and transplanted over dermabraded area. The Results of transplantation were analyzed by three observers on the basis of visual analogue scale (VAS) at 1, 2, 4, and 6 months. These three observations were compared and analysed statistically. The re-pigmentation was graded as excellent with 90% to 100% pigmentation, good with 60% to 89%, fair with 25% to 59%, and poor with 0% to 24% of the treated area. Independent sample t-test was used to The mean differences are highly significant at the 0.001 level of significance ($p<0.001$) The measurement of agreement using Cohen's Kappa was found significant ($p<0.001$).

RESULTS: Three different observers (patient him/her self and two clinicians) reported an average excellent result in 55.57% of the patients and an average good result in 27.8% patients at the end of 6 months according to VAS System.

CONCLUSION: This study revealed >90% re-pigmentation at the end of 6 months after the transfer of non-cultured melanocyte transfer, thus proving the efficacy of the technique as a treatment option in patients with stable vitiligo who have been resistant to other treatment options.

KEYWORDS : Vitiligo, Non cultured melanocyte transfer

INTRODUCTION

Vitiligo is an acquired, hypomelanotic autoimmune inflammatory skin disease where there is an absence, deficiency or dysfunction of melanocytes which is characterized by circumscribed depigmented macules on the skin due to loss of functional melanocytes from the epidermis.[1]The key principle of vitiligo therapy is, facilitating repopulation of depigmented patches of the interfollicular epidermis with active melanocytes that are able to migrate, survive to repopulate the depigmented skin and carry out melanin biosynthesis.[2]

Vitiligo is resistant to treatment and has a high rate of recurrence. Some of the most promising available treatments for the vitiligo are; topical corticosteroids and calcineurin inhibitors, systemic corticosteroids in pulse therapy, combination with UV radiation,Vitamin D analogs, micropigmentation.[3,4,5,6] grafting techniques: noncultured epidermal suspensions [7,8,9] mini punch grafts [10] in-epidermal grafting,[11,12] in-vitro cultured epidermis with melanocytes[13] in-vitro cultured melanocyte suspensions.[14]

METHODS:

This was a prospective study to find out the efficacy of transfer of non cultured melanocytes in vitiligo patients with stability of 6 months or more as a means to improve cosmetic results over a period of 23 months (September 2015 to August 2017) conducted in Central Research Laboratory and department of Dermatology in a tertiary care centre. This was a self-financed project and institutional ethical clearance was taken.

After obtaining written informed consent, 30 known patients, between the age group of 18 to 35 years of age, of stable vitiligo were recruited for the study. Patients with history of bleeding disorders, kediodal/ Koebnerization tendency, lesion over resistant anatomical sites such as palm, sole, glans penis and associated with other hypopigmented disorders were excluded. Patients were diagnosed to have vitiligo based on history, duration, progression of disease, presence of depigmented patch and chalky white appearance on wood's lamp examination. History from all the patients was taken regarding duration of present illness, past history of vitiligo and other systemic disorders, past medical history and family history. Investigations like hemogram, bleeding time, clotting time, fasting blood sugar, HIV and HBsAg status were carried out and recorded.

PROCEDURE

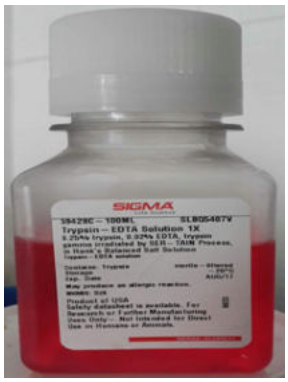
The technique used was described by Mulekar in 2005 [15]

- **DONOR SITE PREPARATION:** The upper thigh region with normal pigmentation was cleaned with povidone-iodine solution and surgical spirit and the area to be harvested was marked with a marking pen. This area was anesthetized with lidocain/epinephrine injected intradermally then held flat and made taut by stretching with the help on an assistant. By holding the cutting edge of razor blade with artery forceps parallel to skin surface it was advanced to cut tangentially to obtain split skin graft of thinnest possible thickness according to requirement of recipient area [Figure 1] The main emphasis was given on the thinness of the graft rather than the continuity. Hemostasis was achieved by pressure applied on the donor area and a betadine dressing was done.

- **PROCSSING OF THE GRAFT:** The grafts were placed on a Petri dish containing 0.2% trypsin solution (sigma aldrich) [Figure 2] and incubated for 30 minutes at 370C. After

washing with normal saline, the epidermis was separated from the dermis and dermis was discarded and the trypsin solution containing melanocyte-keratinocyte mixture[Figure 3] was transferred to a plain vacutainer and centrifuged for 6 min. at 2000 rpm. The cell pellet thus formed [Figure 4,5] was resuspended in a 1mL syringe along with trypsine solution and methyl cellulose (1:1:1)

Figure 1: split thickness graft obtained from the thigh



[Figure 2: 0.2% trypsin solution (sigma aldrich)]

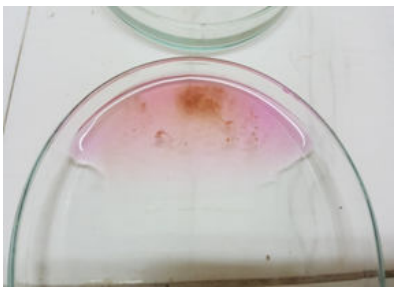


Figure 3: the trypsin solution containing melanocyte-keratinocyte mixture

Figure 4: cell pellet

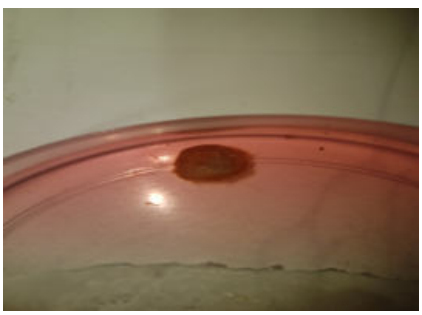


Figure 5: cell pellet mixed with trypsin EDTA solution

- **RECEPIENT SITE PREPARATION:** The recepient area was cleaned with povidone-iodine solution and surgical spirit and the area to be dermabraded was marked with a marking pen. The area was anesthetized with lidocain/epinephrine which was injected intradermally and was submitted to low-speed dermabrasion.

The cell suspension mixed with methyl cellulose and then spread uniformly over the dermabraded area and covered with a collagen dressing, cotton pads and crepe bandage. Only one session is carried out with each patient. Patient was immobilised for 30 minutes and instructed to avoid vigorous activities, friction and pouring water over the dressing. The patient was instructed to perform only restricted movements for next one week. Oral antibiotics (amoxicillin and clavulanic acid 625mg TDS), analgesics and anti-inflammatory drugs (paracetamol+acelofenac) along with probiotics were prescribed for seven days. Donor area dressing was changed every day. Recipient area dressing was opened after seven days. The patient was instructed not to scrub the recipient area for another one week and prescribed with topical 0.01% tacrolimus and oral antioxidants. The consecutive follow ups were done at one month, two months, four months and six months. A record of complications like bleeding, secondary infection, hyperpigmentation and scarring was made and managed accordingly

Assessment and analysis:

To assess the clinical response photographs were taken at the beginning of the procedure and at each follow-up. Results of transplantation were analyzed by three observers on the basis of visual analogue scale (VAS). The first observation was carried out by the patient (observer 1), the second and third observations were conducted by two clinicians (Clinician 1 and Clinician 2). These three observations were compared and analysed statistically in order to determine the measurement of agreements between the judgments of the three observers to determine the actual degree of re-pigmentation. The re-pigmentation was graded as excellent with 90% to 100% pigmentation, good with 60% to 89%, fair with 25% to 59%, and poor with 0% to 24% of the treated area.[16]

Statistical software, SPSS version 17.0 Trial was used for analysis. Prevalence of an outcome variable along with 95% confidence limits was calculated. Independent sample t-test was used to identify the significance of differences in VAS scoring percentage between 1st and 2nd month, 2nd and 4th month, 4th and 6th mont, and 1st and 6th month. Cohen's kappa statistic was used to measure the agreement between categories of re-pigmentation judged by three observers (patient, clinician 1 and clinician 2). This gives a score of how much homogeneity or consensus, there is in the ratings given by three judges. The mean differences are highly significant at the 0.001 level of significance ($p<0.001$)

RESULTS AND OBSERVATIONS

Out of total 30 stable vitiligo patients, At the 1st week, when the bandage was opened, healing was seen in approximately all 27 (90.0%) cases and crusting was recorded in only three (10.0%). The first observer experienced improved and better re-pigmentation after every follow up. Only one of the patients produced poor pigmentation noted after end of last follow up. The re-pigmentation was found to be good at 2nd month in 26.7% (8) patients and the proportion ratio to 60.0% (18) after 4 months and excellent at 4 months among 20.0% (6) patients and ratio to 56.7% (17) at the end of 6 months. At last follow up 26.7% noted good pigmentation and 56.7% observed excellent pigmentation indicating the success of non cultured melanocyte transfer. [Table 1: Patient as Observer 1]

Table 1 shows the degree of re-pigmentation using VAS at the four follow ups by the patient (observer 1).

Scoring with Category		Opinion of Observer 1 (Patient)							
		At 1st month		At 2nd month		At 4th month		At 6th month	
		n	%	n	%	n	%	n	%
0-24 %	Poor	10	33.3	1	3.3	1	3.3	1	3.3
25-59 %	Moderate	20	66.7	21	70.0	5	16.7	4	13.3
60-89 %	Good	0	0.0	8	26.7	18	60.0	8	26.7
90-100 %	Excellent	0	0.0	0	0.0	6	20.0	17	56.7
Total		30	100.0	30	100.0	30	100.0	30	100.0

The observer 2 shared that the earliest pigmentation found to be good at 2nd month after procedure in 26.7% (8) patients and the proportion ratio to 60.0% (18) at 4th month and excellent at 4th month was obtained among 20.0% (6) patients and ratio to 56.7% (17) at the end of 6th months. At last follow up the extent of re-pigmentation had improved and better almost among all treated cases as compared to previous three follow ups (1st month, 2nd month, 4th month) which was just similar and correlated to the experience of observer.[Table 2]

[Table 2: shows the degree of re-pigmentation using VAS at the four follow ups by Clinician 1 as 2nd observer]

Scoring with Category		Opinion of Observer 2 (Clinician 1)							
		At 1st month		At 2nd month		At 4th month		At 6th month	
		n	%	n	%	n	%	n	%
0-24 %	Poor	11	36.7	3	10.0	2	6.7	2	6.7
25-59 %	Moderate	19	63.3	19	63.3	4	13.3	3	10.0
60-89 %	Good	0	0.0	8	26.7	18	60.0	8	26.7
90-100 %	Excellent	0	0.0	0	0.0	6	20.0	17	56.7
Total		30	100.0	30	100.0	30	100.0	30	100.0

The third observer (clinician 2) also reported the same degree of re-pigmentation as indicated by observer 1 and observer 2. The earliest pigmentation found to be good at 2nd month after surgery was in 23.3% (7) patients and the proportion ratio to 66.7% (9) recorded at 4th month, 13.3% (4) at 4th month and 53.3% (16) noted at the end of 6th month. At last follow up, less than one-third (30.0%) patients noted good pigmentation and more than half (53.3%) observed excellent pigmentation; indicated the success of non cultured melanocyte transfer in stable vitiligo [Table 3].

[Table 3: shows the degree of re-pigmentation using VAS at the four follow ups by Clinician 2 as 3rd observer]

Scoring with Category		Opinion of Observer 3 (Clinician 2)							
		At 1st month		At 2nd month		At 4th month		At 6th month	
		n	%	n	%	n	%	n	%
0-24 %	Poor	10	33.3	2	6.7	1	3.3	1	3.3
25-59 %	Moderate	20	66.7	21	70.0	5	16.7	4	13.3
60-89 %	Good	0	0.0	7	23.3	20	66.7	9	30.0
90-100 %	Excellent	0	0.0	0	0.0	4	13.3	16	53.3
Total		30	100.0	30	100.0	30	100.0	30	100.0

Table 4 shows measurement of re-pigmentation and colour match between follow ups by observer 1 (patient) and observer 2 (clinician).

Group & Parameter		Follow up	Scatter (%)	Mean Diff	t-statistic	LOS
			Mean ± SD			
Patient's (Observer 1) perception using VAS (%)	Re-pigmentation at	1st month	25.00±12.87	23.83 %	11.74	p<0.001#
		2nd month	48.83±15.57			
		2nd month	48.83±15.57	20.17 %	9.68	p<0.001#
		4th month	69.00±20.98			
		4th month	69.00±20.98	9.67 %	5.76	p<0.001#
		6th month	78.67±24.70			
		1st month	25.00±12.87	53.67 %	13.10	p<0.001#
		6th month	78.67±24.70			
Clinician 1 (Observer 2) perception using VAS (%)	Re-pigmentation at	1st month	23.67±13.32	25.33 %	13.11	p<0.001#
		2nd month	49.00±17.98			
		2nd month	49.00±17.98	20.83 %	11.12	p<0.001#
		4th month	69.83±22.23			
		4th month	69.83±22.23	9.34 %	6.18	p<0.001#
		6th month	79.17±25.60			
		1st month	23.67±13.32	55.50 %	14.38	p<0.001#
		6th month	79.17±25.60			

The mean differences are highly significant at the 0.001 level of significance.

[Table 5: comparison of re-pigmentation judged by observer 3 (clinician 2) based on VAS between follow ups.#]

Group & Parameter		Follow up	Scatter (%)	Mean Diff	t-statistic	LOS
			Mean±SD			
Clinician 2 (Observer 3) perception using VAS (%)	Re-pigmentation at	1st month	23.67±10.82	24.00 %	11.85	p<0.001#
		2nd month	47.67±15.91			
		2nd month	47.67±15.91	22.00 %	9.70	p<0.001#
		4th month	69.67±21.05			
		4th month	69.67±21.05	8.33 %	7.05	p<0.001#
		6th month	78.00±23.84			
		1st month	23.67±10.82	54.33 %	14.40	p<0.001#
		6th month	78.00±23.84			

On comparing the judgments by three observers, it was noticed that approximately similar results were reported by observers; 1 and 2 were found to be in agreement with observer number 3 that also showed statistically significant improvement using Kappa statistics in further tables [Table 6, 7, 8]

[Table 6: the measurement of agreement using Cohen's Kappa was found to be 0.94 and (p<0.001) between the perceptions of the two investigators]

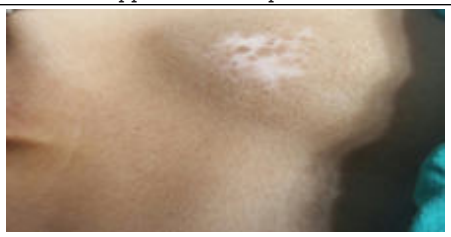
Treatment Outcome		Re-pigmentation at 6 month(Observer 1)				Total
		Poor	Moderate	Good	Excellent	
Re-pigmentation at 6 month (Observer 2)	Poor	1 3.3%	0 0.0%	0 0.0%	0 0.0%	**1 3.3%**
	Moderate	1 3.3%	3 10.0%	0 0.0%	0 0.0%	**4 13.3%**
	Good	0 0.0%	0 0.0%	8 26.7%	0 0.0%	**8 26.7%**
	Excellent	0 0.0%	0 0.0%	0 0.0%	17 56.7%	**17 56.7%**
	Total	**2 6.7%**	**3 10.0%**	**8 26.7%**	**17 56.7%**	**30 100.0%**
Kappa is 0.94and p<0.001#						

[Table 7: The measurement of agreement using Cohen's Kappa was found to be 0.94 and (p<0.001) between the perceptions of the two investigators, observer 1 and observer 3.]

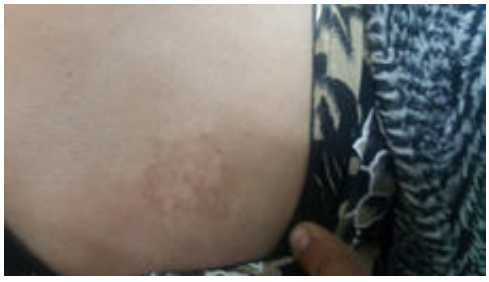
Treatment Outcome		Re-pigmentation at 6 month (Observer 1)				Total
		Poor	Moderate	Good	Excellent	
Re-pigmentation at 6 month (Observer 3)	Poor	1 3.3%	0 0.0%	0 0.0%	0 0.0%	**1 3.3%**
	Moderate	0 0.0%	4 13.3%	0 0.0%	0 0.0%	**4 13.3%**
	Good	0 0.0%	0 0.0%	8 26.7%	0 0.0%	**8 26.7%**
	Excellent	0 0.0%	0 0.0%	1 3.3%	16 53.3%	**17 56.7%**
	Total	**1 3.3%**	**4 13.3%**	**9 30.0%**	**16 53.3%**	**30 100.0%**
Kappa is 0.94and p<0.001#						

[Table 8: the measurement of agreement using Cohen's Kappa was found to be 0.89 and (p<0.001) between the perceptions of the two investigators, observer 2 (clinician 1) and observer 3 (clinician 2).]

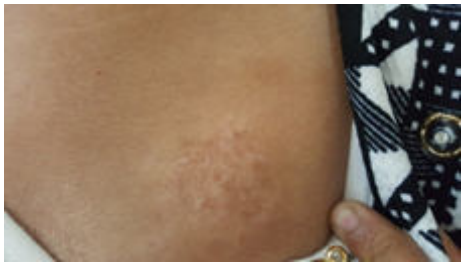
Treatment Outcome		Re-pigmentation at 6 month (Observer 2)				Total
		Poor	Moderate	Good	Excellent	
Re-pigmentation at 6 month (Observer 3)	Poor	1 3.3%	1 3.3%	0 0.0%	0 0.0%	**2 6.7%**
	Moderate	0 0.0%	3 10.0%	0 0.0%	0 0.0%	**3 10.0%**
	Good	0 0.0%	0 0.0%	8 26.7%	0 0.0%	**8 26.7%**
	Excellent	0 0.0%	0 0.0%	1 3.3%	16 53.3%	**17 56.7%**
	Total	**1 3.3%**	**4 13.3%**	**9 30.0%**	**16 53.3%**	**30 100.0%**
Kappa is 0.89and $p<0.001^{\#}$						



[Figure 6.1: a girl with vitiligo patch over right breast]



[Figure 6.2: Re-pigmentation after 4 months]



[Figure 6.3: Re-pigmentation after 6 months]

DISCUSSION:

In present study the efficacy of non cultured melanocyte transfer as a treatment modality in patients of both sex, above 18 years of age who had vitiligo at various sites of the body with disease stability of 6 months or more and the re-pigmentation evaluation at 1, 2, 4, and 6 months by three observers was done.

Table 9 shows stability duration criteria in different studies. [16,17,18,19,20]

author	year	Period of stability
Latheef ENA	2017	1 year
Das SS, Pasricha JS	1992	4 months
Boersma BR, Westerhof W	1995	6 months
Kumar S, Misra RK	2003	6 months
van Geel	2015	1 year

[Table 9: stability duration criteria]

In our study at last follow up, the first observer (patient) observed excellent results in 56.7% patients, second observer (clinician 1) observed excellent results in 56.7% patients and third observer (clinician 2) observed excellent results in 53.3% patients which is similar to study done by Pandya et al[21] who reported that an excellent response was seen in 52.17% cases with non-cultured epidermal suspension technique. similar results were noticed by Van Geel et al[20] and Mulekar et al [22] in segmental vitiligo. However in our study, one patient with stable vitiligo patch over forehead had poor results at the end of 6 months. This case is important to highlight because the patient had a long history of using topical tacrolimus as well as a single sitting of punch grafting technique, both of which were treatment failures (no re-pigmentation except for grafts).

In our study 3 patients with vitiligo patch over the lips showed poor to moderate results. One of these patients had bandage displacement and the other had herpes labialis. Repigmentation of these "lip-tip" areas was more successfully achieved by van Geel[23] with mini-grafting, suggesting that the dermal component of the graft may influence treatment outcome. Mulekar et al reported good results with epidermal cellular grafting on "more difficult-to-treat sites" too (fingers, toes, elbows etc.), but they mentioned that multiple sessions were often necessary. [24]

CONCLUSION

More than half of the patients that were included in the study showed >90% re-pigmentation at the end of 6 months after the transfer of non-cultured melanocytes, thus proving the efficacy of the technique as a treatment option in patients with stable vitiligo who have been resistant to other treatment options.

Declaration of patient consent:

The authors certify that they have obtained written informed consent from all the patients. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understood that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

Financial support and sponsorship - Nil.
Conflicts of interest - There are no conflicts of interest.

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