Original Article

Non-cultured melanocyte transfer in the management of stable vitiligo

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Abstract

Background and Aims: Present study aimed to determine the clinical outcome for non-cultured melanocyte transfer in the management of stable vitiligo. **Methods:** A hospital based prospective study was conducted including 50 stable unresponsive patients of vitiligo undergoing non-cultured melanocyte transplant. Re-pigmentation was analyzed on the basis of baseline photographs after 6 months post procedure. Degree of re-pigmentation was estimated to the nearest of one of the following percentages and the final outcome of re-pigmentation for statistical analysis was graded as: >70% re-pigmentation: Good; 30-69% re-pigmentation: Fair and; <30% re-pigmentation: Poor. **Results:** The mean age of study group was 29.79 ± 13.8 with 52% males and 48% females. Out of total 50 patients, 31 (62%) patients showed good re-pigmentation, 10 (20%) showed fair re-pigmentation while 9 (18%) patients showed poor re-pigmentation. Patches over face, lips, trunk and legs showed good re-pigmentation, however patches over acral areas and bony prominences had poor re-pigmentation. **Conclusion:** Autologous non-cultured melanocyte transfer have an edge over the other modalities, however, proper patient selection, proper technique and good laboratory set up is required. It has an advantage over conventional split skin thickness grafting as it requires very little donor site skin.

Keywords: Non-cultured melanocyte transfer, repigmentation, stable vitiligo

Introduction

Vitiligo is a pigmentary disease of unknown cause, which is characterized by depigmented or hypopigmented macules which results from absence or reduction in the number of epidermal melanocytes in skin and/or mucous membranes. It has immense socio-psychological ramifications in addition to its cosmetic disability.[1] Vitiligo affects 1% of the world's population, with highest incidence of the condition has been reported in India, followed by Mexico and Japan.[2,3]

Many medical treatment modalities are currently used for vitiligo, such as psoralen plus ultraviolet A (PUVA), narrowband ultraviolet B (NB-UVB), excimer lasers, topical steroids, topical immunomodulators and calcipotriol, which may also be used in combination. Mode of therapy is based on decreasing the activity, thereby achieving stability and later inducing pigmentation. Many a times, medical therapy, alone is not helpful. The vitiliginous areas may remain static without showing any re-pigmentation or depigmentation. Such type of patients who are stable for more than 1 year duration are considered suitable for surgical treatment options including transfer of autologous melanocytes.[1]

These surgical techniques are based on a common principle: to transplant autologous melanocytes from a normal pigmented donor skin to depigmented area. Many surgical techniques for re-pigmenting vitiligo have been devised over the years and can be broadly divided into tissue and cellular grafting. Tissue grafts include full-thickness punch grafts, thin dermoepidermal

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grafts, and suction epidermal grafting. With these tissue grafts, only a limited surface area can be treated per treatment session. Cellular grafts include cultured pure melanocytes suspension and non-cultured epidermal cellular suspensions (mixture of melanocytes and keratinocytes). These epidermal cells can also be co-cultured to epithelial sheet grafts. The major advantage of these suspension and culturing techniques is that they permit treatment of affected skin manifold larger than the donor area.[4]

However, culturing techniques are time consuming, expensive due to the culturing time of several weeks, and require highly trained personnel and well-equipped tissue laboratories. Furthermore, the use of specific growth factors and additives in the culture medium (e.g., 12-O-tetradecanoyl-phorbol 13-acetate/TPA), pose safety concerns. These limitations were overcome with the introduction of the non-cultured cellular grafting techniques in 1992 by Gauthier and Surleve-Bazeille.[5] With this technique, a cellular suspension is used without first expanding the cells in culture. Larger areas (8- to 10-fold size of donor skin) can be treated and the procedure can be completed in several hours in an outpatient basis. A double-blind placebo-controlled study, published in 2004, demonstrated that re-pigmentation was primarily induced by the transplanted melanocytes and not by the skin abrasion.[6] This has also been reported earlier by Olsson *et al*.[7,8] This technique can be used in small centres providing primary care at grass root level with imparting simple and adequate practise of the technique procedure, thus making it a primary care intervention as well.

Since the description of the original procedure in 1992, many reports followed including several modifications and further simplifications. This procedure is being done in a limited number of centres in our country and no reliable data exists to ascertain clinical outcome and patient satisfaction for non-cultured melanocyte transfer. This study would be able to provide data that could further help to ascertain efficacy of this procedure as a method of treatment in vitiligo as well as piebaldism, post burn leucoderma, chemical leucoderma, naevus depigmentosus and halo naevus.

Methods

A hospital-based prospective study was conducted at Department of Dermatology of a Tertiary Care Hospital. Study included 50 stable unresponsive patients of vitiligo who have undergone the non-cultured melanocyte transplant at our hospital. Patients with unstable vitiligo, less than 12 years of age, undergoing other surgical treatment for vitiligo, existing co-morbidities, involvement of acrofacial sites, active infection at the local site/ systemic infections, keloidal tendency and history of psoriasis/ lichen planus were excluded.

Methodology

An initial baseline assessment was done for all the patients, during which a medical history was noted and physical examination was carried out. Vitiligo was categorized according to extent of involvement, stability and severity. To calculate the size of vitiligo area, margins of rectangular lesions and radius of circular lesions were measured with a centimetre scale. Irregular big lesions were first divided in to nearest geometrical shapes and then area was calculated. The pigmentation is compared to the baseline after six months post procedure. Baseline standard photographs were taken for comparison.

Procedure

Following were the steps of the procedure:

1. **Informed consent** was taken, both donor and recipient areas were shaved and the area to be obtained for grafting (1/3 to 1/10th of the recipient area) was calculated.
2. **Donor site**:
   a. Site: Normally antero-medial aspect of thigh.
   b. Surgical preparation (cetavlon, spirit, povidone iodine), marking with skin marking pen.
   c. Anaesthesia: LA - 1% xylocaine given as four quadrant infiltration deep dermal and subcutaneous Or; TA - (Topical anaesthesia) EMLA cream under occlusion applied for at least one hour.
   d) The cutting edge of the Humby's knife or the razor blade on Silver's knife was adjusted to take thin (0.2-0.3 mm) graft and its cutting edge and the donor site was lubricated with KY jelly.
   e) **Technique**:
   - Stretching: Donor site was held flat and made taut by stretching. Assistants stretched the skin behind and from below the moving knife while the operator stretched the skin in front with palm of left hand.
   - Harvesting of graft by free hand method: The cutting blade was held parallel to the skin surface and cut tangentially with a to and fro, shaving and sliding movement so as to obtain thin translucent grafts (0.2-0.3 mm).
   - The grafts were transferred to a petridish, containing 0.9% normal saline to wash away the blood clots and then transferred to another petridish (containing 8 ml of 0.2% w/v Trypsin and 0.25% w/v EDTA in trypsin EDTA medium- Hi Media T001).

   f) Haemostasis was achieved by pressure and the donor site was dressed with antibiotic ointment under pressure dressing.
3. **Preparation of Cell suspension:**
   a. The epidermal grafts were washed and then transferred to the petridish with the added trypsin/EDTA (mentioned above).
   b. The grafts were completely soaked by repeatedly turning it back and forth. Finally, the epidermal surface was kept upwards.
   c. The petridish along with the grafts was incubated in an incubator at 37°C for 50 minutes.
   d. The grafts were transferred into a petridish containing 8 ml of melanocyte nourishment medium i.e. Dulbeccos modified eagle medium/F12- DMEM. This media also acted as a diluting agent to wean off the trypsin action.

All the subsequent steps were performed in a laminar air flow bench under strict aseptic conditions.

e. The epidermis was teased gently and separated from the dermis with forceps.
f. The dermal pieces were discarded and the epidermal pieces were retained. The epidermal pieces were scraped, so that they did not have any pigment left on their surface.
g. The transparent epidermal pieces were discarded and the remaining solution was transferred into a centrifuge tube.
h. The tube was centrifuged for 06 minutes at 3000 rpm. The cell pellet settled down to the bottom.
i. The floating epidermal pieces along with supernatant fluid were discarded leaving the cell pellet at the bottom.
j. The pellet was resuspended in a total volume of 0.8ml DMEM medium and transferred gently in steps to a spatula.

4. **Recipient site (Vitiliginous area)**:
   a. Surgical preparation (cetavlon, spirit, povidone iodine), marking the area (2-3 mm more than the border of the lesion), LA (1% Xylocaine).
   b. The vitiliginous areas were dermabraded down to dermoepidermal junction with a diamond fraise wheel. Ideal level was when pinpoint bleeding spots appeared. The denuded areas were covered with gauze pieces moistened with normal saline/isotonic sodium chloride solution after achieving adequate hemostasis.
   c. The cell suspension was applied evenly on the denuded area and spread uniformly with spatula.
   d. The areas were covered with a collagen dressing Collacor CX or Vaseline gauze.
   e. This was covered with sterile gauze pieces moistened with DMEM (Dulbecco's Modified Eagle Medium)/F12 and held in place by Tegaderm transparent dressing.
   f. Patient was immobilized for 30 minutes (elevation of part if required - foot) and then allowed to go with the instructions for restricted movement and to avoid vigorous activities for the next seven days.

Statistical analysis

Data was analyzed using SPSS software ver. 21.0. Appropriate statistical tests were used to determine the significance of the results as per type and distribution of data. The following outcome measures were analyzed:

1. Percentage of patients with different site specific vitiligo patches as over face, lips, trunk, legs, acral and bony prominences.
2. Percentage of re-pigmentation after six months of site specific vitiligo patches as over face, lips, trunk, legs, acral and bony prominences as per photographical improvement.
3. Re-pigmentation was analyzed on the basis of baseline photographs taken and subjective and objective evaluation by patient and doctor. Degree of re-pigmentation was estimated to the nearest of one of the following percentages and the final outcome of re-pigmentation for statistical analysis was graded as good, fair and poor (Wilcoxon sign rank test):
   - >70% re-pigmentation: Good
   - 30-69% re-pigmentation: Fair
   - <30% re-pigmentation: Poor.
4. Percentage occurrence of different complications as related to donor site (depigmentation, keloid formation, infection and pain post procedure) and recipient site (colour mismatch, depigmented halo, cobble stoning pattern, keloid formation and milia formation).

Results

The mean age of study group was 29.79 ± 13.8 with 72% of them were between 11-40 years of age. Only 10% subjects were above 50 years of age. Out of total 50 subjects, 52% were males and 48% females. Among the study groups vitiligo vulgaris was present in 64% patients, localized vitiligo was present in 12% patients, segmental in 8% patients, mucosal in 10% patients and 6% patient had acro- facial vitiligo. Out of the 50 patients, predominant involvement of face was seen in 14%, lips in 8%, trunk in 20%, arms in 4%, elbows in 2%, hands in 10%, legs in 18%, ankles in 10% and feet in 14% [Tables 1 and 2]. The extent of the re-pigmentation process was measured by comparing the respective preoperative photographs at the end of every month, till the sixth month and after 6 months of procedure, percentage of re-pigmentation was calculated. Out of the total 50 patients, 31 (62%) patients showed good re-pigmentation, i.e. more than 70% re-pigmentation, 10 (20%) patients showed fair re-pigmentation, i.e. 30-69% re-pigmentation while 9 (18%) patients showed poor re-pigmentation, i.e. less than 30% re-pigmentation [Figure 1]. Patches over face, lips, trunk and legs showed good re-pigmentation, however patches over acral areas and bony prominences had poor re-pigmentation [Table 3]. Erythema was noted at donor site in 3 subjects, which was resolved after a week of antibiotic course. At the recipient's site, erythematous achromatic area was seen in 1 subject while infection was noted in 1 subject. Both the cases were resolved within a week of oral antibiotics without any sequelae.

Discussion

Melanocyte culture is a state of the art procedure that requires expertise and is being practiced in a few centres only. While the advantage of autologous melanocyte transfer technique over the cell culture method is that it is simple and does not require expensive culturing conditions and high-tech laboratories. There was no statistical difference between these two groups, and it was found that both therapeutic projects were beneficial in more than 50% of the cases, but inferior result in cell culture method was seen because of the delay in transplantation.[9] Nevertheless, basal cell layer suspension method requires a dermatologist who is familiar with the separation of human skin after trypsinization. The potential advantage of techniques based on cell separation is to allow the treatment of larger lesions than techniques based on whole tissue such as minigrafts, suction blister grafts, except split thickness grafts which had many disadvantages.

Table 1: Distribution of subjects based on Type of Vitiligo

Type of Vitiligo	*n*	Percentage
Vulgaris	32	64.0%
Localized	6	12.0%
Mucosal	5	10.0%
Segmental	4	8.0%
Acro-facial	3	6.0%
Total	50	100.0%

Table 2: Distribution of subjects based on Site of Vitiligo

Site of Vitiligo (Predominant)	*n*	Percentage
Face	7	14.0%
Lip	4	8.0%
Trunk	10	20.0%
Arm	2	4.0%
Elbow	1	2.0%
Hand	5	10.0%
Leg	9	18.0%
Ankle	5	10.0%
Foot	7	14.0%
Total	50	100.0%

Table 3: Association between Site of Vitiligo and Improvement

Site of Vitiligo	*n*	Improvement		
		Good	Fair	Poor
Face	7	6	1	-
Lip	4	3	-	1
Trunk	10	6	1	3
Arm	2	1	1	-
Elbow	1	-	-	1
Hand	5	2	-	3
Leg	9	6	3	-
Ankle	5	3	1	1
Foot	7	4	3	-
Total	50	31	10	9

Non-cultured epidermal suspension is indicated mainly for segmental vitiligo and for stable non-segmental vitiligo that does not respond to medical treatment.[10] Present hospital-based prospective study was conducted with the aim of determining the clinical outcome for non-cultured melanocyte transfer in the management of stable vitiligo.

At first follow-up, i.e. after seven days, soon after the removal of dressing the treated area appeared bright pink. Re-pigmentation was first seen after 2-3 weeks after the procedure and was completed in up to 6 months. It was almost of a uniform color.

In a few cases, there was initial hyperpigmentation that subsequently faded to match the normal skin color. This hyperpigmentation may be caused by hyperactivity of transplanted cells from the culture or oversupply of growth factors and melanogenic peptides such as β-FGF during wound healing.[11,12] In most patients, we observed pigmentary islands irrespective of leukotrichia or paucity of hair follicles.

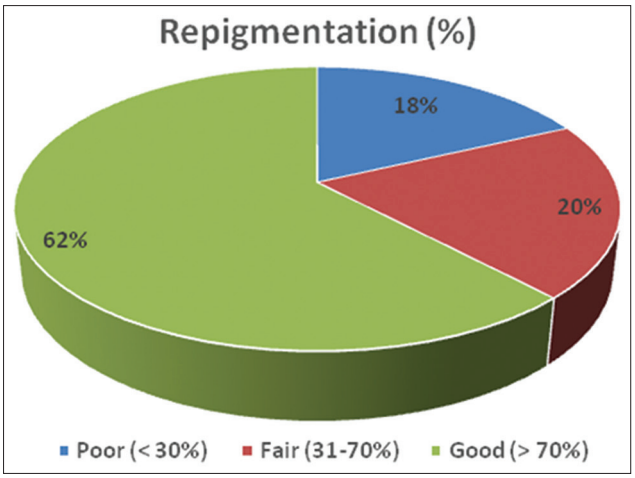

Figure 1: Distribution of subjects based on Percentage of Re-pigmentation

The extent of the re-pigmentation process was measured by comparing the respective preoperative photographs at the end of every month, till the sixth month and after 6 months of procedure, percentage of re-pigmentation was calculated. Out of total 50 patients, 31 (62%) patients showed good re-pigmentation, i.e. more than 70% re-pigmentation, 10 (20%) patients showed fair re-pigmentation, i.e. 30-69% re-pigmentation while 9 (18%) patients showed poor re-pigmentation, i.e. less than 30% re-pigmentation.

A similar study was conducted by Pandya V, *et al.* to evaluate the extent of repigmentation after autologous melanocyte transplantation in patients with stable vitiligo. An excellent response was seen in 52.17% cases with the autologous melanocyte rich cell suspension (AMRCS) technique. They concluded that Autologous melanocyte transplantation can be an effective form of surgical treatment in stable vitiligo.[13] Our study results were also comparable with results found by Hassan I, *et al.*[14] and Mulekar *et al.*[15] In another similar study of 27 patients, Lontz *et al.* reported excellent response in 40.7%, good response in 7.4%, and moderate response in 51.8%.[16]

Patches over face, lips, trunk and legs showed good repigmentation, however patches over acral areas and bony prominences had poor repigmentation. The percentage of repigmentation and final outcome was dependant on the site of the vitiligo lesions. As per the study by Pandya V, *et al.*[13] the results were most favorable on the legs, feet, face and forearms, and poor on the elbows and acral areas of the hand. As compared to the other studies in literature, repigmentation was poor over bony prominences and non-hairy acral areas.[13,17]

In a study, Lontz *et al.* emphasize that the anatomical location is the major factor that determines the response.[16] The fingers,

knuckles and elbows were the most difficult areas to repigment, in part because of the relative uncertainty in controlling the depth of dermabrasion of such heavily cornified areas and also because of the high mobility of the skin covering these joints. Olsson and Juhlin have also made a similar observation.[18]

Erythema was noted at donor site in 3 subjects, which was resolved after a week of antibiotic course. Erythema and Infection was noted in one subject each at recipient's site. Both the cases were resolved within a week of oral antibiotics without any sequelae.

In a study by Pandya V, *et al.*[13] two patients had infection at the donor area and three developed infection at the recipient surface. Only one patient developed Koebner response at the donor area. Similarly, in a study by Nanda S, *et al.*[19] in 2006, using various surgical modalities for eyelid vitiligo, no complication was reported at donor's or recipient's site.

Conclusion

Autologous non-cultured melanocyte transfer techniques is one of the novel surgical modality in the armamentarium of a dermatosurgeon. It is safe, simple and as far as results are concerned, has an edge over the other modalities. However, proper selection of the patient, proper technique in taking graft and good laboratory set up is required. It has an advantage over conventional split skin thickness grafting in that it requires very little donor site skin. Patients are generally satisfied with the results as the quality of the pigmentation is superior. We recommend further large scale randomized studies in comparison with other modalities especially with melanocyte cultured methods, to confirm the efficacy of autologous non-cultured melanocyte transfer technique.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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