COMPARATIVE STUDY OF EFFICACY OF NON-CULTURED MELANOCYTE TRANSFER TECHNIQUE AND PUNCH GRAFTING TECHNIQUE IN THE MANAGEMENT OF STABLE VITILIGO

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ABSTRACT

BACKGROUND

Vitiligo is a common depigmenting disease of skin characterized by the loss of melanocyte of possible autoimmune aetiology and with hereditary basis. The disease causes immense emotional stress and social stigma to the patient because of disfigurement.

AIM

To comparative study of efficacy of non-cultured melanocyte transfer technique and punch grafting technique in the management of stable vitiligo.

MATERIAL AND METHODS

Fifty patients of both sexes aged between 15-50 years clinically diagnosed stable vitiligo, not more than 30% of body surface area were included in the study and randomly divided in two groups. Group A was treated by autologous non-cultured melanocyte rich cell suspension with PUVASOL and Group B was treated by punch grafting technique with PUVASOL. Response was assessed after 6 months followed by every month visit on the basis of investigator assessment and photographic assessment.

STATISTICAL METHODS

Fisher test, Chi square test.

RESULTS

An excellent response was seen in 91.20% cases with the Autologous Non-Cultured Melanocyte Rich Cell Suspension (AMRCS) technique and in 82.40% with punch grafting technique.

CONCLUSION

Autologous non-cultured melanocyte transfer technique can be an effective form of surgical treatment in large surface area of stable vitiligo within short duration by homogenous repigmentation is better than punch grafting technique.

KEYWORDS

Non-Cultured Melanocyte Transfer Technique (NCMT), Punch Grafting Technique, Fisher Test.

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INTRODUCTION

Vitiligo is a pigmentary disease of unknown etiology, 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. That is immense socio-psychological ramifications in addition to its cosmetic disability. 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 repigmentation or depigmentation. Such types of patients who are stable for more than 1 year duration are considered suitable for surgical treatment options including transfer of autologous melanocytes.¹ Replenishing autologous melanocytes selectively within vitiliginous macules is a novel and promising treatment.² This can be carried out by either culture or non-culture techniques, each having its advantages and disadvantages.^{3,4} We undertook this study to compare the two methods of melanocyte transplantation in stable vitiligo, namely non-cultured melanocyte transfer technique and punch graft transfer techniques for replenishing melanocytes.

MATERIAL AND METHODS

This was a comparative study, where in 50 stable patients each were operated upon by the 2 modalities, i.e. punch graft transfer technique and non-cultured autologous melanocyte transfer technique leading to a total of 250 sites in all over a period of 1 year. The 250 patches of vitiligo were randomized based on simple random sampling method.

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Only cases with stable form of vitiligo (No increase in the size of the lesion for at least 1 year) and with a maximum percentage of body surface area involvement upto 30% were included in the study. The pigmentation was compared to the baseline after 6 months postprocedure. No blinding was done in the study. Sample size determination was done as follows:

$$n = \frac{4P \times q}{d^2}$$

$$n = 4 \times 0.50 \times (1 - 0.50) / (0.01)^2 = 100$$

Therefore $n = 220$. Though the calculated sample size was 220, 250 unresponsive sites were considered for surgery by each of the modalities, leading to a total of 125 sites in all. Pre-operative work-up consisted.

Applying design effect of one, the sample comes out to be $100 \times 2 = 200$.

Applying design effect of 2, the sample comes out to be $100 \times 2 = 200$. Assuming 10% patients lost the follow-up, so new sample size will be 220 of an informed consent, clinical photographs, screening for HIV and Hepatitis-B virus infection and charting of the area to be grafted.

Two techniques were employed; the autologous melanocyte rich cell suspension Non-Cultured Melanocyte Technique (NCMT technique).^{5,6,7} and the punch grafting technique. Both these techniques share a common principle of selective replenishment of pigment cell.³ or melanocytes at the recipient stable vitiligo macules. DONE

Donor Site

About one-tenth the size of the recipient was selected as the donor site, usually on non-cosmetically important sites like the medial aspect of thighs. It was cleaned and draped. The site was anesthetized and a very superficial sample of skin was obtained using Silver's skin grafting knife. The superficial wound was then dressed with Sofra-tulle.

Laboratory Procedure for Cell Separation

The skin graft was immediately transferred to 6ml of 0.25% trypsin-EDTA solution in a petridish. This mixture of skin sample with trypsin-EDTA solution was incubated at 37°C for 50 min. The grafts were then transferred into a petridish containing 8ml of melanocyte nourishment medium, i.e. Dulbecco's Modified Eagle Medium/F12-(DMEM). This media also acted as a diluting agent to wear off the trypsin action. All the subsequent steps were performed in a laminar air flow bench under strict aseptic conditions.

The epidermis was teased gently and separated from the dermis with forceps. 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. The contents of the petridish were transferred into a centrifuge tube and centrifuged for 6 minutes at 3000rpm. The cell pellet settled down at the bottom. The supernatant was discarded and the pellet containing cells from the stratum basale.⁷ and lower half of the stratum spinosum that were rich in melanocytes was taken. The pellet was resuspended in a total volume of 0.8ml DMEM medium and transferred gently in steps to a syringe.

Recipient Site (Vitiliginous Area)

The vitiliginous areas were dermabraded down to the papillary dermis with a diamond fraise wheel after surgical cleaning and infiltration of local anaesthesia.

The cell suspension was applied evenly on the denuded area and spread uniformly with spatula. The areas were covered with a collagen dressing and later with sterile gauze pieces moistened with DMEM/F12 and held in place by Tegaderm transparent dressing. Patient was made to lie down for 30 minutes (Elevation of part if required-foot) and then allowed to leave with the instructions to avoid vigorous activities and to carryout only restricted movements for next 7 days.

Post-Operative Care

Oral antibiotics and analgesics were given for 7 days. Dressing of donor area was changed on alternate days and for the recipient areas it was removed after 7 days. PUVA SOL (1:10) was added for accelerating the repigmentation and was started 2 weeks after the erythema subsided. The patients were followed up at 1, 3 and 6 months after procedure for assessing repigmentation.

Preparation of Mini Punch Graft^{8,9,10,11,12,13}

After proper assessment of the stability status and routine physical examination and investigations. An informed consent is taken from the patient, the donor and recipient areas are surgically prepared. The instruments required are 1 or 3mm punches, small jeweler's or graft holding forceps and a small curved tip scissors.

Then recipient area is prepared first with two percent lignocaine with or without adrenaline is infiltrated as a local anesthetic and minimize the chance of developing any perigraft halo, the initial recipient chambers are made on or very close to the border of the lesion. The punched out chambers are spaced according to the result of test grafting or at a gap of 5-10mm from each other. The donor area is either the upper lateral portion of the thigh or the gluteal area. Punch impressions are made very close to each other, so that a maximum number of grafts can be taken from a small area and same size punches are used for both the donor and recipient areas. The grafts are placed directly from the donor (Buttock/upper thigh) to the recipient areas. This speeds up the procedure and lessens the chance of infection. Care is taken to ensure that the graft edges are not folded and the tissue is not crushed or placed upside down.

The needle of the syringe or the tip of the scissors is used for the proper placement of grafts in the recipient chambers. Hemostasis is achieved by pressing a saline-soaked gauze piece over the area. For the recipient area, the three layers of dressing from inside to out are: Paraffin-embedded, no adherent sterile gauze (Jelonet, sterile Surgipad, and bio-occlusive Micropore. For the donor area only Surgipad and Micropore are used. The recipient area may be immobilized if necessary. Proper instructions for special areas like the lips are necessary.

To secure the recipient area, these patients are advised to be on a liquid diet for the first 24 hours, preferably with a straw. Patients are allowed a normal diet after this period. Some times dressings are opened after 24 hours to look for any dislodgement of grafts, if any are found, they are replaced. Finally after 4 to 7 days the dressings are removed. Then patient will be treated with oral PUVA therapy, will be reviewed fortnightly for 2 months, then monthly for at least 6 months. Pre and post procedure photograph and followup photograph will be taken. Data will be collected and analysed by appropriate statistical method.

Study Group	No. of Patients	% of Patients	No. of Patches	% of Patches
Vitiligo vulgaris	17	68%	90	72%
Frictional depigmentation	03	12%	10	8%
Nevus depigmentation	02	8%	4	3.2%
Mucosal vitiligo	02	8%	8	6.4%
Contact depigmentation	01	4%	13	12.4%
Total	25	100%	125	100%

Table 1: NCMT Group

Study Group	No. of patients	% of Patients	No. of Patches	% of Patches
Vitiligo vulgaris	15	60%	85	68.00%
Frictional depigmentation	4	16%	18	14.40%
Nevus depigmentation	2	8%	4	3.20%
Mucosal vitiligo	2	8%	4	3.20%
Contact depigmentation	2	8%	14	11.20%
Total	25	100%	125	100%

Table 2: Punch Grafting Group

RESULTS

A total of 50 patients were enrolled in the study, which included 250 sites of depigmentation for autologous Non-Culture Melanocyte-Keratinocyte Transfer (NCMT) and 125 sites for punch grafting. In the NCMT group 25 patients were enrolled with 125 unresponsive depigmented sites, 5 patients for vitiligo, 5 patients for postherpetic depigmentation, 5 patients for frictional depigmentation, 5 patients for contact depigmentation and 5 patients for Nevus depigmentation.

In the punch grafting group 25 patients with 125 unresponsive depigmented sites were enrolled, 5 patients for vitiligo, 5 patients for postherpetic depigmentation, 5 patients for frictional depigmentation, 5 patients for contact depigmentation and 5 patients for Nevus depigmentation. In the NCMT group, minimum duration of vitiligo was 1 year and maximum duration was 14 years; mean duration was 7.00 ± 3.43 years. In punch grafting group, minimum duration of vitiligo was 3 years and maximum duration was 14 years; mean duration was 7.83 ± 4.45 . Demographically, both the groups were comparable.

Among the study groups in 25(100%) patients with 125(100%) patches for NCMT was done on, vitiligo vulgaris were present in 17(68%) patients with 90(72%) patches, frictional depigmentation in 3(12%) with 10(8%) patches, nevus depigmentation in 2(8%) with 4(3.2%) patches, mucosal in 2(8%) patients with 8(6.4%) patches and 1(4%) patient with 13(12.4%) patches had contact depigmentation.

Among the study group in 25(100%) patients with 125(100%) patches for punch grafting was done on, vitiligo vulgaris were present in 15(60%) patients with 85(68%) patches, frictional depigmentation 4(16%) patients with 18(14.44%) patches, nevus depigmentation in 2(8%) with 4(3.2%) patches, mucosal in 2(8%) patients with 4(3.2%) patches and 2(8%) patients with 14(11.20%) patches had contact depigmentation.

Post-Operative Evaluation

At first follow-up soon after removal of dressing from the treated area, crusted scabs were seen partially attached to the skin surface leaving behind erythematous achromatic area.

Infection was noted at one of the sites following NCMT technique and 5 sites following punch grafting technique; all of which resolved within a week of oral antibiotics without

any scarring. After 6 months of procedure, percentage of repigmentation was calculated for both the groups and the same has been depicted in Table 1.

Repigmentation	NCMT	Punch Grafting	P- value
Poor (<50%)	9	16	P=0.1044
Fair (51-80%)	2	6	
Good (>80%)	114	103	
Total	125	125	

Table 3: Percentage of Repigmentation

In the NCMT method, 114(91.20%) patches showed good repigmentation, i.e. more than 80% repigmentation [Figs. 1 and 2], 2(1.6%) patches showed fair repigmentation, i.e. 51%-80% repigmentation, 9(7.2%) patches showed poor repigmentation, i.e. less than 50% repigmentation.

Fig. 1: Non-culture melanocyte transplant - Lip vitiligo (a) Pre procedure (b) 6 months after procedure

Fig. 2: Non culture melanocyte transplant - Contact depigmentation of anterior aspect of neck (a) Preprocedure (b) 6 months after procedure

- In the punch grafting technique, 103(82.40%) patches showed good repigmentation, i.e. more than 80% repigmentation [Fig. 3], 6(4.8%) patches showed fair repigmentation, i.e. 51-80% repigmentation, 16(12.80%) patches showed poor repigmentation, i.e. less than 50% repigmentation.

Fig. 3: Punch grafting technique – Vitiligo: right and left side of leg (a) Preprocedure (b) 6 months after procedure

Repigmentation	NCMT	Punch Grafting	P- value
Poor (<50%)	9	16	P=0.1044
Fair (51-80%)	2	6	
Good (>80%)	114	103	
Total	125	125	

Table 4: Percentage of Repigmentation

By using Chi-square test p-value for the degree of repigmentation noted after 6 months was 0.1044, which was >0.05 ; therefore there was no statistical association between treatment methodology and repigmentation. So the final outcome, i.e. repigmentation is independent of the modality of treatment. However, in patients of the NCMT group, more patches showed good results as compared to the punch grafting technique, though they were not statistically significant.

By both the methods patches over face, lips, trunk and legs showed good repigmentation; however, patches over acral areas and bony prominences had good repigmentation by NCMT than punch grafting technique.

DISCUSSION

There are many existing dermatosurgical procedures for treatment of stable vitiligo. These include suction blister grafting, punch grafting, split thickness skin grafting, tattooing, needling and plain dermabrasion. However, they have various deficiencies in the form of cobble stoning, pigment mismatch, stuck-on appearance, inadequate pigment cover and patient discomfort. Thus, there is an increasing trend towards the use of advanced technology in the treatment of this condition. Replenishing melanocytes selectively in vitiliginous macules by autologous melanocytes is one such promising treatment.

Both the autologous melanocyte rich cell suspension (Non-cultured) technique and the punch grafting technique are essentially based on the principle of seeding of melanocytes, i.e. introduction of melanocytes from normal skin into a region of depigmented skin. The distinctive advantages of this technique over pre-existing modalities are a wide recipient area for small donor area, no cobble stoning, good colour match and probably the best efficacy.

Punch grafting technique is a state of the art procedure that requires more expertise as compared to the NCMT technique.¹¹ This was a comparative study of efficacy of non-cultured melanocyte transfer technique and punch grafting technique in the management of stable vitiligo in 250 patches of stable vitiligo, satisfying the inclusion and exclusion criteria. It is an accepted fact that during the initial 7 days of healing, melanocytes and keratinocytes present in the grafted material gets entrapped in the healing recipient site tissue. These cells multiply and repigment the depigmented area. Further pigmentation can be accelerated by topical Psoralen therapy. It seems likely that growth factors and cytokines released

during wound healing phase helps in the transplantation and multiplication of melanocytes.

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 area can pigment a much larger recipient area.

Out of 125 patches operated upon, good results were seen in 91.20% patches by using NCMT method and 82.40% by using punch grafting technique. Similarly fair results were seen in 1.6% patches by NCMT method and 4.8% patches by punch grafting technique. Poor results were seen in 7.2% patches by NCMT method and 12.80% patches by punch grafting technique.

The only study from India comparing punch grafting and non-cultured melanocyte transfer demonstrated 50% improvement results in both groups with no difference between the two groups. However, this study recruited very few depigmented sites. In view of the fact that this study did not show any difference between punch grafting and non-cultured melanocyte transfer, it was necessary to rigorously study these two modalities of cellular and tissue transfer therapy of vitiligo in a larger number of areas of depigmentation to confirm or refute these findings as well as to standardize the protocols for performance of these procedures in our population.

In our study there was no statistically significant difference between the two groups, meaning that both the procedures were definitely beneficial to the patients. However, the NCMT method did show slightly better results. The cumbersomeness of the punch grafting technique as well as the delay in repigmentation and cobblestoning and more time consuming technique could be the reasons behind the inferior results.

The donor:recipient area in the NCMT group was 1:10, i.e. 1cm² of normal skin was sufficient to cover about 10cm² of vitiliginous skin. The donor recipient area in the punch grafting technique group was 1:1, i.e. 2mm² of normal skin was sufficient to cover about 1cm² of vitiliginous skin. This was a distinct advantage as compared with punch grafting technique to the NCMT method. However, punch grafting technique was more time consuming of performance of technique than NCMT.

Limitations of NCMT

- Both techniques require an equipped laboratory setup with trained manpower.
- Taking an epidermal graft requires expertise.
- The pathogenesis of vitiligo is still poorly understood, so the stability of vitiligo and reactivation of disease activity after any surgical technique cannot be predicted.
- Punch grafting transfer techniques are state of the art novel surgeries in vitiligo and the results obtained indicate that the procedures can be valuable in motivated patients, when the extent of vitiligo does not exceed 30% of the total body surface area and when the disease is stable.

CONCLUSION

In patients with stable vitiligo, autologous non-cultured melanocyte transplantation is a simple and effective technique to produce homogeneous pigmentation quickly. Punch grafting technique is time consuming and labour intensive technique, which requires a large surface area for donor site, though it can cover vitiliginous areas 1:5 times the donor area and recipient area ratio.

It is therefore suitable to cover small body surface areas. It has an advantage over conventional split thickness grafting, as it requires very little donor skin.

We propose that though the difference between the two procedures were not statistically significant, the non-culture method is simpler, less time consuming and requires less technical expertise, but punch grafting is less expensive than NCMT. Hence NCMT should be the first choice technique when it comes to not so widespread cases of vitiligo. When area involved is large and in institutions where technical expertise and trained manpower are available, the punch grafting method can be undertaken.

Patients were generally satisfied with the results to both methods, as the quality of repigmentation² was superior to other surgical techniques. Further large scale patient studies are required, especially with punch grafting methods, to confirm the efficacy of punch grafting techniques. The patients also need to be followed up for a longer duration to note the long term complications and the status of repigmentation after a period of time.

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