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Original Article

A comparative study of efficacy of cultured versus non cultured melanocyte transfer in the management of stable vitiligo

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ABSTRACT

Background: Replenishing melanocytes by autologous melanocytes selectively in vitiliginous macules is a novel and promising treatment. With expertise in culturing autologous melanocytes, it has now become possible to treat larger recipient areas with smaller skin samples. To determine the relative efficacy of cultured versus non cultured melanocyte transfer in the management of stable vitiligo.

Methods: The melanocytes were harvested as an autologous melanocyte rich cell suspension from a donor split thickness graft. Cultured or non cultured melanocytes were then transplanted to the recipient area that had been superficially dermabraded. 100 patches of vitiligo in patients reporting to this hospital were randomly allocated into 2 groups to receive either of the interventions.

Results: An excellent response was seen in 62.17% cases with the autologous melanocyte rich cell suspension technique and in 52% with the melanocyte culture technique.

Conclusion: Autologous melanocyte transplantation can be an effective form of surgical treatment in stable but recalcitrant lesions of vitiligo. Large areas of skin can be covered with a smaller donor skin using melanocyte culture technique; however culture method is more time consuming, and a labour intensive process, requiring state of the art equipments with a sterile lab setup.

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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. 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 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]

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 melanocyte rich cell suspension and cultured melanocyte transfer for replenishing melanocytes.

Material and methods

This was a comparative study wherein 50 unresponsive sites, each were operated upon by the 2 modalities, i.e. cultured melanocyte transfer and non cultured autologous melanocyte transfer technique leading to a total of one hundred sites in all, over a period of 1 year. The 100 patches of vitiligo were randomized based on simple random sampling method. 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 up to 30% were included in the study. The pigmentation was compared to the baseline after 6 months post procedure. No blinding was done in the study.

Sample size determination was done as follows: $(1 - \alpha) = 0.98$, $(1 - \beta) = 0.80$, $\pi 1 = 0.81$, $\pi 2 = 0.50$, $\delta = 0.1$and therefore $n = 36$. Though the calculated sample size was 36, 50 unresponsive sites were considered for surgery by each of the modalities, leading to a total of one hundred sites in all. Pre operative work-up consisted 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)[5–7] and the cultured melanocyte technique (CMT technique).[8–10] Both these techniques share a common principle of selective replenishment of melanocytes at the recipient stable vitiligo macules.

Donor site

About one-tenth the size of the recipient area 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 6 ml 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 8 ml of melanocyte nourishment medium i.e. Dulbecco's 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. 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 min at 3000 rpm. The cell pellet settled down at the bottom. The supernatant was discarded and the pellet, containing cells from the stratum basale and lower half of the stratum spinosum that were rich in melanocytes, was taken. The pellet was resuspended in a total volume of 0.8 ml 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 min (elevation of part if required – foot) and then allowed to leave with the instructions to avoid vigorous activities and to carry out only restricted movements for next 7 days.

Post operative care

Oral antibiotics and analgesics were given for 5 days. Dressing of donor area was changed on alternate days and for the recipient areas, it was removed after 7 days. PUVASOL (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.

CMT group

Patients in this group received treatment in the form of autologous, cultured, melanocyte plus keratinocyte grafting followed by topical Psoralen therapy. The initial steps were similar to the ones followed during autologous, non cultured, melanocyte plus keratinocyte grafting (NCMT group), till the formation of a cell suspension pellet. This cell suspension was cultured in tissue culture flasks along with 05 ml of M2