

Original Research Article

A retrospective study of 100 cases of focal vitiligo treated by autologous, noncultured melanocyte-keratinocyte cell transplantation

E. N. Abdul Latheef*, K. Muhammed, Najeeba Riyaz, M. P. Binitha

Department of Dermatology, Medical College Kozhikode, Kerala, India

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***Correspondence:**

Dr. E. N. Abdul Latheef,

E-mail: drlatheef90@gmail.com

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ABSTRACT

Background: Vitiligo is a common skin disease affecting 1%-2% of general population. Even though different modalities of treatment available, it remains as a difficult disease to treat. Many cases remain stable after a period of medical therapy without any further response. Surgical techniques are the only hope in such patients. The aim of this study was to evaluate the response of autologous, noncultured melanocyte keratinocyte cell transplantation in patients with stable focal vitiligo.

Methods: A retrospective study of 100 cases focal vitiligo treated by autologous, noncultured melanocyte-keratinocyte cell transplantation. Serial of photographs were taken on each visit. The patients were followed for a period of 5 years. The results were analyzed on a visual analogue scale.

Results: Out of total 100 patients, 44 had excellent (90 to 100%) response, 18 had good (60 to 89%) response, 12 had fair (25 to 59%) response and 26 had poor response (0 to 24%). Age and sex of the patients and size and duration of lesions, did not show significant influence on results of transplantation.

Conclusions: This is a simple, safe, and effective surgical therapy for replenishing the missing melanocytes in resistant cases of vitiligo. Repigmentation lasts long with very good cosmetic acceptability.

Keywords: Focal vitiligo, Melanocyte-keratinocyte cell transplantation, Management

INTRODUCTION

Vitiligo is an acquired idiopathic depigmentation of the skin characterized by well demarcated white patches due to disappearance of melanocytes in the skin. The condition occurs in 1 to 2% of the general population mostly between the ages of 10-30 years, and as often in males as in females.¹ Vitiligo is a disease of multi factorial etiology. Hereditary factors, autoimmunity, toxic neurochemical mediators released from nerve endings, antioxidant deficiency, reduced growth factors from keratinocyte, fibroblasts and other tissues have been hypothesized. Vitiligo may be either localized or generalized. Localized vitiligo is further divided into 2 types: (1) segmental vitiligo, which is characterized by

unilateral macules in quasi dermatomal distribution (2) focal vitiligo, which is described as depigmented macules in a localized, non dermatomal distribution.² Focal vitiligo could be considered an initial stage of generalized or immunologic vitiligo or may be an abortive form of the segmental type.

Several medical treatments are available for the management of vitiligo including topical corticosteroids, calcineurin inhibitors, photo chemotherapy using psoralen – UVA (ultra violet - A) and narrow band UVB (ultra violet- B).^{3,4} But the results of medical therapies are less satisfactory in many cases. The alternative treatment option for patients in whom the lesion is stable and medical therapy fails is surgical treatment. Currently

available surgical treatments are full thickness graft, punch graft, split thickness graft, ultrathin graft, suction blister graft and cultured and noncultured melanocyte keratinocyte cell suspension graft.⁵⁻⁹ The technique of cell suspension and culture has the advantage of treating large depigmented areas by expanding cell numbers from a small piece of normal pigmented skin and it will produce almost normal colour match¹⁰.

METHODS

In the present study, 100 patients (46 males and 54 females) with focal stable vitiligo (i.e., have no new lesion or no increase in size of old lesion since last one year) were undergone autologous, noncultured melanocyte-keratinocyte cell transplantation in the year 1998 to 2000 were included and were on regular follow up for 5 years thereafter. The duration of vitiligo varied from 2 to 18 years. The maximum area treated in 1 operative session was 350 cm². All of them were on some or other form of medical therapy till the surgery. Patients with following criteria were selected for the procedure at the time of surgery.

Patients of age more than 12 years of not being on any immunosuppressive therapy and having a vitiligo lesion stable for 12 months (i.e. no new lesion or no increase or decrease in size of old lesion) were included in the study. Patients of age less than 12 years, history of keloid and bleeding tendency and more than 30% body surface involvement were excluded.

Equipment

Skin grafting knife (Silver's knife), electrical dermabraders, incubator, centrifuge, spatula, test tubes, marking pen, petri dishes, iris scissors, and jeweller's forceps.

Donor site

A donor area of one fifth to one tenth of the recipient area was marked on the lateral aspect of the gluteal region by a marking pen. Then this area and surrounding skin is cleaned with povidone iodine and 70% ethanol. The area is then anaesthetized with 2% xylocaine injection with adrenaline. The skin is stretched uniformly and graft is taken with silver's skin grafting knife. The wound so created is covered with vaseline gauze.

Preparation of the cell suspension

The skin sample taken from the donor site is transferred to a petri dish containing about 4 ml of 0.2% w/v trypsin solution. The sample is kept in trypsin solution with epidermis upwards and shaken to ensure that it comes in complete contact with the solution. Then the petri dish containing sample is incubated for 50 minutes at 37 degree Celsius in a medical incubator. After incubation the sample is taken out from the incubator and about 2 ml

of trypsin inhibitor is added to petri dish to neutralize the action of trypsin. Then the epidermis is separated from dermis and is transferred to a test tube containing 3 ml of Dulbecco modified eagle medium/F12 (DMEM/F12) and vortex mixed for 15 seconds. The dermal pieces are then discarded. The epidermis in petri dish is broken down into multiple small pieces using forceps, washed with DMEM medium and then transferred to test tube containing the same medium. The test tube is then centrifuged (2000 RPM) for 7 minutes. The cell pellet thus settles to the bottom and is made into a suspension of around 0.5 ml in DMEM medium. The suspension is taken in 1ml syringe with detachable needle. The suspension thus contains a mixture of melanocytes and keratinocytes.

Recipient site

Recipient area is shaved and marked out with a marker pen, cleaned with povidone iodine and 70% ethanol and anaesthetized with 1% xylocaine. The recipient area was abraded with a high-speed dermabrader fitted with a diamond fraise wheel. The dermabrasion continued down till multiple pinpoint bleeding spots appear that indicates the dermoepidermal junction was reached.

Transplantation

The denuded area was covered with gauze pieces and moistened with isotonic sodium chloride solution until the cell suspension was applied evenly on the denuded area and covered with a dry collagen sheet. Collagen helps transplanted cells to remain in place, providing an optimal environment for cellular growth and vascularization. This was then covered with sterile gauze pieces moistened with DMEM/F12, held in place with adhesive tapes. The patient was allowed to go home after dressing. The patient was warned against any vigorous activities for 10 days which could displace the dressing. Absolute immobilization was not necessary. Antibiotics were prescribed for 5 days. Dressing was removed after 1 week. PUVA had been given to all patients after transplantation.

Follow up

Patients were followed up on first and second week, then monthly for six months. Then three monthly for 2 years. Then kept under observation for five years. Serial photographs were taken on every visit to assess the repigmentation.

RESULTS

The treated area appeared bright pink immediately after removal of dressing. The earliest pigmentation is noticed 3 weeks after surgery. Results were analyzed and evaluated on the basis of serial photographs taken during the follow up period. Repigmentation was graded on the basis of a VAS score (visual analogue scoring system) 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. Region wise response to melanocyte-keratinocyte cell transplantation is illustrated in Table 1. At the end of 5 years, out of 100 patients 44% had excellent pigmentation 18% had good, 12% had fair and 26% had poor pigmentation. The results are

illustrated in Table 2. The poor response was mainly over the non-hairy areas and over bony prominences. Four patients had marked pigmentary dilution on follow-up and six patients had undergone retransplantation at 8 months to achieve this result. 10% of the patients failed to produce any pigmentation.

Table 1: Region wise response to melanocyte-keratinocyte cell transplantation.

Region	Response to Treatment*				Total	Male	Female
	Excellent	Good	Fair	Poor			
Face	8	6	3	5	22	10	12
Chest	4	1	1	2	8	6	2
Forearm	8	5	2	5	20	8	12
Hand	5	2	1	4	12	7	5
Thigh	5		1	2	8	2	6
Leg	8	2	3	5	18	7	11
Eyelids	6	1	1	2	10	4	6
Toe	-	1	-	1	2	2	-
Total	44	18	12	26	100	46	54

*Data are given as number of lesions.

Table 2: Retention of pigmentation on follow up for 5 years in patients undergone melanocyte-keratinocyte cell transplantation.*

Year	Excellent	Good	Fair	Poor	Total
1	46	20	6	28	100
2	45	19	9	27	100
3	44	18	11	27	100
4	44	18	12	26	100
5	44	18	12	26	100

*Data are given as number of lesions.

The presence of some colour mismatch at the recipient area (hyper and hypo-pigmentation) was observed in 70% and perilesional hypo pigmentation was observed in 50% of the evaluated patients. But this colour mismatch was not disturbing according to the majority of patients.



Figure 1: Focal vitiligo. a) A 36-year-old man with focal vitiligo on the leg of 10 years duration; b) Same patient 2 years after transplantation.

DISCUSSION

Resistant cases of focal vitiligo may remain as such for many years. Surgical intervention is the only therapeutic

option available for such cases. Non cultured melanocyte-keratinocyte (MK) cell suspension transplantation is a widely used procedure in such cases. Response to MK Cell suspension transplantation varied in different studies. Gauthier and Surleve-Bazeille reported zero repigmentation in 4 of 7 patients with focal vitiligo.¹¹ Falabella observed 2 of 4 patients with focal vitiligo improved with complete repigmentation.¹² Guerra et al reported that of 6 patients with focal vitiligo, 3 had complete and 2 had partial repigmentation.¹³ In the study by Mulekar it is observed that repigmentation failed to be produced in 20% of focal vitiligo group.² In this study 10% of the patients failed to produce any pigmentation.

This is a very simple method and can be done on any anatomical location even though the result varies in different areas. This can be carried out as an outpatient treatment. Depending upon the number of patches, anatomical location and total area under treatment, the time required to complete the procedure is about 2 to 3 hours.¹ Absolute immobilization is not required and rest for one week is usually advised. In case, transplantation fails to produce repigmentation, the procedure can be repeated at same place without scarring. In this study an area of around 350 cm² was transplanted in a single

session. Cosmetic acceptability is very superior as there is no cobble-stoning or scarring compared to other vitiligo surgeries. The greatest advantage is the tenfold increase of recipient areas over donor area.¹⁰

The use of a culture medium while preparing cell suspension, leaving them under occlusive dressing together with release of growth promoting factors during healing may be responsible for multiplication of melanocytes after being applied to recipient area¹. This method does not cure the disease but serves to replenish the missing melanocytes. In the long run, this method to be proved as a cosmetically most acceptable and cost effective therapy.

CONCLUSION

This is a simple, safe, and effective surgical therapy for replenishing the missing melanocytes in resistant cases of vitiligo. Repigmentation lasts long with very good cosmetic acceptability and only very small donor area is needed for transplantation of larger areas. Considering these advantages this technique may replace the present methods of treatment of stable vitiligo but large scale blinded trials are needed for establishing statistically significant results.

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Ethical approval: The study was approved by the institutional ethics committee

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