Objective: To evaluate long-term efficacy and safety of melanocyte-keratinocyte cell transplantation in the management of segmental and focal vitiligo.

Design: A simpler and modified method based on that of Olsson and Juhlin was performed. This method uses a shaved biopsy skin sample up to one tenth the size of the recipient area. The skin sample is incubated, and the cells are mechanically separated using trypsin-EDTA solution and then centrifuged to prepare a suspension. Cell suspension is then applied to the dermabraded depigmented skin area, and a collagen dressing is applied to keep it in place.

Patients: Fifty patients with segmental and 17 with focal vitiligo were treated. One patient with segmental and 2 with focal vitiligo did not attend any follow-up visits. The remaining patients were observed for a period of up to 5 years.

Intervention: Autologous, noncultured melanocyte-keratinocyte cell transplantation.

Main Outcome Measure: Repigmentation was graded as excellent with 95% to 100% pigmentation, good with 65% to 94%, fair with 25% to 64%, and poor with 0% to 24% of the treated area.

Results: In the segmental vitiligo group, 41 patients (84%) showed excellent, 3 (6%) good, and 5 (10%) poor pigmentation, which was retained until the end of the respective follow-up period. In the focal vitiligo group, 11 patients (73%) showed excellent, 1 (7%) fair, and 3 (20%) poor pigmentation, which was retained until the end of the respective follow-up period.

Conclusions: Melanocyte-keratinocyte cell transplantation is a simple, safe, and effective surgical therapy. Patients with segmental and focal vitiligo can experience a prolonged disease-free period, which may extend through the rest of their lives.

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In patients with vitiligo, 2 major clinical types are recognized: (1) unilateral vitiligo, which generally becomes nonprogressive 1 to 2 years after an initial rapid spread, and (2) bilateral vitiligo, in which progressive depigmentation occurs for several years. Localized vitiligo is further divided into 2 types: (1) segmental vitiligo, which is characterized by unilateral macules in quasidermatomal distribution, and (2) focal vitiligo, which is described as depigmented macules in a localized, non-dermatomal distribution. Segmental vitiligo is considered a special type of vitiligo because of its earlier onset, recalcitrant course, and decreased association with autoimmune disease. Focal vitiligo could be considered an initial stage of generalized or immunologic vitiligo or may be an abortive form of the segmental type.

The therapeutic response of dermatomal or segmental vitiligo to oral and/or topical psoralen is poor, and in a trial of 41 cases, there was improvement in only 1 case of vitiligo of 3 months' duration and only 1 case showed a partial response. None of the 5 patients with dermatomal vitiligo responded to topical corticosteroid treatment. In contrast, various investigators have reported excellent repigmentation for patients with localized vitiligo when treated by autologous, cultured, or noncultured melanocyte-keratinocyte cell (MKC) transplantation. Thus, surgical therapies are more effective in producing complete repigmentation in a high percentage of patients. However, several reports advocate the use of surgical procedures as an alternate for treating vitiligo owing to the small number of cases and short follow-up period reported in these studies. The purpose of the
The present article is to report the methods and results obtained in treating patients with localized vitiligo with autologous, noncultured MKC transplantation over a 5-year follow-up period.

**METHODS**

**PATIENTS**

In the present study, 50 patients (17 male and 33 female) with segmental vitiligo and 17 patients (8 male and 9 female) with focal vitiligo were treated and observed between July 1996 and June 2002. I have previously reported the treatment results of some of these patients in the first year of transplantation with autologous, noncultured MKC suspension. In this article we describe long-term observations in these patients with an addition of a few more patients. The duration of vitiligo varied from 2 to 15 years. Patients with stable vitiligo for at least 1 year were selected for the said surgical treatment. Patients younger than 12 years and those receiving concomitant medical treatment were omitted from the study.

One patient with segmental and 2 with focal vitiligo did not return for the follow-up after surgical treatment. The remaining patients were observed up to 5 years after treatment. Results were analyzed in 49 patients with segmental and 15 with focal vitiligo.

Of the patients with segmental vitiligo, to achieve these results, 2 underwent retransplantation at 4 months, 1 at 6 months, 2 at 8 months, 8 at 1 year, and 2 at 2 years after the first transplantation. Two patients with focal vitiligo underwent retransplantation at 1 year after the first transplantation to achieve these results.

Repigmentation was graded as excellent with 95% to 100% pigmentation, good with 65% to 94%, fair with 25% to 64%, and poor with 0% to 24% of the treated area.

We followed a modified method, based on the technique by Olsson and Juhlin. In our method, an ordinary incubator pigmentation, good with 65% to 94%, fair with 25% to 64%, was then anesthetized with 1% lidocaine. The skin was stretched, and a superficial sample 200 μm thick (as estimated with a biopsy sample by a pathologist) was taken with a Silver skin grafting knife (E. Murray & Company, Cork, United Kingdom) or skin grafting knife (Parulekar's Surgicals, Mumbai, India). The superficial wound was then covered with sterile petroleum jelly gauze.

The thin skin sample was transferred to a Petri dish containing about 4 mL of 0.2% (weight/volume) trypsin solution. Care was taken to ensure that the sample was properly soaked in the solution. Finally, it was placed with the epidermis upward. The sample in the Petri dish was incubated for 50 minutes at 37°C. After incubation, about 2 mL of trypsin inhibitor (Life Technologies, Grand Island, NY) was added to the Petri dish to neutralize the action of the trypsin. The dermis was separated from the epidermis, transferred to a test tube containing about 3 mL of DMEM/F12, and vortex mixed for 15 seconds.

The epidermis in the Petri dish was broken down into multiple small pieces. It was then washed with DMEM/F12 and transferred to a test tube containing the same medium. Next it was vortex mixed for 15 seconds. The test tube containing epidermal pieces was then centrifuged for 6 minutes. The epidermal pieces were discarded, and the cell pellet was suspended in DMEM/F12 in a 1-mL syringe with detachable needle. The quantity of the suspension prepared was approximately 0.5 mL. One drop of the cell suspension seen under inverted microscope (original magnification ×100) had about 3 to 4 melanocytes.

**TRANSPLANTATION PROCEDURE**

Recipient site was circumscribed with marker, then cleaned with povidone iodine and anesthetized with 1% lidocaine. The recipient area was abraded down to the dermoepidermal junction with a high-speed dermabrader fitted with a diamond fraise wheel. The ideal level was achieved when pinpoint bleeding spots appeared. 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 collagen (Collcor CX, Lund, Sweden, and Collomedica Laboratories, Bangalore, India). 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 tegaderm (3M Health Care, St Paul, Minn) transparent dressing. The patient was allowed to go home immediately after dressing. The patient was cautioned against any vigorous activities, which could displace the dressing. Dressing was removed after 1 week. Absolute immobilization was, however, not necessary.

**RESULTS**

**SEGMENTAL VITILIGO**

In the present study, 49 patients (16 male and 33 female) with segmental vitiligo were followed up to 5 years (Table 1). The maximum area treated in 1 operative session was 120 cm² and the minimum area, 2 cm². The total area transplanted in 49 patients was 1445 cm², with an average of 29.5 cm².

Hypopigmented borders were noticed in 4 patients. The transplanted area was hypopigmented compared with the normal skin in 6 patients and hyperpigmented in 1 patient.

The percentage of patients with an excellent response was 84%, while 6% of the patients had a good response to treatment; 10% of patients failed to produce

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**Table 1. Retention of Repigmentation in 49 Patients With Segmental Vitiligo Treated by Melanocyte-Keratinocyte Cell Suspension Transplantation**

<table>
<thead>
<tr>
<th>Follow-up Year</th>
<th>Excellent</th>
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<tr>
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<td>9</td>
<td>0</td>
<td>0</td>
<td>4</td>
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</table>

*Data are given as number of patients.*
any pigmentation. Most patients seen for the treatment had lesions on the face followed by the lesions on the trunk. Response to the treatment by region was analyzed (Table 2).

FOCAL VITILIGO

In our study, 15 patients (6 male and 9 female) with focal vitiligo were followed up to 5 years (Table 3). The maximum area treated in 1 operative session was 6 cm², and the minimum area was 1 cm². The total area transplanted in 15 patients was 48 cm², with an average of 3.2 cm². The transplanted area was hypopigmented compared with normal skin in 2 patients.

Of the patients, 73% showed an excellent response, while 20% had poor repigmentation at the end of the respective follow-up period. Response to the treatment on the lips was not encouraging. This could be partially explained by the difficulty in dermabrading lips manually (Table 4).

COMMENT

Patients with segmental vitiligo respond poorly to medical treatment. The condition of most of these patients becomes nonprogressive after an initial rapid spread. Hence, surgical therapies are important in their management. There are several articles reporting a high percentage of patients with complete repigmentation, which is retained during the respective follow-up period with various surgical techniques.1,7-9,11 However, there is no clarity about the choice of surgical technique to treat segmental and focal vitiligo. The parameters of evaluation to choose among various treatments may be simplicity, cosmetic results, area treated in a single operative session, and adverse events. Established surgical techniques include minipunch grafting, blister roof grafting, and split-thickness grafting.

Minipunch grafting, performed with grafts of 2- to 2.5- or even 3-mm diameter, has the distinct advantage of incorporating more melanocytes, thereby extending the pigment spread even up to 10 mm.12 It may lead to cobblestoning at recipient area and superficial scarring at donor site. Falabella1 has used 1.2-mm minigrafts, thus minimizing the chances of a cobblestone appearance, but the pigment spread is restricted. Westerhof and Boersma13 reported that pigment cells apparently do not migrate beyond 5 mm. Blister roof graft produces excellent cosmetic results but is time consuming, needs to be handled delicately, and only small areas can be treated at one time. Split-thickness skin grafts harvested with a dermatome eliminate the chances of scarring but require skill. Immobilization, donor-recipient ratio of 1:1, and use of general anesthesia remain the limiting factors. Procedures based on cultured melanocytes/MKCs produce excellent cosmetic results and treat large areas in 1 operative session. It is simpler compared with procedures based on cultured techniques.

We noticed the appearance of new vitiligo lesions in the same dermatome in 2 patients after they were successfully treated. One patient developed new lesions 6 months after the transplantation. These lesions were treated 6 months after they stabilized clinically. A second patient showed a hypopigmented ring at the margin of the treated and repigmented vitiligo lesion. She developed a new vitiligo lesion adjacent to the margin of the initial lesion when the hypopigmented ring was treated with cell transplantation 6 months after the first trans-
plantation. The new lesion was treated 1 year later, which repigmented completely. Falabella also has reported the appearance of new depigmented lesions in 1 case of segmental vitiligo. Thus, the appearance of new lesions cannot be totally ruled out in segmental vitiligo, though it may be a rare event.

Pigmentation failed to be produced in 5 patients with segmental and 3 with focal vitiligo, despite stability of 1 or more years. Unfortunately, these patients did not offer themselves for another treatment. We have seen earlier that some patients required retransplantation to improve the percentage of pigmentation. Other investigators have also reported failures in patients with segmental vitiligo. Because the exact cause of segmental and focal vitiligo is not known and its clinical behavior is different from that of vitiligo vulgaris, it is difficult to explain failures in clinically stable patients. There are no ultrastructural studies before and after treatment to ascertain the events that may throw some light on the causes of failure.

We have observed that patients with segmental vitiligo have retained the repigmentation achieved at the end of the respective follow-up period (Figure 1). Other investigators have also reported retention of pigment in patients with segmental vitiligo at the end of respective follow-up periods. Thus, it appears that patients with segmental vitiligo can experience a long disease-free period, which can extend through the rest of their lives. If new lesions develop, they can be successfully treated.

Patients with focal vitiligo also have retained the pigment at the end of respective follow-up periods (Figure 2). Gauthier and Surleve-Bazeille reported 0% repigmentation in 4 of 7 patients with focal vitiligo, but all 3 patients with segmental vitiligo improved with complete or near complete repigmentation. Falabella observed that 12 of 13 patients with segmental vitiligo improved with complete or near complete repigmentation, and 2 of 4 patients with focal vitiligo improved with complete repigmentation. Guerra et al reported that of 6 patients with focal vitiligo, 3 had complete and 2 had partial repigmentation, and all 4 patients with segmental vitiligo had near complete repigmentation. In the present study, I have observed that repigmentation failed to be produced in 10% of patients in the segmental vitiligo group and 20% in the focal vitiligo group. Thus, patients with focal vitiligo have a smaller success rate compared with patient with segmental vitiligo when treated by surgical techniques. Further research is required to

Figure 1. Segmental vitiligo. A, A 19-year-old man with segmental vitiligo on the face of 8 years’ duration; B, same patient 5 years after transplantation.

Figure 2. Focal vitiligo. A, A 26-year-old man with focal vitiligo on the scalp of 10 years’ duration; B, same patient 2 years after transplantation.
establish whether focal vitiligo is a partial expression of segmental vitiligo or a precursor of bilateral vitiligo.

In conclusion, MKC transplantation is a simple, safe, and effective surgical therapy. Patients with segmental and focal vitiligo can experience a long disease-free period, which can extend through the rest of their lives.

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