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The effect of simplified autologous melanocyte and keratinocyte grafting on the treatment of refractory vitiligo

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Abstract

Introduction: Vitiligo is a disease with a high prevalence and burden that has a negative impact on various aspects of life. Because most cases of vitiligo are not responsive to medical treatment, we performed simplified autologous melanocyte and keratinocyte grafting to treat refractory stable vitiligo.
Methods: This interventional study was carried out on 32 patients (16 females and 16 males). After local anesthesia at the donor site, we shaved the donor sites to harvest autologous melanocyte material. The harvested paste-like non-trypsinized material was spread over the abraded recipient area. Patient follow-up was performed to track the treatment outcome and possible complications.
Results: Out of 32 patients with 99 lesions, the generalized type accounted for 28 (87.5%) patients, and 46 (46.5%) lesions were seen in the upper extremity as the most common clinical type and site of involvement. Eighteen (18.2%) and 26 (26.3%) patches showed excellent and good re-pigmentation, respectively, and face areas showed significantly better re-pigmentation ($p < 0.001$).
Conclusions: Simplified autologous melanocyte and keratinocyte grafting is a safe, simple, office-based, and low-cost procedure with a modest re-pigmentation outcome in refractory stable vitiligo. Moreover, the face area had a satisfactory outcome with this procedure.

Keywords: refractory vitiligo, autologous melanocyte, grafting, re-pigmentation

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Introduction

Vitiligo is the most common pigmentary disorder and is a common disorder in dermatology, with a 1% prevalence in the general population. This disease, in addition to somatic disorders, has many consequences for various aspects of patients' lives, including psychological, educational, familial, occupational, and sexual aspects (1–3).

Several optional treatments have been suggested for vitiligo, but drug treatment and phototherapy are not effective in refractory vitiligo (RV), especially vitiligo in glabrous areas or in non-glabrous areas with leukotrichia (4–7).

In recent years, simplified autologous (non-trypsinized) and processed (trypsinized) melanocyte grafting as well as homologous melanocyte culture have been suggested for the treatment of stable refractory vitiligo (SRV) (7–22). Simplified autologous melanocyte and keratinocyte grafting (SAMKG) is an uncomplicated, safe, and office-based procedure with variable outcomes in the treatment of SRV (8–12).

Because vitiligo is a disease with a high prevalence and burden and is usually resistant to drug treatment, we performed SAMKG for treatment of SRV.

Materials and methods

Study design and population

This clinical, interventional, cross-sectional study was carried out on 35 patients over a period of 2 years from 2018 to 2020 at the Hajdaie Dermatology Referral Clinic of Kermanshah University of Medical Sciences, Iran. Out of 35 patients, three were excluded from the study because of failure to refer and follow up.

In our study, patients were selected from among those that were referred to our clinic or those that were available. After clinical documentation of vitiligo (Fig. 1), patients with SRV criteria were selected as candidates for SAMKG. When there was clinical doubt of vitiligo, a Wood's lamp was used for diagnosis confirmation. Patients with at least three patches, the absence of a new lesion or enlargement of previous lesions induced in at least the previous 6 months, vitiligo in glabrous areas or in non-glabrous areas with leukotrichia, and unresponsiveness to medical treatment or phototherapy over the past 2 years were included in the study.

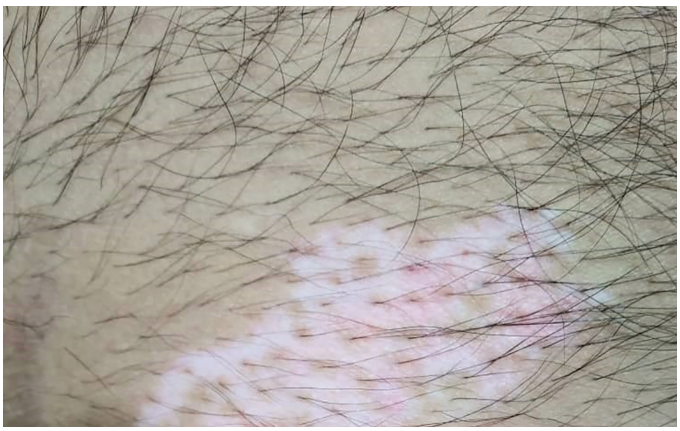

Figure 1 | Patient with a vitiliginous patch on the abdomen.

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The exclusion criteria included patients younger than 13 or older than 60 and a recent history of Koebner phenomena, breast feeding or pregnancy, a history of keloid or hypertrophic scar, instability, very disseminated and universal vitiligo, and active infection at the recipient or donor sites. All participants were informed about this procedure and were asked for their consent, and they were then enrolled in the study.

Demographic data and clinical characteristics of SRV, treatment outcome, and complications were recorded in the questionnaires used in this study.

Procedural technique

The lateral thigh or buttock areas with normal pigmentation were selected as the donor sites. The area of the recipient site selected was four times greater than the donor site.

After anesthetizing the donor site by injection of 2% lidocaine, we used a sharp disposable curette to shave the skin for harvesting the autologous material. We performed a two-step shaving process: first, very light shaving removed the cornified layer, which was discarded, and then shaving was continued until pinpoint bleeding was observed. The harvested material in the second step, which was a paste-like material and contained melanocytes and keratinocytes, was collected with a spatula, transferred to a sterile petri dish, and mixed with a hyaluronic acid gel.

The recipient site was shaved the same as the donor site until pinpoint bleeding was observed, and the harvested materials were discarded. The harvested paste-like mixture from the donor site was spread over the recipient area as a thin layer.

Both the donor and recipient sites were dressed with a semipermeable hydrocolloid sheet. The patients were advised to immobilize the recipient sites for at least 1 week. After 10 days, the dressing was changed at the donor and recipient sites.

The harvested samples were evaluated for the presence of melanocytes, which were prepared as a direct smear and evaluated under a light microscope. The patients were evaluated in the 1st week, 3rd week, and 2nd month, and then every 2 months for 18 months.

Classification of treatment outcome

We classified the treatment outcome based on the comparison of pre- and post-treatment photographs of the treated sites. Assessment was done by two dermatologists that were blinded in this study. Re-pigmentation was graded as excellent (more than 75% pigmentation of the treated regions), good (50%–74%), moderate (25%–49%), and poor (less than 25%).

Ethical considerations

Our study was approved by the Ethics Committee of Kermanshah University of Medical Science and registered in the Iranian registration clinical trial (IRCT) database (IRCT201702016403N7). Participants' personal information was kept confidential.

Statistical analysis

The data were analyzed using SPSS 16.0 software. Frequency tables (frequency and percentage) were used to summarize the quantitative data. Then crosstab, two-dimensional bar charts, and a Pearson chi-square test were used to determine the relationship between qualitative variables. $P < 0.05$ was considered statistically significant.

Results

This study included 99 vitiligo patches in 32 patients, among whom four (12.5%) had segmental vitiligo and 28 (87.5%) had generalized vitiligo, with a 34.3% positive family history of vitiligo. The demographic data and treated sites are presented in Tables 1 and 2. For better statistical analysis and more accurate assessment of the correlation between variables, we classified the treated areas into trunk, face, and upper and lower extremities; this is shown in Table 1.

Table 1 | Demographic and clinical characteristics of our patients.

Variable		Value
Patients, *n*		32
Vitiliginous patches, *n*		99
Mean age of patients (years)		28.03 ± 5.83
Sex, *n* (%):	Female	16 (50.0)
	Male	16 (50.0)
Family history, *n* (%):	Positive	34 (34.3)
	Negative	65 (65.7)
Mean duration of disease (months)		11.2 ± 5.6
Treatment site, *n* (%):	Upper extremity	46 (46.5%)
	Face	26 (26.3%)
	Trunk	18 (18.1%)
	Lower extremity	9 (9.1%)

Overall, 24 (24.2%), 31 (31.3%), 26 (26.3%), and 18 (18.2%) vitiliginous lesions showed poor, moderate, good, and excellent re-pigmentation outcomes at the treated sites, respectively (Fig. 2).

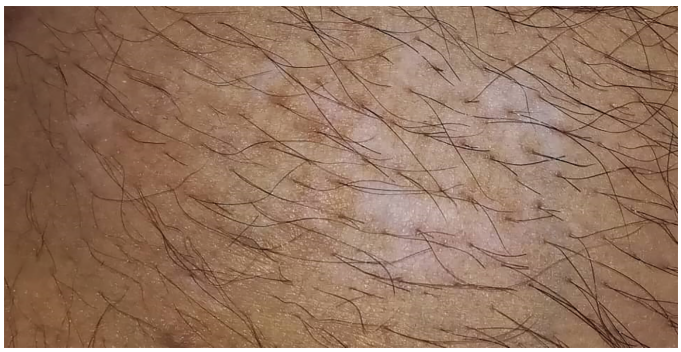

Figure 2 | Excellent treatment outcome after simplified autologous melanocyte and keratinocyte grafting.

In the face areas, three (11.5%), three (11.5%), six (23.1%), and 14 (53.8%) samples showed poor, moderate, good, and excellent re-pigmentation outcomes at the treated sites, respectively (Table 2). The re-pigmentation outcome in other areas is shown in Table 2. There was a statistically significant difference in re-pigmentation among the treatment sites, and face areas showed a significantly better treatment response ($p < 0.001$; Table 2).

With respect to complications, erythema, post-inflammatory hyperpigmentation, folliculitis, and pruritus were seen in 42 (42.4%), 20 (20.2%), 11 (11.2%), and eight (8.1%) cases of vitiliginous treated lesions, respectively. However, all of the complications were transient and improved over a few weeks. We also did not find any complications at the donor sites.

Table 2 | **Treatment sites and outcomes of re-pigmentation in our patients.**

			Outcome of re-pigmentation, *n* (%)				
Treatment site		**Frequency**	**Poor**	**Moderate**	**Good**	**Excellent**	***p* value**
Upper extremity:	Fingers	18 (39.1)	7 (38.9)	8 (44.4)	3 (16.7)	0 (0.0)	
	Dorsum of hand	13 (28.3)	3 (23.1)	6 (46.2)	2 (15.4)	2 (15.4)	
	Forearm	13 (28.3)	4 (30.8)	6 (46.1)	3 (23.1)	0 (0.0)	
	Arm	2 (2.0)	2 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Total	46 (100.0)	16 (34.8)	20 (43.5)	8 (17.4)	2 (4.3)	
Face:	Cheek	10 (38.5)	0 (0.0)	0 (0.0)	2 (20.0)	8 (80.0)	
	Lower lid	6 (23.1)	0 (0.0)	1 (16.7)	1 (16.7)	4 (66.7)	
	Chin	4 (15.4)	2 (50.0)	0 (0.0)	1 (25.0)	1 (25.0)	
	Upper lip	2 (7.7)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0.0)	
	Upper lid	2 (7.7)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	
	Forehead	2 (7.7)	0 (0.0)	0 (0.0)	1 (50.0)	1 (50.0)	
	Total	26 (100.0)	3 (11.5)	3 (11.5	6 (23.1)	14 (53.8)	
Trunk:	Abdomen	9 (50.0)	0 (0.0)	1 (11.1)	6 (66.7)	2 (22.2)	
	Flank	6 (33.3)	1 (16.7)	2 (33.3)	3 (50.0)	0 (0.0)	0.001
	Chest	3 (16.7)	0 (0.0)	2 (66.7)	1 (33.3)	0 (0.0)	
	Total	18 (100.0)	1 (5.6)	5 (27.8)	10 (55.5)	2 (11.1)	
Lower extremity	Dorsum of foot	5 (55.5)	3 (60.0)	1 (20.0)	1 (20.0)	0 (0.0)	
	Shin	4 (44.4)	1 (25.0)	2 (50.0)	1 (25.0)	0 (0.0)	
	Total	9 (100.0)	4 (44.4)	3 (33.3)	2 (22.2)	0 (0.0)	
All areas treated		99 (100.0)	24 (24.2)	31 (31.3)	26 (26.3)	18 (18.2)	

Discussion

Our results showed that SAMKG is generally a simple, office-based, and safe procedure with an acceptable outcome; namely, good and excellent re-pigmentation in vitiligo patches was achieved in 44.4%. However, this procedure was associated with better re-pigmentation in vitiliginous lesions on the face.

In another study, 12 patients were subjected to SAMKG and postoperative psoralen plus ultraviolet light A (PUVA). The results indicated that small vitiliginous patches were associated with cosmetically satisfying outcomes (8). In a study from India, 437 vitiligo patches were treated in a manner similar to our method, which showed significant improvement, especially on the thigh and face, after a 6-month follow-up (9). We believe that rich blood supply and more exposure to ultraviolet from sunlight or post-procedure PUVA therapy can be helpful for a good response on the face or other areas. Machado-Filho et al. (11) used autologous non-trypsinized melanocyte-keratinocyte paste harvested from the donor's normal skin site with a curette. In that study, intense hyper-pigmentation was seen in 40% of patients after 6 months. Forty patients with stable vitiligo were treated with a non-cultured, non-trypsinized epidermal cell grafting method, which was followed by narrowband UVB therapy. At the end of the treatment course, homogeneous re-pigmentation was seen in 65% of patients (10). Lamoria et al. compared the Jodhpur technique or SAMKG and follicular unit transplantation in 30 vitiligo patients. Both methods showed nearly 70% excellent re-pigmentation with minimal side effects (11). However, in many studies trypsinized (7, 13, 15–18) and non-trypsinized (7–11) melanocyte and keratinocyte suspension have also shown a high rate of successful treatment outcome. In contrast, Huggins et al. (19) and Ramos et al. (23) reported treatment results similar to those of the current study.

We believe that patient selection is the first step for successful treatment with an autologous melanocyte graft (14, 16, 23). A better response is achieved in long-term stable vitiligo (25) with a limited age range (i.e., older than teenager and younger than middle age) (9, 16), small vitiliginous patches (8), and limited or segmental types (9, 10, 16, 23). In our study, consistent with most previous studies (9, 10, 15–17, 23), re-pigmentation was significantly better in the face area than in other areas of the body.

However, Sahni et al. (20) reported that the treatment outcome of trypsinized melanocyte and keratinocyte suspension was not affected by the location or size of lesions, type of vitiligo, and duration of disease. Another important step in successful treatment with an autologous melanocyte graft is the correct procedural technique; that is, precise preparation of the recipient site and proper harvesting of the autologous grafting material (9, 13, 16, 17).

In one study, the recipient site was prepared through blister induction with liquid nitrogen (13). We believe that this not only serves as a biologic and clean dressing but also, because of the presence of some growth factors in this situation, promotes melanocyte proliferation and successful grafting. However, the limitation of this procedure is that it is time-consuming, painful, and experience-based.

Proper harvesting of the autologous material must contain melanocytes, especially viable and stable ones (8, 9, 13, 16, 17). Some studies used a directly prepared smear (13), anti-HMB45 staining (24), trypan blue solution, and flow cytometry to assess the existence or viability of melanocytes in the grafting suspension (18).

Some studies have compared follicular and epidermal melanocyte autologous suspension for the treatment of RV. Nevertheless, the epidermal method had a better outcome than follicular melanocyte autologous suspension (12, 21, 22, 24). We abraded both the recipient and donor sites with a sharp disposable curette. We also harvested epidermal melanocytes and melanocyte autologous suspension from the lateral part of the thigh or buttock.

The final step for the satisfactory outcome of an autologous melanocyte graft is appropriate postoperative care. Some studies have emphasized that the fixation of autologous grafting material and immobilization of the recipient site such as the acral areas, surface of the joints, and upper lids are very important for successful treatment (9, 16, 17, 23). Moreover, many studies have suggested postoperative phototherapy (i.e., PUVA or UVB therapy) for better re-pigmentation (8–10, 13). We did not use postoperative phototherapy because we wanted to obtain a pure SAMKG outcome and to rule out confounding factors. Most studies (7–9, 12, 16, 21), consistent with the current study, have reported rare and usually transient side effects; therefore, SAMKG is a safe optional treatment.

Conclusions

SAMKG is a safe, simple, office-based, and low-cost procedure with a modest re-pigmentation outcome in RSV. However, face areas showed significantly better re-pigmentation than other areas of the body. Proper selection of patients, an accurately performed procedure, and appropriate post-procedure follow-up are very important in order to achieve a satisfactory outcome.

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