Non-cultured autologous melanocytes of hair follicle outer root sheath and bulge area transplantation for repigmentation of the stable generalized vitiligo patches: a pilot study

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Background: Different modalities have been tried in order to treat stable vitiligo. Culturing melanocytes is time consuming and expensive. Therefore, new methods using autologous melanocytes are sought. We aimed to compare the mixed non-cultured autologous melanocytes of the outer root sheath and the bulge area of hair follicle transplantation plus dermabrasion with dermabrasion alone in stable generalized vitiligo patches repigmentation.

Method: Eight patients with stable generalized vitiligo were recruited. Two patches were selected and assigned to one of the study groups: one was treated with dermabrasion alone, and the other was treated with dermabrasion and transplantation. Uncultured melanocytes were extracted from the patients' hair follicle outer root sheath and bulge. The patches were assessed monthly for the next six months. The primary outcome was to determine the percentage changes of the depigmented patches from the baseline.

Result: Although the repigmentation changes were statistically significant in transplantation patches, there was only one patch with good and another with fair pigmentation. Furthermore, seven patches without and one patch with poor pigmentation were observed as control lesions. The transplanted patches demonstrated a significantly better repigmentation rate in contrast to their controls (p=0.03).

Conclusion: Although this method is fast and economic, the clinical response was not satisfactory.

Keywords: bulge, melanocyte, outer root sheath, transplantation, repigmentation, vitiligo

INTRODUCTION

Vitiligo is the pigmentary disorder of the skin because of melanin loss with an undetermined etiology that affects 1-3% of the population; male and female subjects are affected equally [1]. Although many theories have been proposed to explain the disease mechanism, the evidence mostly favors an autoimmune mechanism. Vitiligo is not only a dermatologic disease but also a cause of psychological problems. Many patients suffer from lack of self-esteem and depression because of its disfiguring and cosmetic effects that influence their quality of life [2].

Different modalities including topical depigmentation of the unaffected area, laser therapy by excimer laser, narrow-band ultraviolet (NBUVB) therapy, dermabrasion, and skin grafting have been

used to provide repigmentation. During recent years, surgical methods, such as transplantation of non-cultured basal cell layer–enriched epidermal cell suspension, melanocyte-keratinocyte suspension, and cultured melanocytes have been examined for repigmentation of stable vitiligo [3,4].

Hair follicle bulge and outer root sheath (ORS) are reservoirs of melanocytes and melanocyte precursor cells. Although the results of using cultured melanocytes are promising, the sophisticated and expensive modalities of the culture limit its practical implementation. A recent study utilized non-cultured melanocytes in stable generalized vitiligo lesions and reported promising results [5].

In the current study, we aimed to assess the repigmentation rate of vitiligo patches by transplantation of the mixed non-cultured autologous mature and immature (DOPA positive and DOPA negative) melanocytes of ORS and the bulge area of hair follicles plus dermabrasion in contrast to dermabrasion alone.

PATIENTS AND METHODS

Trial design

This interventional controlled study was conducted in Shohada-e-Tajrish and Loghman-e-Hakim University Hospitals from March 2011 through June 2012. The study protocol was designed in accordance with the Helsinki Declaration and was implemented after the approval of the Board Committee of Ethics in Medical Research of Shahid Beheshti University of Medical Sciences.

Participants

We decided to recruit patients with stable generalized vitiligo patches who were between 20 and 55 years old. The eligible patients were those who had the disease for at least four years and their lesions did not change over the last six months. Moreover, the lesions had to be bilateral and resistant to the previous therapeutic measures. Patients with the following conditions were excluded from the study: receiving treatment at the time of screening, history of recurrence after a satisfactory response to NBUVB therapy, existence of the lesions at sun exposed areas, history of keloid formation, pregnancy or lactation, and having any systemic disease.

Interventions

A small area of the occipital skin was determined and anesthetized by injecting 2 ml of 2% lidocaine. To achieve better access for harvesting and providing a good turgor, a tumescent solution prepared by mixing 1000 ml normal saline and 1 cc 0.001% epinephrine was injected into the area. We excised a 3×1 cm ellipse from the prepared area of the scalp and immediately transferred it into an isotonic normal saline solution. The skin was cut into slivers of 1-cm length and 2-mm width by a #10 surgical blade under binocular stereotypic microscope (Mantis, Vision Engineering Inc., USA). Then, it was cut into one unit of hair follicles. The pigmented hair follicles were selected, and the epidermis was removed. Considering the location of the bulge area around the sebaceous gland, the bulge area was determined and separated. Then, the two-third inferior of the hair shaft, which is the ORS, was extracted under a microscope. The fat and hair shaft were separated by a #10 surgical blade. Then, the bulge and ORS were placed in a cold isotonic normal saline solution to keep them wet and cold. The materials yielded from each hair follicle was mixed and considered for transplantation. We used these mixtures for the recipient site in the predetermined areas.

We selected two vitiligo patches located in the same anatomical area of each patient. The patches were chosen from approximately the same size in an area that was not usually exposed to sunlight such as the trunk, thighs, and legs. One patch was considered as the treatment and the other as the control patch. The patches were anesthetized by 2% lidocaine and treated by dermabrasion. Dermabrasion was discontinued once pinpoint bleeding appeared.

Dermabrasion was performed to approximate the transplanted materials to the papillary dermis. The small size of transplant materials made needle a more practical instrument than surgical forceps for transplantation. A 21G needle was used to transfer mixed materials to the recipient site in the treatment patch. According to the patch size, 20 to 30 units of bulge and ORS were transplanted. The exudate during dermabrasion improvement could displace the transplanted materials; therefore, the needle was used to transplant the materials to the favorable site.

On the control patch, a 21G needle was used to make 20 to 30 tunnels in the patch. The dermabrasion and traumatization of the control patch with the needle were done to neutralize the effect of trauma on repigmentation.

The method of tunnel making was the same as making tunnels for hair transplantation, but it was devoid of transplanted materials. The aim of making tunnels in the control patches was to neutralize the effect of trauma on repigmentation and assessing the effect of transferred material.

The process of strip harvest through the completion of transplantation took 30 to 40 minutes for each patient. Each patch was covered by a sterile plastic coverage for two days, and the patients were instructed to avoid washing the intervention site at this time. The dressing was changed on day 2, and sterile gauze was used to cover the area and changed every day by the patients for seven days. Oral cephalexin was prescribed for patients for seven days. The patients were visited 10 days after that to remove the sutures of the scalp. During the next 6-month follow-up period, no other systemic or topical therapy was used for vitiligo treatment.

Outcomes

The primary outcome measure of the study was to determine the percentage changes of the depigmented patch from the baseline. The changes in the pigmentation percentage illustrated the effect of treatment. During the next six months, the lesions were photographed with one-month intervals and examined for possible response to the therapy by a dermatology resident. A Canon digital camera (IXUS 12015, Canon Inc, Tokyo, Japan) was used to take photos of the patches; a ruler was placed near the patches during photography to provide a scale for calculation of the depigmented area (in cm^2). A civil engineer used AutoCAD 2010 (Autodesk Inc. San Raphael, CA, USA) software to calculate the area of the depigmented patches to assess the improvement in lesion pigmentation [6].

Repigmentation was graded as excellent if repigmentation of the treated area was 95% to 100%, good if the repigmentation was 65% to 94%, fair if the repigmentation was 25% to 64%, and poor if it was 1% to 24%.

Another outcome measure of the study was to contrast the pigmentation between patches and their control. Moreover, we assessed the control patches to see whether the dermabrasion and traumatization had any effects on pigmentation. The side effects including keloid formation and infection were registered at each assessment session.

Statistical methods

In this study, percentage changes of the depigmented areas of the patches were calculated as follows:

(Depigmented area of the patch at end point – depigmented area of the patch at baseline) / depigmented area of the patch at baseline × 100.

The Wilcoxon signed rank test was applied to compare the depigmented areas of the patches at baseline and at the end of the study. All tests were two sided, and the level of significance was considered 0.05. All statistical analyses were performed with the statistical software SPSS 16.0.0. (SPSS Inc. Chicago, IL, USA).

RESULTS

Ten patients were assessed, and eight patients (6 women and 2 men) were included in the study. The

Table 1. The percentage change of the depigmented areas (from baseline) in the patches treated with and without transplantation

Patients	Age	Sex	Duration of disease (years)	Stability (year)	area	percentage change of patches *	
						Transplanted	Control
1	30	F	27	27	trunk	-2.46	0
2	30	M	8	8	abdoman	-65.19	0
3	47	M	24	5	trunk	0	0
4	23	F	10	1	abdoman	-8.82	9.96
5	21	F	17	7	leg	-32.2	-9.55
6	48	F	36	6	leg	-10.54	5.39
7	36	F	25	5	trunk	-0.86	0
8	20	F	12	6	trunk	0	0

* Percentage change from baseline (cm^2): (depigmented area at endpoint – depigmented area at baseline) / depigmented area at baseline×100
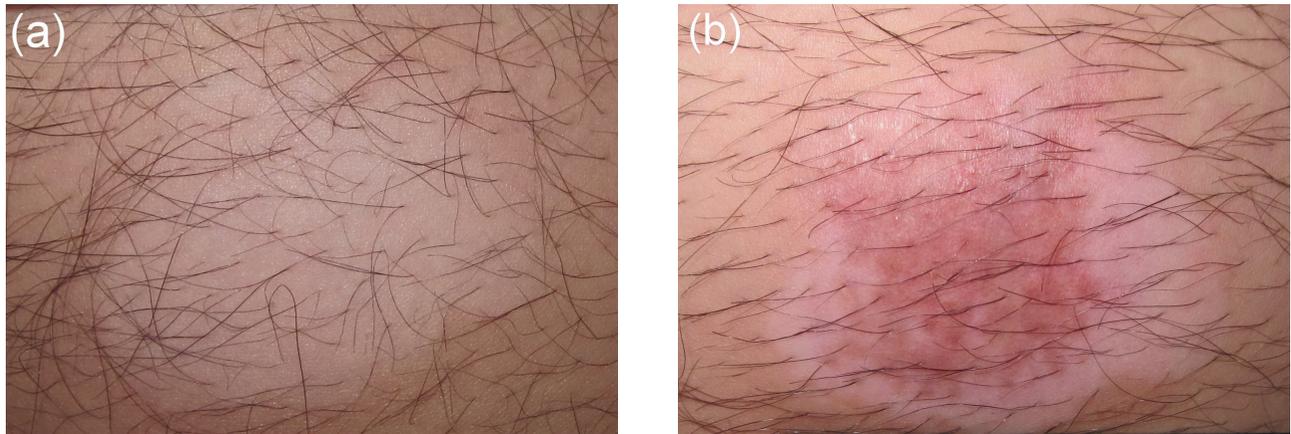
Figure 1. A vitiligo patch on the abdomen; before (a) and 3 months after transplantation (b) with depigmented surface areas of 11.98 and 4.17 cm^2, respectively.

mean age of the participants was 31.9 ± 11.0 years (median: 30.0; range: 20-48 years); the median of the duration of the disease was 20.5 years (range: 8-36 years). The percentage changes of depigmented areas (from baseline) for the patches treated with and without transplantation are presented in table 1. Furthermore, table 2 shows the frequency distribution of the patients classified according to the repigmentation percentage. Of eight patches treated with transplantation, good response was achieved in only one of the patients. Repigmentation was fair in 1 and poor in 4 patients. Moreover, two of the patches showed no repigmentation (Table 2). In these patches, the median percentage reduction of the depigmented area was 5.6% with a maximum of 65.2% reduction. There was a statistically significant reduction in the depigmented area of the transplanted patches from baseline to the 6th month follow-up (p = 0.03).

In seven of eight vitiliginous patches that were

treated without transplantation, no repigmentation was observed, and poor response to treatment was detected in only one of the patients (Table 2). In these eight patches, the percentage reduction of the depigmented area varied from 0% to 9.5%. No statistically significant changes were observed in the depigmented area of the patches from baseline to the end of the study (p = 1). The patches treated with transplantation showed more repigmentation as compared with the patches treated without transplantation (p = 0.03, Table 2).

No side effect was seen other than erythema of the patches that resolved spontaneously after 2 to 4 months.

DISCUSSION

In our study, transplantation of mixed non-cultured autologous melanocytes of the bulge area and ORS after dermabrasion was assessed. The improvement in pigmentation was statistically significant in the patches treated with non-cultured autologous melanocytes transplanting and dermabrasion compared with those treated with dermabrasion alone; however, the clinical results were not satisfactory because good repigmentation was seen in only one patch. On the other hand, with such a small sample size, we cannot draw a definitive conclusion about the results and generalize it.

Hair follicles are important reservoirs of melanocytes [7]. It is demonstrated that single hair grafting can improve acral vitiligo [8]; however, it can cause some cosmetic adverse effects including the

Table 2. Frequency distribution of patients with stable generalized vitiligo classified according to the percentage change of the depigmented area from baseline

Repigmentation percentage*	Transplanted (n=8)	Control (n=8)
No pigmentation or becoming worse	2	7
Poor	4	1
Fair	1	0
good	1	0
excellent	0	0

*based on percentage change of depigmented area from baseline Repigmentation was graded as excellent with 95% to 100% pigmentation, good with 65% to 94%, fair with 25% to 64% and poor with 1-24% repigmentation of the treated area.

growth of hair follicle and granuloma formation [9,10], which makes it a less favorable option. To overcome these obstacles, we decided to use the bulge area and ORS of the hair follicles, which are the main sites of melanocyte production.

Transferring non-cultured melanocytes has been recently examined. Mohanty et al. [5] used non-cultured extracted hair follicle ORS cell suspension for transplantation in vitiligo. They recruited patients with stable patches for three months and applied ORS suspension to the dermabraded area and dressed it for one week. After two to three weeks, they exposed the treated area to PUVA or sunlight and treated patients with oral methoxsalen. They observed repigmentation in nine out of fourteen participants and reported higher percentages of repigmentation in those with more than one year of stable disease.

We used non-cultured melanocytes of the hair follicle ORS transplantation with some differences with the study by Mohanty et al. We treated the other patches with dermabrasion and traumatized them by a needle in the same way that the recipient sites were treated. Traumatization and dermabrasion alone might induce repigmentation and can improve the effect of cell transplantation by activating follicular melanocyte reservoirs, stimulating peripheral epidermal melanocytes, or activating melanocytes that are metabolically inactive [11-14]. For making the effect of transplantation in repigmentation more reliable and neutralizing the role of traumatization, we made tunnels in control patches in the same manner we made tunnels for transferring materials.

We did not use PUVA or methoxsalen because we wished to assess the effect of transplantation; therefore, lower rates of repigmentation were observed in our patients in contrast to the study by Mohanty et al. In fact, by avoiding phototherapy, we may have lost the opportunity to enhance repigmentation with additional UV light therapy.

The effect of PUVA on melanocytes has been demonstrated by histochemical studies. In vitiligo patches, inactive melanocytes below the basal keratinocytes are present in the cytoplasm vacuolation and nuclear pyknosis with peripheral margination of nuclear chromatin suggesting degeneration of melanocytes. Moreover, keratinocyte degeneration with minimal or without nuclear changes is seen in vitiligo [15]. A significant increase

in the number of active melanocytes and recovery of melanocyte and keratinocyte degeneration after PUVA therapy has been reported by previous studies [16,17]. These findings support that PUVA therapy might have led to a better response in the study conducted by Mohanty et al. Leukotrichia and prolonged vitiligo have poor prognosis and unfavorable response to medical treatment [18,19]; however, transplanting melanocytes might cause pigmentation in these patches. Our participants had stable generalized vitiligo, and those with leukotrichia demonstrated a poorer response to therapy.

Our method is not superior to other cell transplantation methods including autologous non-cultured melanocyte-keratinocyte transplantation (MKT). Mulekar et al. [20] used MKT in the treatment of patients with stable vitiligo for at least 6 months. In their study, more than 50% of the patients showed more than 65% repigmentation of the treated areas. In another study that was conducted in our center, non-cultured MKT after dermabrasion was used to treat vitiligo patches. Half of the patients experienced excellent repigmentation (95%-100%), whereas there was no repigmentation in the control patches [21].

Our study had some limitations. A small proportion of vitiligo patients have stable generalized vitiligo, and generally, most of these patients are receiving different modalities of therapy; therefore, stable generalized vitiligo patients were rare in our clinics. Moreover, as a pilot study, we could not recruit many patients because it was a new method of treatment, and the number of patients with stable generalized vitiligo who agreed to participate was small. These factors led to selecting a small number of participants. Another limitation to our study was the follow-up. The patients were treated at the first session but they did not tend to present at the clinic for monthly evaluations.

In conclusion, although transplantation of non-cultured melanocytes of the ORS and bulge of the hair follicle is less difficult and less expensive than cultured melanocytes transplantation, clinical repigmentation is not satisfactory and favorable response in the previous study might be due to the use of PUVA therapy. In our study, patches that were resistant to other forms of therapy were treated, which might obscure the effect of this

method on lesions with a more benign course. Further studies with larger sample sizes and longer follow-ups are needed to establish it as a universal option in the treatment of vitiligo.

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