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Comparison Treatment of Vitiligo by Co-culture of Melanocytes Derived from Hair Follicle with Adipose-Derived Stem Cells with and without NB-UVB

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Abstract

Objective: The aim of this work is to evaluate treatment of vitiligo by co-culture of melanocytes derived from hair follicle with adipose-derived stem cells with (NB-UVB) and without (NB-UVB).

Patients and methods: In this study, we used co culture of adipose derived stem cell with melanocytes derived from hair follicle in treating different types of stable resistant vitiligo, by two methods transplantation: group (A) exposed to (NB-UVB), group (B) did not expose to (NB-UVB). They are followed up for 3 months.

Results: At the end of the follow up period which was 3 months group (A) showed better pigmentary response than group (B) and it was highly statistically significant. Stability, size, site and onset of vitiligo appeared to be important factors affecting treatment results. Using (NB-UVB) also after injection of the treatment showed more improvement in the treatment results.

Conclusion: Co-culture of adipose derived stem cell with melanocytes derived from hair follicle could be a safe and effective method of treatment for stable localized vitiligo in patients resistant to other methods of therapy.

Keywords: Vitiligo; Skin transfers; Depigmentation; Topical steroids; Cellular grafting

Introduction

Vitiligo is a common acquired disorder of pigmentation that causes a tremendous impact on the quality of life in affected patients. It occurs worldwide, with a prevalence of 0.1%–2% in various populations [1].

The etiology of vitiligo is uncertainly though genetic, immunological, biochemical (including oxidative stress) and neurogenic factors may interact to contribute to its development [2]. Vitiligo can be classified into segmental and non-segmental vitiligo. Segmental vitiligo has depigment edmacules arranged in a dermatomal or unilateral distribution, which does not cross the midline. It differs from non-segmental vitiligo in terms of clinical features, natural history, and also treatment response [3]. The treatment can be classified into medical treatment, light-based treatment, surgical treatment, and camouflage and depigmentation therapy. Medical treatment includes the use of immune-modulating drug such as systemic corticosteroids, vitamin supplements (especially vitamin B12 and folic acid). Light-based treatment includes psoralen photo chemotherapy (PUVA) and NB-UVB [2].

Surgical treatments can be classified as procedures involving complete skin transfers (e.g. partial split-thickness grafting, punch grafting and blister grafting) and cell transplantations which are further divided into culture and non-culture techniques. These surgical procedures basically donate some viable melanocytes to the affected area of depigmentation [5]. These viable melanocytes are then stimulated by different means like PUVA, narrowband UVB (NBUVB), topical steroids, and even excimer laser treatment to cause melanin production and thereby causing a repigmentation of the recipient or treated area [6].

Cellular grafting includes, co-culture of melanocytes with adipose-derived stem cells [7]. But, till date none of the medical or surgical therapeutic choices could assure guaranteed success in all the cases. This is primarily because of the obscure etiopathogenesis and elusive activity profile of the disease itself. Proper selection of cases is of paramount importance. The specific criteria for selection have been well defined [8-11]. This study aims to evaluate treatment of vitiligo by co-culture of melanocytes derived from hair follicle with adipose-derived stem cells with (NB-UVB) and without (NB-UVB).

Materials and Methods

The study included 20 patients complaining of vitiligo 11 males and 9 females of different age groups. Aged between 10-60 years old, with Fitzpatrick skin color III and IV. They were selected from patients attending the Dermatology clinic of Benha University Hospital. From May 2013 to May 2014. Consent to participate in the study was obtained from all the patients after explaining the aim of the study to them. Those who refused to participate in the study were excluded. All patients were submitted to history taking and general and local examinations.

Personal history

Including name, age, sex, residence.

Present history

Including the onset, course, duration, associated disorders.

Precipitating factors

Including psychic stress, trauma and sun burn.

Family history

Of vitiligo or other autoimmune disorders.

Examination

Complete clinical examination to detect the disease extent.

Investigation

Routine investigations were performed for each patient in the form of: complete blood picture, erythrocyte sedimentation rate, liver function test, kidney function test, fasting blood sugar and two hours post prandial, urine analysis, PT, PTT and bleeding time.

Biopsy

Punch biopsy 4 mm was obtained from hair follicle and preserved it in saline 0.9%. Another 6 mm Punch biopsy was obtained from adipose connective tissue (abdomen or buttocks) and preserved it in saline 0.9% and then the two biopsies were preserved in ice box and work up within 24 h so as not to be infected and damaged (Figure 1).

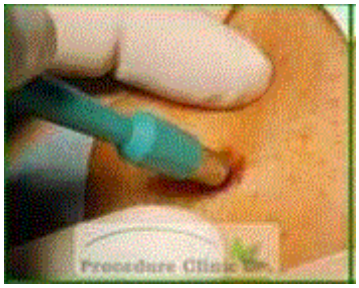

Figure 1: Punch biopsy technique [9].

Culture

Skin specimens that obtained from areas contain hair follicles (scalp, axilla and pubic area) were used for the cultures to isolate melanocytes (A). The epidermis was separated from the dermis after treatment with 2.4 U/ml of collagen for 1 hr. The epidermal sheets were treated with 0.05% trypsin for 10 min to produce a suspension of individual epidermal cells (B). The cells were suspended in fibroblast medium supplemented with fetal bovine serum (FBS), hydrocortisone, bFGF and gentecin [10]. The cells were kept in humidified 5% CO_2 incubator at 37°C for 3 days.

Adipose-derived stem cell preparation

Human ADSCs were taken from the abdomen by punch biopsy. The biopsy was cut into small pieces and subjected to 2 steps, digested with collagenase for one hour at 37°C followed by trypsin for 30 min at 37°C. The digested tissue was passed through sterile mesh to isolate single cell suspension. The cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% FBS, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Adipose derived MSC were examined by inverted microscope and when reached 80–90% confluence. Plate Passage1 (P1) and Passage2 (P2) at density (2×10^5) in 6 well cultures Plate until the adherent cells (MSCs) reached 80-90% confluence. Cells were passed 2 Passage; Passage 3 MSCs were used in the Study.

Isolation of epidermal melanocytes and hair follicle associated melanocytes

1-punch of skin biopsies from hairy areas were used .biopsies were treated with 2-4 u/ml collagenase for 1 h 2-epidermes were separated from dermis, epidermal sheets were digested by addition of 0.05% trypsin for 10 min. 3-specimen were passed through 100 M cell straine. Cell suspension was cultured in fibroblast media supplemented with 10% FBS, hydrocortisone, b FGF (20 ng/ml) and gentecin to prevent over growth of keratinocytes. 4-cells were kept in a humidified 5% CO_2 incubator at 37°C. Media changes were done every 3 days.

Adipose–derived MSCs isolation

Human ADSCs were taken from the abdomen by punch biopsy. the biopsy was cut into small pieces and subjected to 2 steps, digested with collagenase for one hour at 37°C followed by trypsin for 30 min at 37°C, The digested tissue was passed through sterile mesh 100 mm nylon mesh to isolate single cell suspension. After washing by centrifugation at 400 g for ten min, cells were plated into 6 well culture plates. The cells were cultured in low-glucose Dulbecco's Modified Eagle's Medium supplemented with 10% FBS, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Adipose derived MSC were examined by inverted microscope and the media were changed every three days until the adherent cells reach when 80–90% confluence. Non adherent cells were removed after three days by changing the medium after washing two times with PBS.

Plate Passage1 (P1) and Passage 2 (P2) at density (2×10^5) in 6 well culture plates until the adherent cells (MSCs) reached 80-90% confluence. Cells were passaged 2 passages, passage 3 MSCs were used in the study.

Co-culture of adipose-derived stem cells with melanocyte culture

Harvested adipose SC and melanocytes were co-cultured in Tissue Culture Flask with Filter Cap, PS, 25 cm^2 in complete media for 7–12 days. The culture medium was changed every 3 days. Treat the cells with fresh prepared trypsin/EDTA, after which both non-adherent and adherent cells were harvested, washed by PBS prepared for injection.

In similar study, we examined both cell population separately for melanin secretion using Dopa reaction. Co-culture cells were suspended at a conc. 200,000 cell/ml. Cells were plated at a density of 15-20 $\times 10^3/cm^2$ in 14 Patient and 6 Patients were plated at a density of 8-12 $\times 10^3/cm^2$. Patients were characterized by Fitzpatrick skin color, all 19 patients was between III and VI and only one patient had skin type V.

Treatment

The treatment area was cleansed of debris using 70% ethyl alcohol, the sample was then injected intra epidermal of the vitiligo lesions, using a disposable syringe 24 G needle, both sides of the body were

injected intra epidermal, the right side which was injected followed by NB-UVB, But the left side was injected with stem cell and melanocytes only.

The NB-UVB sessions were given 3 times/week, as the initial UVB dose was determined according to the system devised in the phototherapy unit of the Dermatology Department Benha University for vitiligo using phototherapy tables (Machine: UV1000 Waldmann lighting). Criteria of clinical response were based on development of perifollicular pigmentation or tanning of the lesions (Figure 2). Narrow band -UVB schedules can be tailored according to patient's skin type and local experience, by determination of the individual's minimum erythema dose (MED) [12].

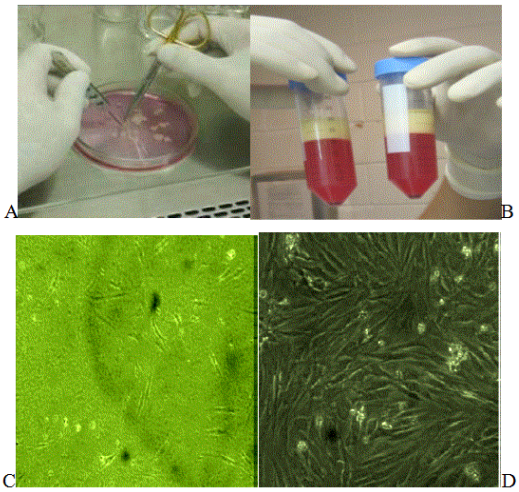

Figure 2: Isolation and Culture of human adipose-derived cells. Human adipose-derived cells were cultured in media (DMEM containing 10% FBS) for 7 days after isolation by; (A) explants or (B)collagenase treatment, showing low proliferation rate of cultured cells isolated by explants method (C) in relation to high proliferation rate of these isolated by collagenase enzyme (D).

The patients are instructed not to receive any natural or artificial UV light to this region of the skin during the next 24 h and asked to return to the phototherapy center in 24 h. The area of the photo-testing should be identified by ink marking at the different dosage sites. A positive reading is considered as identifiable erythema within the margins of the photo testing port. If bright red erythema develops or blistering occurs at the site of any of the photo testing sites, topical corticosteroids can be used to treat the area (Figure 3) [14].

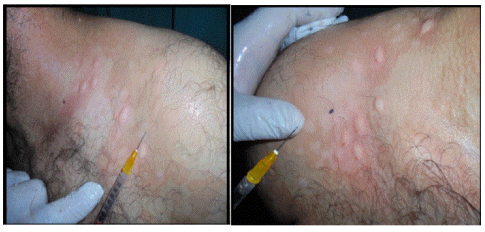

Figure 3: Intra epidermal injection of stem cell.

Once MED has been determined, the treatment protocol is usually percent based. Often 70% of the MED value is used for the first treatment; thereafter therapy is given three times or more weekly with 40%, 20% or 10% increments depending on local experience, erythema response and skin type tolerance [15]. Side effects noticed in few patients after phototherapy in the form of erythema and burning sensation for which the patients were given topical steroids and antihistamines.

Results

The vitiligo area scoring index (a quantitative tool) was used to evaluate the extent of vitiligo and the degree of repigmentation during and after therapy, improvement was recorded as no response (0), minimal (<25%), moderate (26–50%), marked (>50%) or complete (100%) (Table 1) [16].

Anatomic Structure	Surface Area	
	Adult	Child
Anterior head	4.50%	9%
Posterior head	4.50%	9%
Anterior trunk	18%	18%
Posterior trunk	18%	18%
Anterior leg, each	9%	6.75%
Posterior leg, each	9%	6.75%
Anterior arm, each	4.50%	4.50%
Posterior arm, each	4.50%	4.50%
Genitalia/perineum	1.00%	1.00%

Table 1: Role of nine in adult and in child.

Total body surface area (TBSA) is an assessment of injury to or disease of the skin, such as burns, psoriasis or vitiligo. Role of nine can be used to determine the total percentage of area diseased for each major section of the body (Figure 4) [17].

Figure 4: UV1000 Waldmann phototherapy unite.

This study was conducted on twenty patients were complaining of vitiligo; 11 males (55%) and 9 females (45%). The age of them ranged

from 10 to 57 years old, the mean age (32.6+13.6). The occupations of the patients are 5 patients works as employers (25%), 5 patients students (25%), 6 patients housewives (30%), 2 patients nurses (10%), one patient driver (5%), one patient driver (5%). The occupation of patients showed no specific improvement (Table 2).

Site of lesions	Patients		Improved	
	N	%	N	%
Hands	10	50	10	100
Elbows	5	25	4	80
Trunk	6	30	0	0
Breast	3	15	2	66
Upper limbs	5	25	1	20
Lower limbs	6	30	1	16
Knee	2	10	2	100
Feet	6	30	1	16
Genitalia	1	5	-	0
Scalp	1	5	-	0
Wrist	1	5	1	100
Neck	4	20	3	75
Axilla	1	5	-	0
Face	4	20	4	100
X^2	4.326			
P value	0.009*			

Table 2: Improvement according to the site of the lesion.

According to Fitzpatrick skin color classification, 5 patients are skin type III (25%), 14 patients are skin type IV (70%), one patient are skin type V (5%), there was significant improvement. Duration of vitiligo varied between 1-40 years with mean duration (6.25+2.36). The extent of the lesions ranged from 4-70% of the body with mean extent (24.45+11.41%), size of the lesions ranged from 0.8-11.5 cm with mean size (4.03+2.92 cm). Hair color in the vitiligo lesions was noticed as 14patients had black hair in the vitiligo lesions (70%), 2 patient shad grey hair in the lesions (10%), 2 patients had black /grey hair in the lesions (10%), one patient has yellow hair in the lesion (5%) and one patient has grey/white hair in the lesions (5%). This study showed significant improvement (Tables 3 and 4), on the other hand the course of lesions showed significant improvement, as 9 patient share progressive (45%) while 11 patients are stable (55%) as shown in Figure 5. The duration of vitiligo varied between 1-40 years with mean duration (6.25+2.36). The extent of the lesions ranged from 4-70% of the body with mean extent (24.45+11.41%), Size of the lesions ranged from 0.8-11.5 cm with mean size (4.03+2.92 cm).

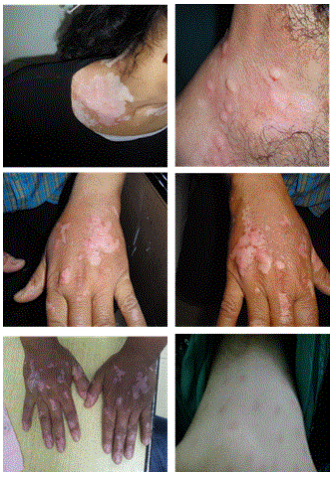

Figure 5: Erythema and edema after stem cell injection.

	Site of responding lesion	
	With narrowband	Without narrow band
%	70	45
X2	9.325	
p. value	0.001	

Table 3: Improvement of vitiligo lesion with and without NB-UVB (degree of response).

Time of follow up	Rt side	Lt side
After 3 weeks	20%	10%
After 6 weeks	45%	25%
After 10 weeks	70%	45%
X^2	0.44	
P value	0.801	

Table 4: Degree of repigmentation according to duration of follow up of the disease.

Hair colour in the vitiligo lesions was noticed as 14 patients had black hair in the vitiligo lesions (70%), 2 patients had grey hair in the lesions (10%), 2 patients had black/grey hair in the lesions (10%), one patient has yellow hair in the lesion (5%), one patient has grey/white hair in the lesions (5%).This study showed significant improvement. On the other hand the course of lesions showed significant improvement, as 9 patients are progressive (45%) while 11 patients are stable (55%). This study showed significant improvement as regard to sun exposure, 12 patients are exposed to sun (60%) while 8 patients didn't expose to sun (40%).

It also showed significant improvement as regard to psychological factors as 9 patients is exposed to psychological factors (45%) while 11 patients are not exposed to psychological factors (55%). It showed significant improvement as regard to family history, as 11 patients

(55%) has positive family history of vitiligo while 9 patients (45%) has negative family history of vitiligo (Figures 6 and 7).

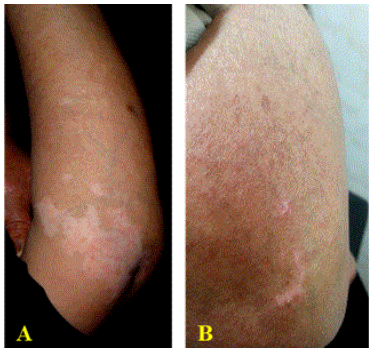

Figure 6: Elbow before treatment A and after treatment B.

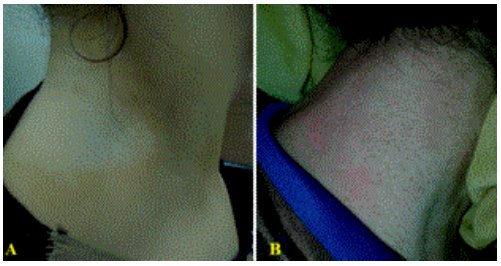

Figure 7: Back of the neck before A and after treatment B.

In this study all patients are exposed to narrow band on the right side of the body ranged between (13.41-73.61 Jules/cm^2) with mean (39.73+18.67 Jules/cm^2).

Discussion

Melanocytes provide the pigment melanin to keratinocytes in the skin epithelium. These cells are neural crest in origin and recent research suggests that skin melanocytes are derived from the same population that Schwann cells are derived [18]. The role of ADSCs has not been reported previously but a study showed that co-culturing of melanocyte with ADSCs increased the number of melanocytes proliferation and migration more than monocultures [7].

Stem cell research has a vast, unexplored potential in the treatment of vitiligo patients. Differentiation and amplification of pluripotent cells of outer root sheath (ORS) of the hair follicle can provide an unlimited supply of melanocytes for cell based treatment. Development in melanocyte and stem cell research has identified various cytokines, growth factors and regulators involved in proliferation migration and differentiation of melano blasts to mature melanocytes. These growth factors can be explored in the future for in situ melanocyte regeneration. Advancement in molecular biology has made the future prospect of vitiligo treatment bright and hopeful [19]. Our study we wanted to assess the efficacy and safety of treating vitiligo by co culture of adipose tissue derived stem cells (ADSCs) with melanocytes derived from hair follicle, by using cultured stem cell and melanocyte and to compare this efficacy with and without (NB-UVB).

In this study, 20 patients with vitiligo, not on medical treatment for at least one month; patients were 11 (55%) male and 9 (45%) female patient, with age ranging from 10 to 57 years old, the mean age (32.6+13.6), duration of the disease ranging from 10 months to 40years, 5 patients (25%) were generalized vitiligo while 15 patients (75%) were focal and acral type. Unfortunately this study hasn't been done before on human but similar study was done on skin animals, in a study made by Lim et al. [20] on lab animals showed that, Grafting of melanocytes co cultured with ADSCs increased melanocyte number in nude mice compared to melanocyte monocultures.

All patients in this study are treated by co-culture of adipose tissue derived stem cell with melanocyte derived from hair follicle, the 20 patients are divided into Right side and Left side, the right side is group (A) while the left side is group (B), group (A) exposed their right side of the body to (NB-UVB), while group (B) did not expose to (NB-UVB). Age, sex of the patients and onset of the disease were statistically insignificant which indicates that they had no effect on their pigmentation response. Skin type of the patients showed statistically significant value (p=0.048), hair color in the lesion also showed statistically significant value (p=0.024) when compared to results. As the black hair in the lesion showed more improvement in the pigmentation response than the white, yellow and grey hair.

In the study, Stability of the disease appeared to be one of the major factors affecting the degree of repigmentation. The results of the work showed that patients with longer stability time had better response and this difference was statistically significant (p=0.008), also type of vitiligo as 5 patients (25%) were generalized vitiligo showed only 3 patients improved,15 patients(75%) were focal and acral type showed 12 patients improved.

In the study, some factors appeared to be highly significant, as sun exposure showed statistically significant value (p=0.009), psychological factors showed statistically significant value (p=0.008) and family history of vitiligo showed statistically significant value (p=0.008). Site of the lesions showed statistically significant value (p=0.027) while site of responding to the treatment appeared to be one of the major factors affecting the degree of repigmentation. It showed highly statistically significant value (p<0.001).The results of the work showed the degree of improvement to the patients, hands (100%), face (100%), knee (100%), wrist (100%), elbows (80%),neck (75%), breast (66%), upper limb (20%), lower limb (16%) and feet (16%). In the study, Stem cell concentration appeared to be one of the major factors affecting the degree of repigmentation. The results of the work showed that patients injected with full concentration had better response and this difference was statistically highly significant (p=0.008). In this study, the (NB-UVB) showed highly statistically significant in repigmentation value (p<0.001), the right side of the body which injected by stem cell and followed by narrow band showed highly significant improvement than the left side which is injected by Stemcells only. As NB-UVB accelerates the repigmentation time and shorten the time needed for improvement but not affect the degree of the improvement after completes the time off follow up of the patients. When compared the result of improvement of the right side and the left side it showed that after 3 weeks of injection of the treatment both sides showed minimal response, after 6 weeks of injection the right side showed moderate improvement while the Lt side showed minimal improvement, after 10 weeks of injection the right side showed marked improvement while the left side showed moderate improvement. So the NB-UVB accelerates the time of response.

In group (A) 15 patients showed pigmentation (75%), while in group (B) 13 patients showed pigmentation (65%). Although there was difference in response between the two groups and it was statistically highly significant value ($p<0.001$) as group (A) which is the right side used NB-UVB after injection showed 70% of repigmentation to the sites of the lesions which injected, while group (B) which is the left side followed by NB-UVB showed only 45% of repigmentation to the sites of the lesions which injected. In a study by Kim et al. [7], they used Co-culture of adipose-derived stem cells with melanocyte culture and found that, co-culturing with ADSCs stimulated both proliferation and migration of melanocytes, ADSC co-culturing also increased the number of melanocyte precursor cells. These findings may be used to improve treatments of pigmentation disorders associated with the loss of melanocytes, although this was to a lesser degree compared with co-culturing with keratinocytes, this results match with our results, but we had no co-culturing with keratinocytes and we used (NB-UVB) after culture.

Another study by Zhou et al. [21], investigated the efficiency of autologous melanocyte transplantation of 23 vitiligo patients by focusing on peri lesional skin homing CD8+ T lymphocytes, and studied the potential effect of dermal mesenchymal stem cells (DMSCs) on CD8+ T cell activities in vitro. Out of 23 patients with the autologous melanocyte transplantation, 12 patients (52.17%) had an excellent re-pigmentation (>90%), 6 patients (26.09%) had good re-pigmentation (50-89%), 5 patients (21.74%) had fair (20-49%) or poor (<20%) repigmentation. It was also demonstrated significant CD8+ T cells infiltrating in the peri lesional area of vitiligo patients undergoing melanocyte transplantation. The efficiency of vitiligo patients 'autologous melanocytes transplantation is closely associated with skin-homing CD8+ T cell activities. DMSCs inhibit CD8+ T cells proliferation, induce those apoptosis and regulate their cytokines/chemokine production. Our results suggest that vitiligo patients 'autologous melanocytes transplantation efficiency may be predicted by peri lesional skin-homing CD8+ T cell activities, and the immune regulatory DMSCs might be used as auxiliary agent to improve the efficacy. This study match with our study, but we had no Culture of Human Skin Homing CD8+ T Cells or Assay of *in vitro* human skin CD8+ T cells proliferation when co-cultured with DMSCs.

Verma et al. [22] compared the efficacy of autologous melanocyte seeded amniotic membrane versus cultured melanocyte cell suspension in the treatment of stable vitiligo. They treated 30 patients with at least 1 year of stable vitiligo, patients were divided into three groups; group (1) treated with cultured melanocyte suspension, group (2) melanocytes on amniotic membrane, group (3) no melanocyte (placebo). They used CO_2 laser in preparing lesion sites. Sun exposure was recommended after culture. Follow up was for 6 months. A significant difference was seen between cellular grafts and placebo group. In group (1) and (2) repigmentation of at least 75% in treated areas, 51% and 95% in group (1) and (2) respectively. Indicating that amniotic membrane groups showed better response. This results matches with our results, but we had no melanocytes on amniotic membrane group or placebo group and we used (NB-UVB) after culture. Compared to another study by Pianigiani et al. [23] in which they used autologous melanocyte culture and narrow-band ultraviolet B in the surgical treatment of vitiligo. Cells were cultured on hyaluronic acid membranes using medium supplemented with patient's serum. Cell cultures were grafted onto laser-abraded depigmented areas. Patients underwent narrow-band UVB therapy 3weeks after grafting. All patients were evaluated 3, 6, 12 and 18 months after grafting. At the 18-month follow-up, repigmentation was observed in 75% of patients with focal and segmental vitiligo and in 30% of patients with generalized vitiligo.

Compared to our study, follow up after 3 months, repigmentation was seen in 60% with focal and acral vitiligo and 15% of patients with generalized vitiligo. The results of both studies revealed that the type of vitiligo is an important factor affecting the results of surgical procedures. In another study by Pandya et al. [24] which included 27 patients they used autologous melanocyte rich cell suspension (uncultured), and cultured melanocytes suspension. Excellent response was seen in (50%) with the melanocyte culture (MC) technique and this result match with our study as excellent response was seen in (50%) of patients. In a retrospective study from Taiwan, by Chenet al. [25] 120 patients with vitiligo resistant to ultraviolet light therapy underwent autologous melanocyte culture transplantation into some vitiliginous areas after epidermal stripping with a resurfacing CO_2 laser. Excellent results (90% to 100% repigmentation) were obtained in 84% of patients with stable localized vitiligo. Age and sex of the patients, size and location of the lesions, did not show significant influence on the results of transplantation. Compared to our study which had 20 patients, 9 patients (45%) showed repigmentation response with stable vitiligo, 7 patients (35%) generalized type showed repigmentation. This difference in results may be related to the smaller number of patients in our study.

Age and sex of the patients, also did not show significant influence on the results of transplantation in our study, but the site and size of lesion influenced the response, as the acral lesions showed marked response, the smaller the lesion the good response. Also the concentration of the suspension full concentration showed better response as Co-cultured cells were suspended at a concentration of 200,000 cell/ml. Cells were plated at a density of 15-25 $\times 10^3/cm^2$ in 14 patients and 6 patients were plated at a density of 8-12 $\times 10^3/cm^2$. A randomized controlled trial by Hamzavi et al. [26] established that NB-UVB is an effective treatment for vitiligo. Additionally, both Yones et al. [13] and Kumar et al. [27] confirmed efficacy of NB-UVB, but showed a better repigmentation percentage in patients during treatment phase compared to our results. This is probably due to the higher number of exposures which reached 48 sessions with Yones et al. [13] and up to 12 months with Kumar et al. [27] whereas in our study the treatment period did not exceed 30 sessions.

Another retrospective study by Scherschun et al. [28] also confirmed the fact that NB-UVB is a useful and well-tolerated therapy for vitiligo. This study showed a higher repigmentation extent with a lower number of treatments (19 exposures) compared to our study. This could be explained by the fact that this study used a starting dose of 2.8 mJ/cm^2, with 15% dose increments at each subsequent treatment, whereas in our study the initial dose was 0.25 J/cm^2 with increments of 0.1 J/cm^2 (up to a maximum of 3 J/cm^2). In a study by Lontz et al. [29] 27 patients were treated after superficial dermabrasion with application of suspensions of autologous cultured melanocytes reported excellent response in 40.7%, good response in 7.4% and moderate response in 51.8%. They emphasize that the anatomical location is the major factor that determines the response. As in our study the anatomical location was important factor to determine the response but we had not dermabrasion. In a study by Awad [30], by investigated the effectiveness of dermabrasion alone in managing stable vitiligo. Ten patients with vitiligo were candidates in this study. Superficial dermabrasion was carried out using proper diamond fraises. Biopsy was obtained after 10days of the procedure and examined pathologically. The patients were followed up for 3 months.

Dermabrasion was able to repigment vitiliginous patches completely in six patients and partially in two patients, while two cases failed to repigment at all. Spindle melanocyte precursors were demonstrated in the epidermis 10 days after abrasion with regaining normal thickness of keratin layers. Dermabrasion and repigment vitiligo through stimulation of melanocyte stem cells and elimination of hyperkeratosis. Westerhof and D'Ischia [31] mentioned NBUVB causes the repigmentation of vitiligo via: (a) immunosuppression to stop melanocyte death, and (b) restoring pigmentation via increasing the numbers of melanocytes. UVB irradiation can induce T-regulatory (suppressor) cell activity and IL-10, produced in the epidermis following UVR irradiation and may be important for the differentiation and the activity of populations of T-regulatory cells in the periphery [32].

In a study by Kanwar et al. [33] showed that dark skin (Fitzpatrick type IV and V) requires lesser number of exposures and cumulative dose to achieve 25-75% repigmentation when compared with white skin (Fitzpatrick I, II and III). This result match with our study, skin type has statistically significant value (p value 0.048) as skin type IV showed faster and good response than skin type III. Stability of the disease and location of the recipient site were the major determinant of the outcome acral parts including the dorsal aspects of the hands and feet, and skin over the joints were good responsive, 10 patients with lesions on the hands all showed repigmentation varying from age of patients and duration of the disease and the course of the lesion, 6 patients with lesions on the feet only one patient showed repigmentation, 5 patients with lesions one elbows showed repigmentation in 4 patients,2 patients with knee lesions showed repigmentation in all lesions, also 4 patients with lesions on the face all showed repigmentation. As regards to the side effects they were mild and did not necessitate cessation of therapy, infection, erythema, itching, burning sensation, rigors and fever was the most common seen side effects. Some minor complications were also observed in Pandya et al. [34]. Two (10%) patient shad infection at the donor area and one (5%) developed infection at the recipient surface. Infection occurred probably because patients did not comply with the antibiotic treatment. All patients had erythema, rigors and fever at the day of injection of stem cell treated by antipyretic and bed rest. Three (15%) patients developed depigmentation of re pigmented sites and Koebner phenomenon during the follow-up period of 10 weeks from NB-UVB [27] and stop the treatment and used sunscreen in spite of UVB.

In our study, co culture of adipose tissue derived stem cell with melanocyte derived from hair follicle in treating vitiligo proved to be effective and safe method. It has an advantage it requires very little donor skin usually only one tenth of the recipient site. Different methods of co culture were used as co culture of keratinocyte with melanocyte showed better response than co culture of ADSCs with melanocyte as Cell-to-cell interactions between melanocytes and keratinocytes increase the proliferation and migration of melanocytes, the effect of ADSCs was less powerful than that of keratinocytes. However, this may require taking an amount of skin tissue large enough to leave scars [7]. At the end, a question arises whether the repigmentation induced by co-culture methods is permanent or not? Since co culture of ADSCS with melanocytes does not treat the underlying cause in vitiligo, reactivation of the disease may lead to a secondary failure of the treated skin and to the development of the Koebner phenomenon at the donor sites.

Conclusion

From the previous discussion it can be conclusion that co-culture of adipose derived stem cell with melanocytes derived from hair follicle could be a safe and effective method of treatment for stable localized vitiligo in patients resistant to other methods of therapy.

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