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Studying the effect of adding growth factors to the autologous melanocyte keratinocyte suspension in segmental vitiligo

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Abstract

Addition of different growth factors to the medium used in autologous melanocyte-keratinocyte transplantation procedure (MKTP) was reported in the literature. The aim of the current study was comparison of response to MKTP in segmental vitiligo (SV) with and without adding growth factors to the suspension medium. Eighteen cases with SV were randomly divided into two groups. In group A: Ham F12 medium was used for suspension and in group B: 5 ng/mL recombinant basic fibroblast growth factor (bFGF) and 25 mg/500 mL 3′5′ cyclic adenosine monophosphate (cAMP) were added to the medium. All cases received NB-UVB twice weekly for 24 weeks. The area of vitiligo lesions was measured before and after therapy by point-counting technique and complications were recorded. Excellent response (90%-100% repigmentation) occurred in 5/9 cases (56%) in group A and 7/9 cases (78%) in group B (with growth factors). A significant decrease in the area of treated lesions before and after therapy was found in both groups A and B (P = .0012 and .0004, respectively), however, a higher percentage of reduction in area of vitiligo was seen in group B cases (70% in group A vs 90% in group B; P value: .028). Marginal halo was seen in five cases in group A and six in group B. In conclusion addition of bFGF and cAMP to MKTP medium improved the results of the procedure. It could be considered if economically feasible.

KEYWORDS

3′5′cAMP, bFGF, cell suspension, CO_2 laser, melanocyte-keratinocyte transplantation procedure

1 | INTRODUCTION

Segmental vitiligo (SV) is characterized by rapid onset and involvement of the hair follicle pigmentary system resulting in early occurrence of leucotrichia.[1] Stabilized cases with extensive leucotrichia are the best candidates for surgical therapy which replaces the depleted melanocytes with ones from a normally pigmented autologous donor site.[2] Non-cultured autologous melanocyte keratinocyte transplantation procedure (MKTP) was first described by Gauthier and Surleve-Bazeille in 1992.[3] Simplification of the technique was done over the years[4,5] making it more popular among dermatologists for treating cases of SV being relatively easy to perform with good cosmetic outcome. Basically, MKTP involves processing normal donor skin from the patient through incubation at 37°c in 0.25% trypsin followed by discarding the dermal tissue and separation and fragmentation of the epidermis. A concentrated cellular suspension is obtained by centrifugation. This suspension; composed of melanocytes and keratinocytes; is then applied to the selected dermabraded vitiligo recipient area.[4]

A few authors reported addition of different growth factors to the medium in which the cellular graft was suspended in an attempt to

enhance melanogenesis.[4-6] These additions were omitted later to simplify the procedure and reduce the expenses.[7-9] More recently, phosphate-buffered saline (PBS) was used during suspension preparation to further cut the cost.[10] On reviewing the literature, no comparative studies assessing the value of adding such growth factors were found. In the present comparative study, we wanted to assess the effect of adding two of the key factors that stimulate the melanocytes, namely basic fibroblast growth factor (bFGf) and 3′5′ cyclic adenosine monophosphate (cAMP), on the outcome of MKTP in SV cases.

2 | METHODS

2.1 | Patient recruitment and clinical assessment

Eighteen cases of stable SV were recruited during the period from January 2014 to January 2015. Inclusion criteria were resistance to medical therapy for a minimum of 6 months and/ or presence of leucotrichia. Exclusion criteria were disease activity during the past year or keloidal tendency. Detailed history taking and clinical assessment were performed using vitiligo European task force (VETF),[11] vitiligo area and severity index (VASI),[12] and vitiligo disease activity (VIDA) scores.[13] Surface area of the lesions to be treated were calculated by point counting technique.[14] Approval of the Dermatology Research Ethical Committee was obtained. All patients (or guardians of minors) signed informed written consents prior to surgical treatment. Cases were randomized using envelope-concealed method into two groups A and B, each including nine patients.

2.2 | Surgical Therapy

2.2.1 | Donor tissue

A split thickness graft with an area 1/5 the area of the recipient site was obtained using a hand dermatome from the patient's gluteal area or front of thigh after local anesthesia by intralesional Mepecain L (carpule: Mepivacaine HCL 2% and Levonordefrin 1:20000) (Alexandria Co. for Pharmaceuticals & Chemical Industries, Alexandria, Egypt). The donor site was covered by sterile petrolatum jelly gauze and adhesive tape.

2.2.2 | Preparation of suspension

The skin graft was washed by saline and immersed in 0.25% trypsin-EDTA (GIBCO) solution for 40 minutes at 37°C. The sample was removed from trypsin and washed three times with ringer lactate solution in a petri-dish. The epidermis was separated from the dermis which was discarded. Then the epidermis was cut into tiny pieces, transferred to a sterile falcon tube and centrifuged for 20 minutes at 1000 rpm to obtain a cellular pellet. The cell pellet was re-suspended in 1-2 mL of medium according to the area to be treated (1 mL/20 cm^2 recipient area). In group A: The cellular pellet was suspended in Ham F12 medium while in group B: The cellular pellet was suspended in HAM F12 medium with addition of 5 ng/mL recombinant bFGF and 25 mg/500 mL cAMP.

2.2.3 | Recipient site preparation

Full resurfacing was carried out using CO_2 laser equipped by a scanner at 12 W and 600 milliseconds dwell time (DEKA, Florence, Italy). One to three passes were done until the epidermis was removed uniformly. The suspension was then applied by a pipette and covered immediately by collagen sheets (NeüSkin, New Delhi, India). The recipient area was covered by sterile petrolatum jelly gauze, thick gauze, and adhesive tape. Patients were instructed to lie flat after the procedure for 30 minutes to allow successful attachment of cells.

2.2.4 | Post-procedural care

The dressings were removed after 7 days during which prophylactic oral broad spectrum antibiotic was administered.

2.3 | Phototherapy

All patients received narrow band ultraviolet B 311 nm (NB-UVB) sessions twice weekly for a period of 24 weeks, (UV 100 L; Waldmann GmbH, Villingen-Schwenningen, Germany). Phototherapy was started 2 weeks after removal of the dressings at a starting dose of 0.5 J/cm^2 which was increased by 0.3 J/cm^2 every other session until faint erythema was achieved and the dose was fixed then.

2.4 | Follow-up

Patients were followed up monthly for a total duration of 6 months. Clinical examination and digital photography were done each visit. The senior researcher who performed the final clinical assessment was blinded. Percentage of repigmentation was assessed through reversed VASI scoring[12] such that: 0%: no repigmentation, 10%: specks of pigmentation, 25%: depigmented area > pigmentation achieved, 50%: pigmented area equivalent to the residual depigmented area, 75%: pigmentation achieved > residual depigmentation, 90%: few depigmented specks left and 100%: full repigmentation. The area of the lesion at the end of the follow-up period was calculated by point counting and percentage of reduction was compared between both groups. Color match and homogenecity as well as any complications were recorded.

2.5 | Statistical Analysis

Comparisons between two groups regarding the age, sex, disease stability, site, VETF, VASI, and mean area of vitiligo before and

after MKTP were done by Student's *t* test using graphpad prism 5 for windows (San Diego, CA). *P* value ≤.05 was considered significant.

3 | RESULTS

There were 14 females and 4 males, aged 14 to 50 years. Table 1 shows demographic and clinical data of cases in both groups with similar baseline characteristics. In group A: 90% to 100% repigmentation occurred in 5/9 cases (Figure 1); 75% in one; 50% in one and 10% repigmentation in two cases respectively while in group B: 90% to 100% repigmentation occurred in 7/9 cases (Figure 2) while 10% repigmentation occurred in the remaining two cases. A total of 32 lesions (15 over the face and neck, 10 over the trunk, and 7 over the proximal extremities) were treated in all cases. Due to the small number of lesions, statistical analysis of response according to site treated was not performed; however, we noticed that lesions over the upper part of the face, trunk and proximal extremities responded better than those over the lower face and neck.

There was a significant decrease in the area of treated lesion before and after MKTP in both groups, however a higher percentage of reduction in area of vitiligo was seen in group B cases (70% in group A vs 90% in group B; *P* value: .028; Table 2).

Color match and homogenecity was good in all cases in both groups with only one case in group A showing transient hyperpigmentation in two of three treated lesions. Marginal halo was seen in five cases in group A and six in group B (Figure 2B). Infection at the

TABLE 1 Demographic and clinical data of cases

	Group A (no growth factors) 9 cases; 17 lesions	Group B (with growth factors) 9 cases; 15 lesions	*P* value
Age[a]	20 (14–50)	20 (14-31)	.3
Sex[a]	7 Females, 2 males	7 Females, 2 males	1
Disease duration (yrs)[a]	4 (1.5-15)	6 (1.5-15)	.6
Stability (yrs)[a]	2 (1–8)	2 (1–10)	.5
Skin type	III: 7 cases; IV: 2 cases	III: 6 cases; IV: 3 cases	.4
Family history of vitiligo	+ve in 1 case	+ve in 2 cases	NA
Site Head & neck Trunk Proximal limbs	 9 3 5	 6 7 2	 .2
VETF area[a]	4 (0.25-10)	4 (0.5-10)	.72
VETF stage[a]	3 (2-8)	4 (2–9)	.71
VASI[a]	3 (1-5.25)	3 (0.75-5.25)	.62
Leucotrichia	+ve 4 cases	+ve 5 cases	.13
Area treated (cm^2)[a]	4-60 (12)	5-80 (12)	.82
Donor area (cm^2)[a]	2–12 (10)	4-20 (6)	.78

Abbreviations: VASI, vitiligo area and severity index; VETF, vitiligo European task force.
[a]Median & range.

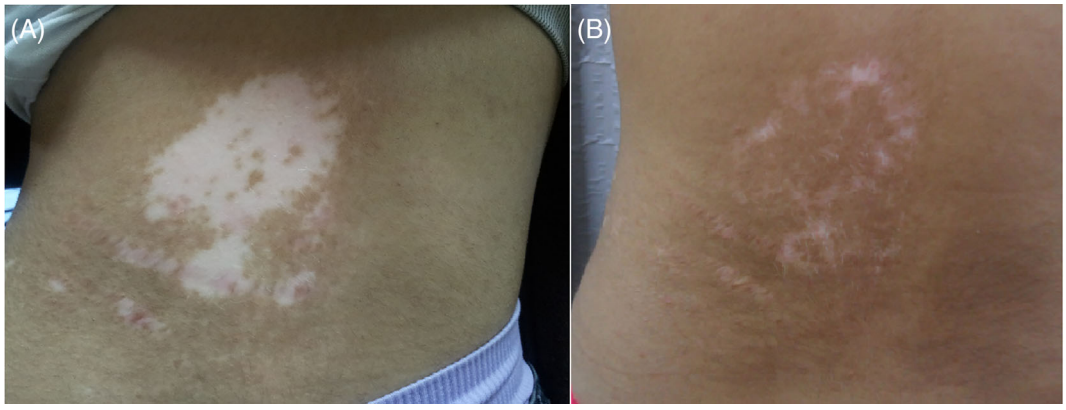

FIGURE 1 Group A (no growth factors); A, Before therapy and B, 95% repigmentation 6 months after surgery with marginal halo

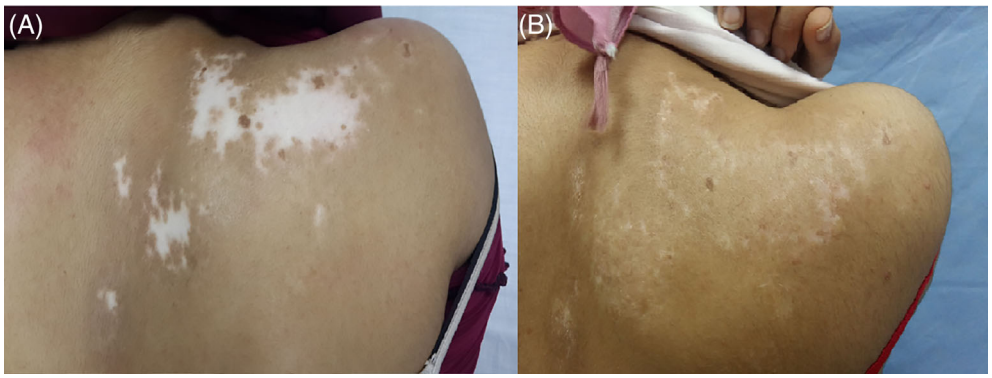

FIGURE 2 Group B (with growth factors); A, Before therapy and B, 100% repigmentation 6 months after surgery with faint marginal halo

TABLE 2 Area of treated lesions before and after therapy in both groups

	Group A (no growth factors) 9 cases; 17 lesions	Group B (with growth factors) 9 cases; 15 lesions
Area in cm^2 before MKTP; median (range)	12 (4–60)	12 (5–80)
Area in cm^2 after MKTP; median (range)	3.5 (0.5-45)	2 (0–72)
P value	0.0012*	0.0004*
Percentage reduction in area treated; Median (range)	70 (0-90)	90 (10-100)
P value	0.028*	

Abbreviation: MKTP, melanocytes keratinocyte transplantation procedure.
*$P < .05$ is statistically significant.

recipient site occurred in two cases in group A and was followed by complete repigmentation with coloring of white hair in the first and hyperpigmentation of lesions in the second case.

4 | DISCUSSION

Modifications occurred in different aspects of MKTP including tissue harvesting, trypsinization method, medium used for suspending the cells, recipient site preparation and dressings used. Omitting the addition of growth factors to the suspension medium was first done in 2003[7] and was widely adopted in most of the subsequent studies.[15-17] We believe all these procedural related changes have an impact on the outcome of MKTP. Studying and comparing each and every aspect of these modifications can lead to reaching the optimum technique. Several studies compared the effect of donor tissue used,[18,19] method of recipient site preparation,[18,20]and even dressing used postoperatively.[20] On reviewing literature, no comparative studies were performed to assess the impact of adding or omitting growth factors to the suspension medium.

The aim of this work was to explore this point to determine the effect of adding growth factors to the suspension on the outcome of MKTP.

In the present study, two groups of cases with matched clinical and demographic characteristics were treated by MKTP using Dulbecco Modified Eagle Medium (DMEMF) 12 medium to which bFGF and cAMP was added in one group of cases compared to medium only in the other group. All surgical steps were unified to ascertain that the addition of growth factors was the only variable. Both groups showed a favorable response with good color matching and homogenecity and no serious side effects. However, in group B cases where growth factors were used, 78% of patients showed excellent response as compared to 56% in group A and more significant reduction in the area of treated lesions was found ($P = .028$), denoting that these growth factors may have a beneficial impact after all.

In the original technique, saline was used to suspend the cell pellet[3] with 4/11 cases of vitiligo (two SV and two focal) achieving 90% to 100% repigmentation. A few years later, Olsson and Juhlin[4] used a modified technique to treat 26 cases of leucoderma of which 20 were vitiligo patients (7 SV and 13 NSV). Dermabrasion was used to prepare the recipient site and the cell pellet was suspended in M2 melanocyte medium; which is a highly balanced serum-free medium, suitable for melanocytes, based on DMEM/F-12, 1:1, vol/vol and a 15 mmol/L HEPES buffer system; supplemented by bFGF.[4] An average repigmentation rate of 100% in SV and 79% in NSV cases was achieved. Poor response was only reported in two cases.[4] Subsequently, other authors used different additions with variable repigmentation rates ranging from 92% and 88% in SV and NSV cases, respectively,[5] to 50-60% in another study where most of the lesions were acral.[6]

A few years later, Mulekar simplified the technique by eliminating all additions and using DMEM F12 medium only and achieved excellent response in 56% (80/142) of patients while poor response occurred in

24%.[8] Although this modification relatively reduced the cost of performing the technique, it could be noticed that the percentage of excellent responders was reduced compared to other authors where growth factors were added.[4,5] This confirms the results achieved in the present study where addition of bFGF and cAMP lead to more excellent responders and more reduction of the area of vitiligo after MKTP.

This could be explained by the fact that bFGF is a mitogen for melanocytes normally produced in the human body by keratinocytes of the skin and the pituitary glands. It has been used safely as a component of melanocytes culture medium.[21]

Melanocytes are stimulated to proliferate and to become more dendritic by factors secreted by keratinocytes such as endothelin-1, alpha melanocyte stimulating hormone (α-MSH) and adrenocorticotropic hormone.[22,23] After α-MSH binds to its receptor melanocortin-1 (MCR1), cAMP is essential for the formation of protein kinase A which later forms cAMP-response element binding protein involved in microphthalmia-associated transcription factor (MITF) upregulation.[24] MITF dictates the pigment cell phenotype by regulating melanocyte-specific proteins and genes involved in melanoblast survival, lineage commitment, and melanocyte proliferation and survival.[25] Rho proteins become active when they bind guanosine triphosphate and inactive when binding guanosine diphosphate. Hence, when Rho is activated, dendrites retract; while when its family member Rac is activated, dendrites form. By increasing cAMP levels, α-MSH inhibits Rho, enhancing melanocyte dendricity.[26]

In this study, a significant improvement was noted in all treated cases that may be explained by the fact that several cytokines including bFGF and α-MSH are released from neighboring keratinocytes and fibroblasts during the healing process.[27-29] A slightly better outcome occurred in cases where growth factors were added at the time of cellular grafting in the present work. This finding is interesting as it raises questions of whether cutting the costs may have slightly compromised the outcome of MKTP. However, the decision to add growth factors remains influenced by economical factors especially in developing countries where vitiligo has major psychological impact in populations with skin type III and IV.

All our cases received NB-UVB phototherapy after MKTP which is the routine regimen used in our center where the majority of cases are Caucasians with skin types 3 and 4. The use of phototherapy after MKTP was adopted in several studies to enhance repigmentation[6,19,30,31] despite the fact that no controlled comparative studies were performed addressing its value.

Limitations of the present work are the small number of cases and combining two growth factors instead of testing each separately.

In conclusion, addition of bFGF and cAMP to MKTP medium improved the results of the procedure. It could be considered if economically feasible.

CONFLICT OF INTEREST

None.

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