Zagazig University Medical Journal
www.zumj.journals.ekb.eg
https://doi.org/10.21608/zumj.2024.328961.3647 Volume 31, Issue 1, January. 2025
Manuscript ID: ZUMJ-2410-3647
DOI: 10.21608/ZUMJ.2024.328961.3647
ORIGINAL ARTICLE

A study of Non-Cultured Epidermal Suspension Grafting Using Trypsinized Epidermal Grafting for Stable Vitiligo

Samia A. Ibrahim, Sara Mohamed Ibrahim Mohamed*, Khaled Gharib
Dermatology, Venerology & Andrology Department, Faculty of Medicine, Zagazig University, Zagazig, Egypt

***Corresponding author:**
Sara Mohamed Ibrahim Mohamed
Email:
sara.ibrahim.k1@gmail.com

Submit Date: 16-10-2024
Accept Date: 02-11-2024

ABSTRACT

Background: The loss of functioning melanocytes and a dysregulated immune system led to the persistent depigmenting illness known as vitiligo. The objective of this research was to examine the efficacy of Noncultured epidermal suspension (NCES) transplantation as a therapy for persistent vitiligo. **Methods**: The investigation was a clinical trial. There were twenty-four patients with stable vitiligo. The degree of repigmentation and color match were assessed 16 weeks after surgery. **Results**: The percentage of improvement in NCES group, on 8 days, 2 (8.33%) patients had been improved with an improvement percentage of 2%, after 4 weeks 16 (66.67%) patients had been improved with an improvement percentage of 10%, after 8 weeks 14 (58.33%) patients had been improved with an improvement percentage of 30%, after 12 weeks and after 16 weeks, the studied patients showed stable improvement where 12 (50%) patients in both times had been improved with an improvement percentage of 62%. **Conclusions**: For individuals with stable vitiligo, NCES is the recommended course of treatment due to its great effectiveness. the rate of repigmentation was high in NCES.

Keywords: Vitiligo; Noncultured epidermal suspension; Stable Vitiligo

INTRODUCTION

About 1% of people worldwide suffer with vitiligo, a prevalent condition that has a significant morbidity rate and psychological consequences. The disease has been divided into five categories by an international consensus definition: segmental, non-segmental, focal, universal, mucosal, and unclassified. Less frequently occurring, segmental vitiligo typically manifests as a unilateral, band-shaped distribution. The hallmark of non-segmental vitiligo is typically bilateral or symmetrical dispersion over the body [1].

The classic method of histopathologically diagnosing vitiligo is based on the fact that the skin biopsy lacks melanocytes and melanin. Nonetheless, pathologists may have difficulties in verifying the complete lack of these components. As such, it is crucial to take a variety of aspects and variables into consideration while considering the possibility of a biopsy for a vitiligo lesion. Thyroid problems are the most common autoimmune issue that is often connected to this condition [2].

The primary pigment responsible for determining the color of the skin is melanin. Melanosomes help the melanocyte synthesize melanin, which is then eventually transported to the keratinocytes [3]. Although vitiligo's precise etiology is uncertain, several theories have been proposed. The a etiopathogenesis of vitiligo is commonly thought to be caused by an autoimmune, neural, biochemical, intrinsic structural fault, or genetic theory [4].

The pathophysiology of vitiligo has been linked to an imbalance in the skin's antioxidant defense and oxidative stress systems. Elevated levels of oxidative stress have been linked to melanocyte destruction and apoptosis, which in turn can cause depigmentation [5].

One of the trickiest dermatological disorders to treat is vitiligo. Topical steroids, either by itself or in conjunction with topical calcineurin inhibitors, are advised as the first line of treatment. For unstable lesions that progress quickly, systemic steroids or immunosuppressants are utilized to stop the autoimmune damage. The immunomodulatory impact and melanocyte regeneration can be further enhanced by monochromatic excimer laser treatment and narrowband ultraviolet B phototherapy. Individuals who have tried non-surgical

approaches yet still have stable, resistant lesions may choose to undergo surgery using melanocyte transplant procedures [6].

Transferring melanocytes from unaffected skin to the stable vitiligo patch in the form of a tissue or cellular transplant is the fundamental surgical technique. Among the surgical treatments, suction blister epidermal grafting (SBEG), a tissue transplantation technique, and autologous noncultured epidermal cell suspension grafting (NCES), a cellular transplantation technique, yield encouraging results [7].

Aim of the work

This study set out to investigate the efficacy of transplanting noncultured epidermal suspension (NCES) in the management of stable vitiligo.

METHODS

In this clinical trial investigation, 24 patients with stable vitiligo were enrolled between December 2023 and April 2024 from the dermatology, venereology, and andrology department's outpatient clinic at Zagazig University. The ethics committee of the Zagazig University Faculty of Medicine granted approval for the study design, and the study was authorized by the outpatient clinic of the department of dermatology, venereology, and andrology at Zagazig University Hospitals. (IRB#10317-3-12-2023). The work was finished in compliance with the World Medical Association's code of ethics for research involving human subjects, the Declaration of Helsinki.

The inclusion criteria include any patient age between (18-55) years old of both sexes that diagnosed clinically with stable vitiligo and patient who has resistance to treatment for vitiligo. "Having no new lesions, no lesions progressing, and no active signs for at least a year" is the definition of stable vitiligo. There are two types of vitiligo: segmental and non-segmental. Patients with active infections or skin cancer, those who are pregnant or nursing, any skin pathology or condition that could interfere with the evaluation or necessitate the use of interfering topical or systemic therapy, patients with keloidal tendency, patients with a history of koebnerization, and patients taking immunosuppressive medications or anticoagulants are among the exclusion criteria.

Sample Size

Assuming that 71% of patients had excellent repigmentation, compared to 27% in the NCES, so the sample size will be 24 cases, calculated using OpenEpi, with power 80 % and CI 95%.

Every patient had their history taken. Taking into account the past of persistent illness, previous therapy to vitiligo, any skin diseases or drug intake, and precipitating factors such as stress.

Technique of non-cultured epidermal cell suspension grafting

The donor site was chosen to be around tenth the size of the recipient area. The lateral side of the thigh's skin was chosen as the ideal donor site. After cleaning, shaving, and 2% lignocaine anesthesia, the donor area was prepared. Straight artery forceps and a shaving blade were used to remove a split-thickness skin graft. Under aseptic conditions, the skin specimen was moved to a container filled with regular saline. The graft was placed in a solution of trypsin–ethylenediamine tetra-acetic acid (EDTA) (0•25% trypsin and 0•02% EDTA) and incubated for 40 minutes at 40°C in 5% CO2. To separate the cells from the tissue, phosphate-buffered saline (PBS) was added and thoroughly pipetted after the trypsin-EDTA solution was removed. The tissue waste that was solid was taken out. The pellet, which contained cells from the stratum basale and the lower part of the stratum spinosum, both of which are rich in melanocytes, was then removed after the supernatant was discarded. The pellet of melanocytes was mixed with 1-3 milliliters of PBS, depending on the recipient area, to create a suspension of nonculture epidermal cells. After shaving, the recipient site was cleaned with surgical spirit and betadine, and 2% lignocaine was used to induce anesthesia. Using a motorized dermabrader, dermabrasion was performed until punctate bleeding was observed. In order to avoid the halo effect, the dermal graft was stretched 5 mm past the margins. Using an 18-gauge needle and a tuberculin syringe, the NCES was carefully moved to the recipient site. Over the recipient site, a surgical dressing made of collagen, Vaseline-chlorhexidine gauze, and a sterile surgical pad was applied.

Follow-up

Follow-up and evaluation were performed at each visit. In order to identify any results following the transplant operation, patients are asked to check up at the clinic on day 8 as well as weeks 4, 8, 12, and 16. At follow-up visits, response to treatment was evaluated. Partial responders were defined as patients who had cleared 50% or more of the lesions, whereas non-responders were characterized as patients who had cleared less than 50%. Following session, evaluation of immediate and late adverse effects such as flu-like symptoms, regional pain, swelling, vesiculation, and discoloration at the site of injection was assessed.

Statistical analysis

SPSS v28 (IBM Inc., Armonk, NY, USA) was used for the statistical analysis. The mean and standard deviation (SD) of quantitative variables were displayed. The frequency and percentage (%) of the qualitative variables were reported, and when applicable, the Fisher's exact test or the Chi-square test were used for analysis.

RESULTS

Table1; showed that there was an insignificant difference regarding the demographic data (Age, Sex, Weight, HT, BMI, waist circumference, Residence, Special Habit, Family History. There was an insignificant difference regarding disease duration and previous treatment. All cases had stable disease.

Table 2; showed that there was an insignificant difference as regard VASI score before treatment, whereas after treatment, VASI was significantly low in NCES cases.

Repigmentation start period was significantly prolonged in NCES cases (Table 3).

Table 4; showed that there were insignificant differences between Koebner, pigmentation and sepsis (at donor site and recipient site). Scar in donor site was significantly Low in NCES cases .

In terms of satisfaction, in NCES cases, there were 18 (75%) very satisfied patients, 4 (16.67%) satisfied patients, and 2 (8.33%) unsatisfied patients. Satisfaction was significantly high in NCES cases as shown in Table 5.

Regarding satisfaction, in NCES cases ; 3 patients(12.5%) were not satisfied, 5 patients (20.83%) were satisfied, and 17 patients (70.83%) were very satisfied. Satisfaction was significantly high in the NCES group. (Table 6)

Table 7; Sowed that there was an insignificant difference between the number of improved patients on 8 days, after 4, 12 and 16 weeks, however, the number of patients who had improved after 8 weeks was noticeably higher in NCES cases. The percentage of improvement in NCES cases, on 8 days, 2 (8.33%) patients had been improved with an improvement percentage of 2%, after 4 weeks16 (66.67%) patients had been improved with an improvement percentage of 10%, after 8 weeks14 (58.33%) patients had been improved with an improvement percentage of 30%, after 12 weeks and after 16 weeks, the studied patients showed stable improvement where 12 (50%) patients in both times had been improved with an improvement percentage of 62%.

Table 1: Demographics of the studied cases.

		NCES cases (n=24)
Age(years)	**Mean± SD**	31.92± 10.67
	Range	19-50
Sex	**Male**	2(8.33%)
	Female	22(91.67%)
Weight(kg)	**Mean± SD**	75.31± 16.11
	Range	48-98
Height (m)	**Mean± SD**	1.6± 0.08
	Range	1.5-1.76
BMI(kg/m^2)	**Mean± SD**	29.38± 6.03
	Range	18.14-38.28
Waist circumference(cm)	**Mean± SD**	81.42± 26.97
	Range	55-128
Residence	**Urban**	18(75%)
	Rural	6(25%)
Special Habit		0(0%)
Family History		14(58.33%)
Clinical data		
Disease Duration	**Mean± SD**	7.71± 4.15
	Range	2-18
Previous Treatment	**Topical**	14(58.33%)
	Topical & NUVB	9(37.5%)
	Topical & Steroids	1(4.17%)
Vitiligo Type	**Segmental**	5(20.83%)
	Non-Segmental	19(79.17%)

BMI: body mass index.

Table 2: VASI Score of the studied cases

		NCES cases (n=24)
VASI before	**Mean± SD**	8.74± 2.07
	Range	73-161
VASI after	**Mean± SD**	4.57± 2.14
	Range	0.7-8.8

VASI: Vitiligo Area Scoring Index. *:

Table 3: Re-pigmentation of the studied cases

		NCES cases (n=24)
Re-pigmentation start period	**Mean± SD**	6.54± 0.51
	Range	6-7

Table 4: Outcomes of the studied cases

	NCES cases (n=24)	
	Donor site	**Recipient site**
Koebner	0(0%)	0(0%)
Scar in Donor site	11(45.83%)	0(0%)
Pigmentation	2 (8.33%)	6(25%)
Sepsis	0 (0%)	0 (0%)

Table 5: Objective satisfaction (double blind by dermatologist)

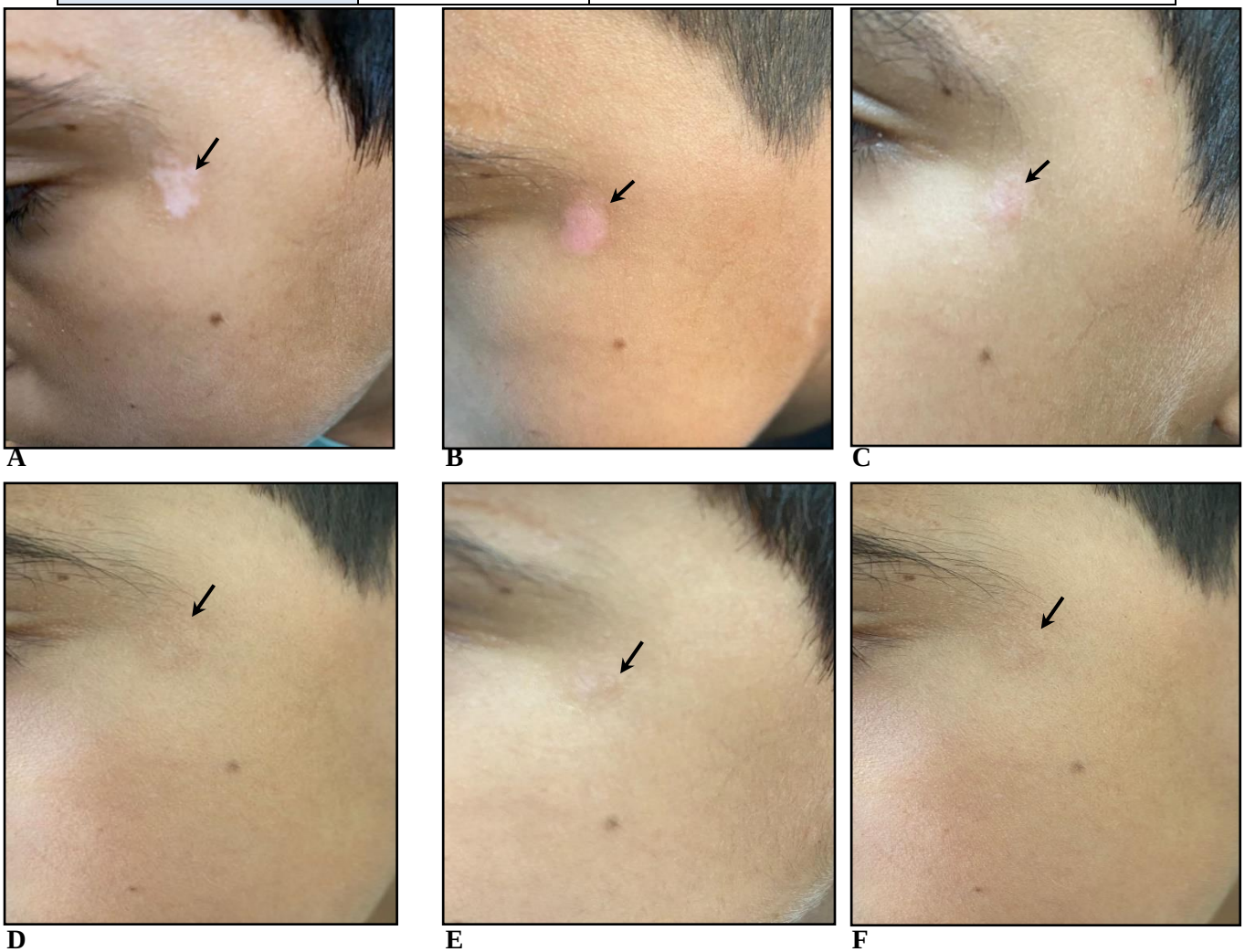
	NCES cases (n=24)
Very satisfied	18 (75%)
Satisfied	4 (16.67%)
Dissatisfied	2 (8.33%)
Very Dissatisfied	0 (0%)

Table 6: Subjective satisfaction of the studied cases

	NCES cases (n=24)
Very satisfied	17 (70.83%)
Satisfied	5 (20.83%)
Dissatisfied	3 (12.5%)
Very Dissatisfied	0 (0%)

Table 7: Percentage of improvement(repigmentation) of the studied cases

		NCES cases (n=24)
On 8 days	**Improved**	2 (8.33%)
	Not improved	22 (91.67%)
After 4 weeks	**Improved**	16 (66.67%)
	Not improved	8 (33.33%)
After 8 weeks	**Improved**	14 (58.33%)
	Not improved	10 (41.67%)
After12 weeks	**Improved**	12 (50%)
	Not improved	12 (50%)
After16 weeks	**Improved**	12 (50%)
	Not improved	12 (50%)



Case 1 NCES: A case of Vitiligo (19 years old Male). lesion 1*1 cm in size on left side of cheek**. (A)** Before VASI score = 0.1 **(B)** 1week after VASI score = 0.08 **(C)** 4weeks after VASI score = 0.05 **(D)** 8weeks after VASI score = 0.03 **(E)** 12weeks after VASI score = 0.01 **(F)** 16 weeks after VASI score = 0.01.

Case Presentation:

Case 1 NCES: A case of Vitiligo (19 years old Male). lesion 1*1 cm in size on left side of cheek. (A) Before VASI score = 0.1 (B) 1week after VASI score = 0.08 (C) 4weeks after VASI score = 0.05 (D) 8weeks after VASI score = 0.03 (E) 12weeks after VASI score = 0.01 (F) 16 weeks after VASI score = 0.01.

DISCUSSION

The purpose of this study was to determine whether transplanting noncultured epidermal suspension (NCES) is a useful treatment for stable vitiligo.

We matched the patients in our trial based on their age, sex, type of vitiligo, stability of vitiligo, and length of illness. There was an insignificant difference between VASI score before treatment, whereas after treatment, VASI was significantly low in NCES cases.

Regarding the repigmentation of vitiligo lesions we detected that the start period was significantly prolonged in NCES cases.

There were insignificant differences between Koebner, pigmentation and sepsis (at donor site and recipient site). But scar in donor site was significantly low in NCES cases.

Concerning satisfaction, it was significantly high in NCES cases in NCES cases; 4 (16.67%) patients were satisfied, 2 (8.33%) patients were disappointed, and 18 (75%) patients were very satisfied. Regarding the percentage of improvement, there was an insignificant difference between the number of improved patients on 8 days, after 4, 12 and 16 weeks, however, in NCES cases, the number of better patients was much higher after 8 weeks.

The percentage of improvement in NCES cases, on 8 days, 2 (8.33%) patients had been improved with an improvement percentage of 2%, after 4 weeks 16 (66.67%) patients had been improved with an improvement percentage of 10%, after 8 weeks 14 (58.33%) patients had been improved with an improvement percentage of 30%, after 12 weeks and after 16 weeks, the studied patients showed stable improvement where 12 (50%) patients in both times had been improved with an improvement percentage of 62%.

A retrospective study employing image analysis was conducted to assess the clinical efficacy of autologous NCES transplantation treatment for 41 patients with stable vitiligo, which aligns with our findings. Following a follow-up period of 6 to 9 months, 80.5% of the patients exhibited a favorable response, with 17.1% displaying complete or almost complete repigmentation [8].

In a comparison study, 10% of the NCES group had exceptional pigmentation, 10% had good pigmentation, 40% had fair pigmentation, and 40% had poor pigmentation. The difference was not significant in terms of statistics. In NCES, moderate scarring affected 20% of donors, while hyperpigmentation affected 40% of patients [9].

After a 12-week follow-up, Tyagi et al. [10] discovered that NCES was safe, efficacious, and equally effective at attaining even repigmentation in patients of stable vitiligo. Following a 12-week period of observation, 12 lesions (60%) showed outstanding repigmentation, 7 lesions (35%) showed good repigmentation, and 1 lesion (5%) showed fair repigmentation. These results agreed with our results with priority to our results as we follow up after 16 weeks, as the studied patients showed stable improvement where 12 (50%) patients in both times had been improved with an improvement percentage of 62%.

Percentage re-pigmentation with both ECS (non-cultured epidermal cell suspension) improved over time, with the former being considerably better at weeks 4 and 16, according to Challa et al. [11]. Over 50% of the individuals demonstrated great response, and over 60% of the subjects had good re-pigmentation at the 36-week mark. The longer follow-up period and larger participant count in this study contributed to the greater result compared to ours.

When treating patients with stable vitiligo, suction blistering graft, mini punch graft, and hair follicle transplant were compared. The results showed that the percentage of vitiligo repigmentation in suction blister technique was significantly higher than in mini punch and hair follicle techniques (p values.0001 and.001, respectively). Regarding the DLQI score following surgery, there was a statistically significant difference between the three procedures (p value =.0001). According to the results of this study, suction blistering is a safer and more successful method of treating stable vitiligo than hair follicle and small punch techniques [12].

According to Gao et al. [13], 47 (76%) of the lesions in the SBEG group were graded as very good (≥ 75%), 5 (8%) as good (50–74%), 1 (2%) as fair (25–49%), and 9 (15%) as poor (0–24%) for the degree of repigmentation following grafting using the PGA scale. 22 (39%) of the lesions in the ABEM group were rated as very good, 12 (21%) as good, thirteen (23%) as fair, and 9 (16%) as poor. Compared to the SBEG group, the ABEM group had a considerably lower degree of repigmentation ($p<0.001$). The SBEG group demonstrated a substantially greater improvement in the VASI from baseline when

compared to the ABEM group ($p < 0.001$). That outcome was approved in light of our findings.

Ding et al. [14] disagreed with our findings, claiming that tiny punch grafts (MPGs) are significantly simpler, quicker, and have fewer adverse effects at the donor site. Graft pigmentation rates were 99.3% (272/274) in SBEG (dermabrasion for recipient site), 98.7% (312/316) in MPG, and 98% (49/50) in SBEG (blister for recipient site). There was no statistically significant difference between MPG and SBEG in the relative melanin index (RMI) or relative erythema index (REI) at various time intervals. In SBEG, cobblestone appearance was the most common problem. For MPG, there were no overt side effects at the donor sites and a minor scar developed in two cases at the recipient locations.

In comparative research, 93.3% of patches that had suction blister epidermal grafting (SBEG) and 97.8% of patches that received ultrathin skin grafting (UTSG) showed good repigmentation. At one, two, and three months following the grafting surgery, there was no discernible difference between the two procedures' RMI and REI. 4.4% of the recipient site's patches that had UTSG showed incomplete fall-off of the graft, while 66.7% of the patches that underwent SEBG showed a "cobblestone appearance." Compared to SBEG, UTSG resulted in fewer problems at the donor site. Faster than SBEG, UTSG produces better cosmetic results at the donor and recipient sites [15].

CONCLUSIONS

That NCES techniques is simple and effective treatment technique for vitiligo and suitable for all skin types. Patients with stable vitiligo should be treated with NCES since it is much more successful than other options. The rate of repigmentation was high.

Recommended that:

To compare the efficacy of non-cultured epidermal suspension (NCES) transplantation in the treatment of stable vitiligo, more research with bigger sample sizes is required. It is advised to do larger-scale research with longer follow-ups to further confirm the findings' dependability. Additional histological and special stain investigations to assess the efficacy of transplanting noncultured epidermal suspension (NCES) in the management of stable vitiligo.

Conflict of interest: The authors declare no conflict of interest.

Financial disclosures: This study was not supported by any source of finding.

Sources of funding: No specific grant was obtained for this research from governmental, private, or nonprofit funding organizations.

REFERENCES

1- Nissan X, Larribere L, Saidani M, Hurbain I, Delevoye C, Feteira J et al. Functional melanocytes derived from human pluripotent stem cells engraft into pluristratified epidermis. Proc Natl Acad Sci. 2011;108(36):14861-6.

2- Syed HA, Masood S. Poroma. InStatPearls [Internet] 2024 Feb 14. StatPearls Publishing. Publishing; 2024 Jan– PMID: 32809744.

3- Huang CL, Nordlund JJ, Boissy R. Vitiligo: a manifestation of apoptosis? Am J Clin Dermatol. 2002; 3(5):301-8.

4- Ongenae K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo, Pigment cell Res. 2003; 16(2):90-100.

5- Chang WL, Ko CH. The Role of Oxidative Stress in Vitiligo: An Update on Its Pathogenesis and Therapeutic Implications. Cells. 2023;12(6):936.

6- Felsten LM, Alikhan A, Petronic-Rosic V. Vitiligo: a comprehensive overview Part II: treatment options and approach to treatment. J Am AcadDermatol. 2011; 65(3):493-514.

7- Mysore V, Salim T. Cellular grafts in management of leucoderma. Indian Journal of Dermatology. 2009 ;54(2):142-9.

8-Mysore and Salim T:Cellular grafts in management of leucoderma. Indian J Dermatol. 2009; 54(2): 142-9.

9- Liu B, Chen HH, Liu ZH, Liang JF, Xue RJ, Chen PJ et al. The clinical efficacy of treatment using the autologous non-cultured epidermal cell suspension technique for stable vitiligo in 41 patients. *J Dermatolog Treat*. 2019; 31: 1-5.

10- Hamza A, Hussein T, Shakshouk HR. Noncultured Extracted Hair Follicle Outer Root Sheath Cell Suspension versus Noncultured Epidermal Cell Suspension in the Treatment of Stable Vitiligo. J Cutan Aesthet Surg. 2019;12:105

11- Tyagi S, Malhotra SK, Kaur T. Comparative Evaluation of Efficacy of Non-Cultured Epidermal Cell Suspension and Epidermal Curettage in Stable Vitiligo. J Cutan Aesthet Surg. 2021 Jan 1;14(1):32-40.

12- Challa A, Chauhan S, Pangti R, Arava SK, Prajapati S, Pandey A et al. Evaluation of clinical efficacy and laboratory indicators of non- cultured epidermal cell suspension and hair follicle cell suspension in surgical management of stable vitiligo: A randomized comparative trial. J Cosmet Dermatol. 2022; 21(12):6958-64.

13- Ezz-Eldawla R, Abu El-Hamd M, Saied SM, Hassanien SH. A comparative study between

suction blistering graft, mini punch graft, and hair follicle transplant in treatment of patients with stable vitiligo. J Dermatolog Treat. 2019; 30(5):492-497.

14- Gao PR, Wang CH, Lin YJ, Huang YH, Chang YC, Chung WH et al. A comparative study of suction blister epidermal grafting and automated blister epidermal micrograft in stable vitiligo. Sci Rep. 2022; 12(1):393.

15- Ding X, Zhao M, Li M, Du J. A self-controlled comparative study of mini punch graft versus suction blister epidermal graft in the treatment of stable vitiligo. J Dermatol Treat. 2021; 32(6):585-9.

16- Ding X, Sun Y, Wang F, Du J. Ultrathin Skin Grafting Versus Suction Blister Epidermal Grafting in the Treatment of Resistant Stable Vitiligo: A Self-Controlled Comparative Study. Dermatol Surg. 2023; 49(7): 659-63.

Citation

Ibrahim, S., Ibrahim Mohamed, S., Gharib, K. A study of Non-Cultured Epidermal Suspension Grafting Using Trypsinized Epidermal Grafting for Stable Vitiligo. *Zagazig University Medical Journal*, 2024; (76-83): -. doi: 10.21608/zumj.2024.328961.3647