Original Article

Comparison of Smash Skin Grafting and Autologous Non-cultured Epidermal Cell Suspension in Re-pigmentation of Stable Vitiligo

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Abstract

Background: Vitiligo is the most common depigmenting dermatosis causing immense psychosocial concern. When medical therapies fail to cause re-pigmentation, surgical modalities are developed to combat the same in stable vitiligo patients. Here we are comparing two such surgeries: smash skin grafting (SSG) and autologous non-cultured epidermal cell suspension (NCES). **Aims and Objectives:** The aim of this article is to compare the efficacy of SSG and NCES in re-pigmentation of stable vitiligo and to know the feasibility of both the surgeries. **Materials and Methods:** It is an open, randomized, and prospective study conducted in dermatology outpatient department at a tertiary care center. Thirty patients with single stable vitiligo lesion were randomized into two groups: 15 each in Group A (SSG) and Group B (NCES). Following the surgery, excimer lamp phototherapy was initiated twice weekly. Patients were followed up till 16 weeks of surgery. Photo-documentation was done every month. Grading was performed for the response in the form of re-pigmentation as excellent (>75%), good (50–75%), fair (25–50%), and poor (<25%). The χ^2 test was used to analyze statistical significance. **Results:** Both the surgeries showed initial specks of re-pigmentation at 10–14 days post-surgery. Excellent response (>75% re-pigmentation) was observed in 10 (66.67%) patients in Group A and 9 (60%) patients in Group B. Both the surgeries showed equal response and uniform texture of re-pigmentation. **Conclusion:** SSG is equally effective when compared with NCES, in causing re-pigmentation. Also, SSG is simple, easy to perform, faster learning curve, less time-consuming, and cost-effective when compared with NCES.

Keywords: Autologous non-cultured epidermal cell suspension, smash skin grafting, vitiligo

Introduction

Vitiligo is an acquired condition resulting from the progressive loss of melanocytes. It is characterized by milky-white sharply demarcated macules.[1] It affects 0.5–2% of world's population with no sexual or racial predominance.[2] The highest incidence of the condition has been reported in India. It causes severe cosmetic disability and has immense socio-psychological concern.

Various treatment modalities for vitiligo include medical line of management with topical and systemic steroids, immunosuppressants, and phototherapy, which includes psoralen plus ultraviolet A, narrow band ultraviolet B therapy, targeted phototherapy devices. This mode of therapy helps in achieving stability and then in inducing re-pigmentation. Many a times, medical therapy alone does not suffice. The lesions remain static without any depigmentation or re-pigmentation. Such patients who are stable for more than a year are aptly suitable for surgical treatment.

The surgical treatment helps in transfer of autologous melanocytes from a normal pigmented donor skin to depigmented area. The surgical line of management is broadly classified into tissue and cellular grafting methods. Tissue grafts included punch grafts, suction blister grafting, split thickness grafting, and smash skin grafting

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(SSG). Cellular grafts include culture pure melanocyte suspension and non-culture epidermal cellular suspension (NCES).

To study early re-pigmentation of stable vitiligo patches, we employed two surgical techniques: SSG and NCES.

SSG is a new technique in vitiligo surgery. It is a modification of split thickness grafting. The split thickness graft obtained from donor site undergoes "smashing" before applied onto recipient area. The amount of graft needed is roughly 1/10th the size of the recipient area. The advantage of this new method is simplicity of the surgery, less time-consuming, faster learning curvature, and is very economical to patients.[3]

For NCES, the split thickness graft obtained from the donor site is treated with trypsin and incubated to separate melanocytes from keratinocytes. A pellet rich in melanocytes is obtained at the end of the procedure, which can be used to treat larger areas. The advantages of this technique are as follows: it is less time-consuming, less expensive, and does not need well-equipped tissue laboratories as in culturing techniques.[4] However, when compared with SSG, this method is more time-consuming, needs sophisticated equipment for incubation and centrifugation, and is comparatively costly.

Materials and Methods

It was an open, randomized, and prospective study conducted in Dermatology Outpatient Department at a tertiary care center over a period of 8 months from November 2020 to June 2021. Informed written consent for participation in the study and consent for photo-documentation were obtained. Patients were screened for the following inclusion and exclusion criteria.

Inclusion criteria

1. *Patients with stable vitiligo*. Stable vitiligo is defined as a patient reporting the absence of new lesions, absence of the progression of existing lesions, and absence of Koebner phenomenon in the previous 1 year[5];
2. Patients with age >15 and <60 years;
3. Patients refractory to topical and systemic treatment;
4. Patients not on any treatment 3 months prior to the start of the study.

Exclusion criteria

1. Patient with vitiligo involving >20% body surface area involvement;
2. Patients with bleeding and clotting disorders;
3. Pregnant females;
4. Patients with systemic disorders;
5. Patients having tendency for keloid formation;
6. Patients with reactive serology (HIV, HBsAG, and VDRL);
7. Patients with history of photodermatosis or history of skin cancer;
8. Patients not willing for the procedure and regular follow-up;
9. Patients with unrealistic expectations.

A wash-out period of 3 months was given to patients on prior treatment. Thirty patients with a single vitiligo patch were selected under study after considering the inclusion and exclusion criteria. Fifteen patients were randomly allocated to SSG (Group A) and NCES (Group B) each. Random allocation was done by computer-generated random numbers.

Methodology

1. Demographic data including age, sex, address, occupation, socio-economic status are obtained.
2. Site, size, extent, stability of the vitiligo patch are noted.
3. Investigations performed:
   - Complete hemogram, bleeding, and clotting time;
   - Random blood sugar;
   - Serology tests: HIV, HBsAG, and VDRL.

Procedural technique: for obtaining tissue from donor site

The anterolateral aspect of the thigh was chosen as the donor site. The donor site was surgically cleaned using 95% ethanol and povidone iodine solution. Local anesthesia using lidocaine 2% and normal saline in 1: 1 ratio was given at the donor site. Donor tissue was obtained using a sterile razor blade held at the middle along the longitudinal axis using a straight artery forceps. Very thin skin sheets 1–2 cm wide devoid of much dermis are removed by to and fro blade movements at the donor site [Figure 1]. The amount of donor tissue removed was only 1/10th of the recipient area. The tissue obtained was transferred to a bowl with normal saline. The donor site

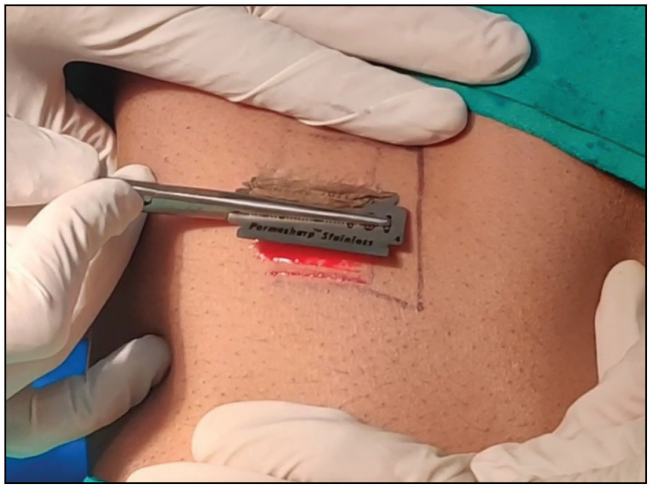

Figure 1: Harvesting the split thickness skin grafting

was then cleaned and bandaged using povidone iodine-impregnated gauze piece.

Preparation of recipient area

The recipient area was surgically cleaned and anesthetized similar to the donor area. It was then dermabraded using a manual dermabrader till minute bleeding points were observed. The dermabraded area was covered with a normal saline-soaked gauze piece.

Procedure of SSG

The bowl with the donor tissue was drained of excess normal saline, so that only a little bit of saline was left to keep the donor tissue moist. The tissue was then cut into very minute pieces using a sterile curved scissor [Figure 2]. This process of smashing the skin was continued for 15–20 min to make a fine homogeneous donor material. The smashed graft was applied to the recipient area by a sterilized spatula. Care was taken to cover up the periphery of the lesion. After applying the smashed skin, the recipient site was left open for 15–30 min for the exudates to dry.

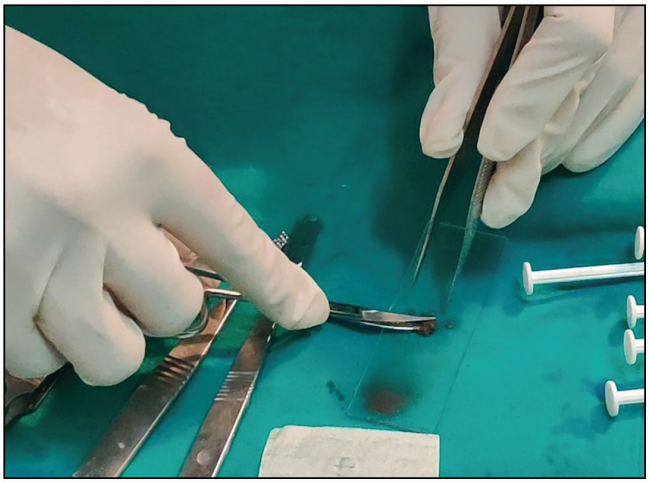

Figure 2: Smashing of the graft tissue with curved artery forceps

The recipient area was given a dressing using povidone iodine impregnated gauze and with dressing pads over it.

Procedure of NCES

After obtaining the donor tissue, it was combined with a solution of 0.25% trypsin and Dulbecco's modified eagle medium (DMEM) in a conical test tube [Figure 3(A)]. It was incubated at 37°C for 50 min in a dry bath incubator [Figure 3(B)]. Meanwhile, 5 cc of patient's blood was drawn into a plain test tube and was centrifuged at 2000 rpm for 10 min to obtain the serum [Figure 3(C)]. Excess trypsin was neutralized with patient's serum. The dermis was separated from the epidermis using a spatula to release cells from the basal layer of the epidermis into the saline [Figure 3(D)]. Transfer the cell suspension rich in basal cell layer with patient's serum and DMEM into a conical test tube, which was further centrifuged at 2000 rpm for 10 min. The supernatant fluid was then discarded, and the pellet containing melanocytes was mixed with carboxymethyl cellulose solution [Figure 3(E) and (F)]. This mixture was placed on the dermabraded recipient area and was followed by collagen and betadine gauze dressing in two layers.

Prophylactic therapy

Patients were on a course on oral antibiotic therapy and oral NSAIDS for 1 week following the procedure. Patients were advised to keep the recipient area immobile for a period of 1 week. The recipient area and the donor site were opened after a period of 7 days post-surgery.

Follow-up

Patients were started on twice weekly 308 nm excimer lamp from 7 days post-procedure. Photo documentation of recipient area and donor area and watching for any side effects were performed monthly. All patients were followed up for 4 months.

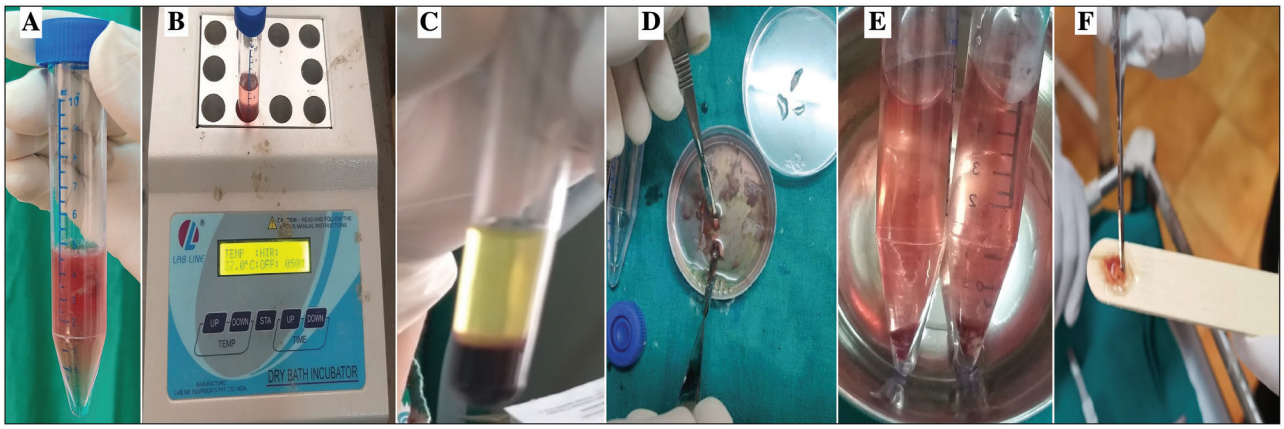

Figure 3: Steps in autologous NCES. (A) Graft tissue suspended in trypsin solution and DMEM in a conical test tube. (B) It is incubated in a dry bath incubator for 50 min at 30°C. (C) Serum obtained after centrifugation of patient's blood. (D) Separating the basal cells from the graft using spatula. (E) Melanocyte pellet in the conical test tube. (F) Mixing melanocyte pellet with carboxymethyl cellulose

Outcome measures

At the end of the study, depending on the percentage of re-pigmentation, final outcome was recorded as follows:

Grade 1 = < 25% of pigmentation (poor response);

Grade 2 = 26–50% of pigmentation (fair response);

Grade 3 = 51–75% of pigmentation (good response);

Grade 4 = 76–100% of pigmentation (excellent response).

Statistical analysis

The data obtained were entered in Microsoft Excel and were analyzed statistically by using software SPSS version 23. The χ^2 test was applied for to know statistical significance. $P < 0.05$ was considered as statistically significant.

Table 1: Demographic data of study patients

Characteristics	Smash skin grafting ($n=15$ patches)	Autologous non-cultured epidermal cell suspension ($n=15$ patches)
Sex		
Male	9 (60%)	9 (60%)
Female	6 (40%)	6 (40%)
Age range (years)	30.2 ± 11.05	31.3 ± 13.55
Duration of disease (years)	11 ± 9.8	13.3 ± 11.5
Stability of the disease (years)	6.4 ± 5.4	10.8 ± 9.8
Types of vitiligo		
Non-segmental	15	15
Segmental	0	0
Medical treatment in past	15	15
Family history of vitiligo	2 (13.3%)	6 (40%)
Leukotrichia	7 (46.7%)	6 (40%)

Results

Thirty patients were recruited into the study, of which 12 are female and 18 male patients with stable vitiligo. The mean age group of patients was 30.2 ± 11.05 years in Group A and 31.3 ± 13.55 years in Group B. All patients completed 4 months of follow-up.

All patients had a history of prior treatment with phototherapy, topical corticosteroids, oral corticosteroids, topical tacrolimus, and ayurvedic medications. The socio-demographic profile of the patients is mentioned in Table 1. Stability of the disease ranged from 1 to 25 years in both the treatment groups.

All patients had non-segmental vitiligo, upper extremity being most common site [Figure 4]. Area of vitiligo patches on which the surgery was performed ranged from 1×2 cm sq. to 7×8 cm sq. Leukotrichia was noted in 13 patients (7 in Group A and 6 in Group B).

All the patients were called after 7 days of surgery for the removal of dressing. Initially, we noticed erythematous areas with minimal crusting on removal of dressing from the donor site in all the patients. The recipient area in most patients was epithelialized completely in 7 days, and no further dressings were needed. The appearance of specks of pigmentation over the donor site was seen at 10–14 days post-surgery in both groups.

At the end of 16 weeks, the status of re-pigmentation was analyzed in both the groups. Excellent (>75%) re-pigmentation was noted in 10 (66.67%) patients of Group A [Figure 5] and 9 (60%) patients of Group B [Figure 6], which was statistically not significant in both the groups. Both the procedures gave similar re-pigmentation. Good (50–75%) re-pigmentation was observed in four (26.67%) patients each in both groups.

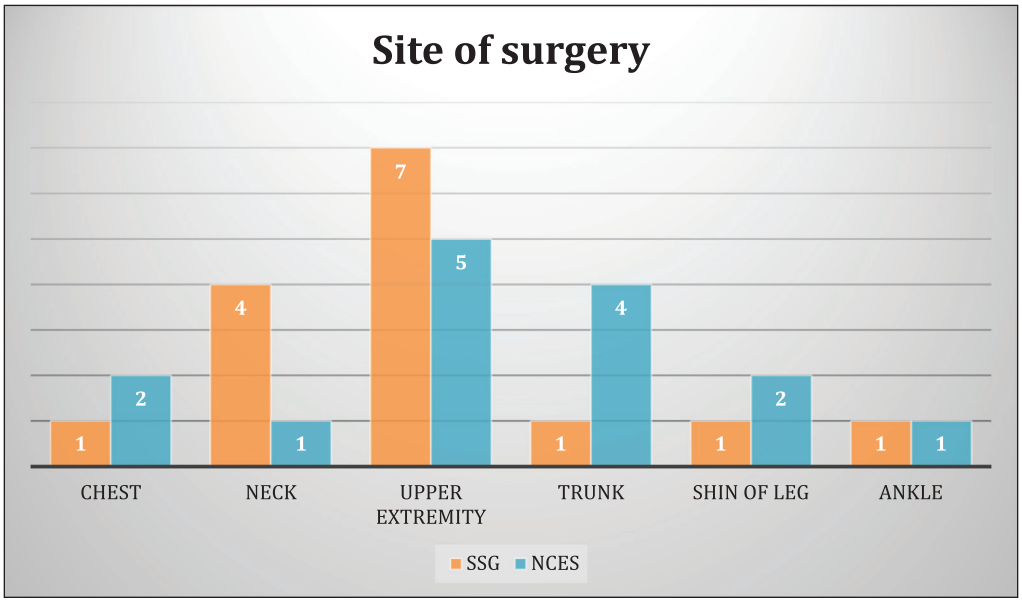

Figure 4: (Graph depicting number of patients with different sites of surgery in both groups

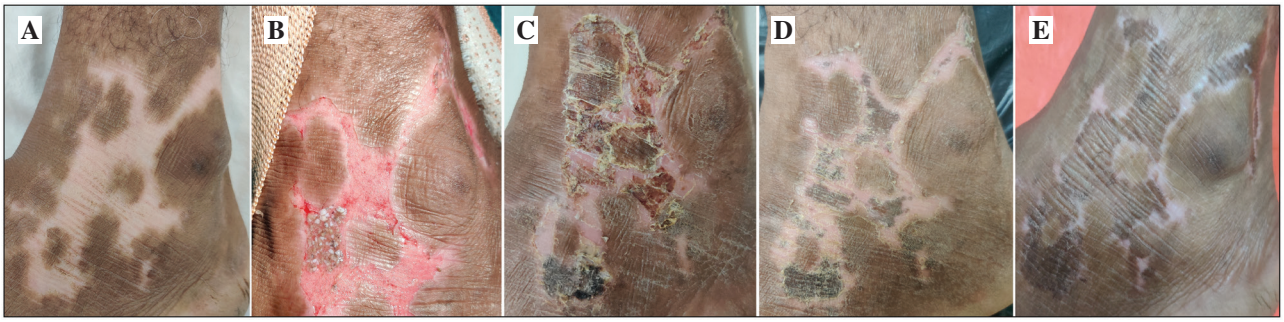

Figure 5: (A) Vitiligo patch over ankle. (B) Re-pigmentation at 16 weeks after SSG

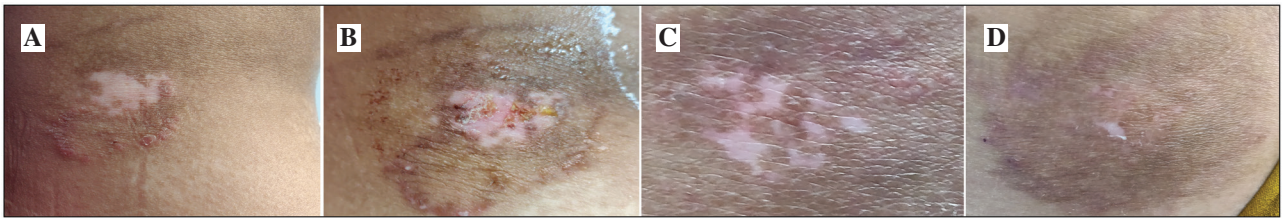

Figure 6: (A) Vitiligo patch over lower back. (B) Re-pigmentation at 16 weeks after autologous NCES

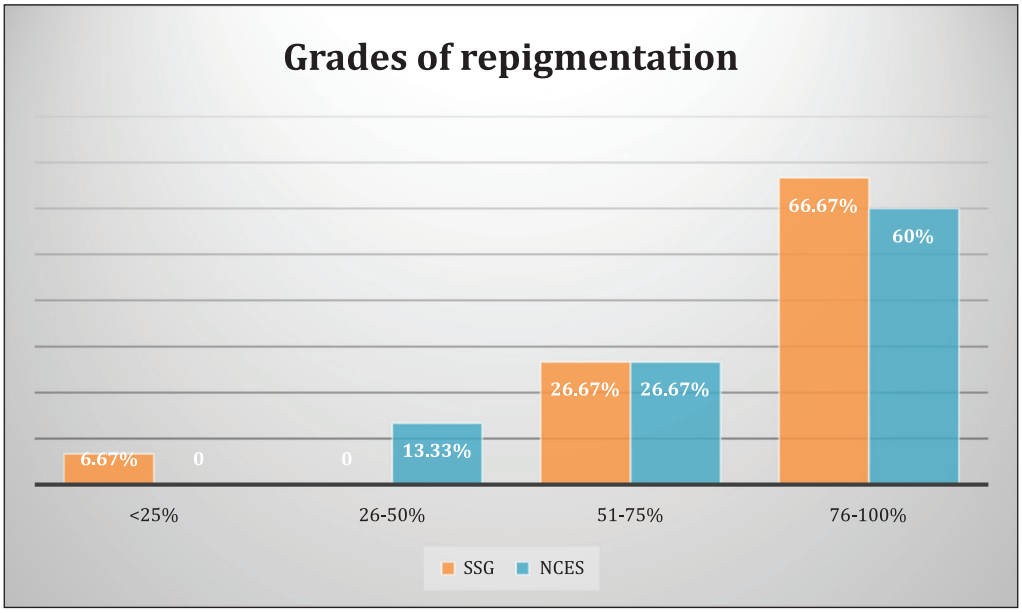

Figure 7: Graph depicting percentage of patients achieving different grades of re-pigmentation in both groups

Fair re-pigmentation (25–50%) was observed in two (13.33%) patients in Group B and poor response (<25% re-pigmentation) in one (6.67%) patient in Group A [Figure 7].

Minor complications like secondary infection were found at the donor site in three lesions (one in Group A and two in Group B), for which patients were started on oral and topical antibiotics. Contact dermatitis due to application of adhesive bandage, color mismatch with hyperpigmentation of the recipient area, and peri-graft halo were other complications [Figure 8].

The donor site re-pigmentation was completed within 1–4 months. In few patients, the donor area healed with hyperpigmentation.

Discussion

As there are numerous theories in the pathogenesis of vitiligo, there are various modalities of its treatment. They are classified as medical, light-based, surgical treatments, and depigmentation therapy. For those lesions which do not respond to medical therapy in stable vitiligo, surgical therapy is indicated.

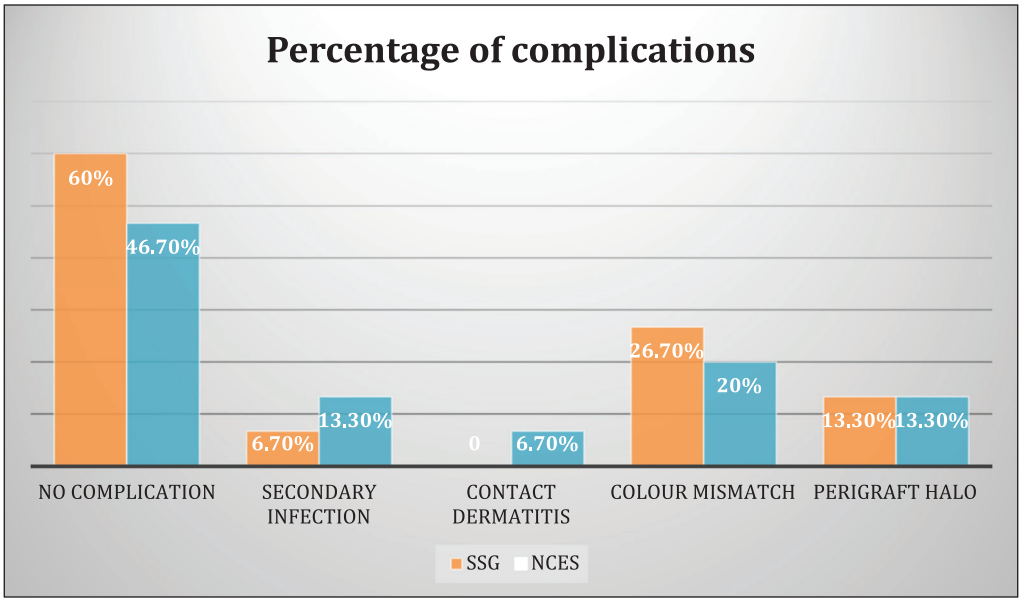

Figure 8: Graph depicting percentage of patients with complications in both groups

In the present study, a comparison between two different surgical techniques for stable vitiligo, that is, SSG and NCES, was performed. All the patients recruited in this study were resistant to previous medical therapies. In both the techniques, we noticed the initial specks of pigmentation around mean duration of 2–3 weeks following surgery.

At the end of 16 weeks of follow-up, we observed that maximum lesions achieved excellent re-pigmentation, that is, 10 (66.67%) lesions of Group A and 9 (60%) lesions of Group B. Thus, SSG gave equally similar re-pigmentation response to NCES.

In Group A, all the patients showed the peri-marginal type of re-pigmentation, where the pigment spread was seen circumferentially around the grafts which later on turned coalesced with adjacent grafts. In Group B, most of the lesions showed re-pigmentation that began from the margins which spread centripetally and few patches showed initial peri-follicular re-pigmentation that later coalesced. This signifies that the re-pigmentation was due to transplanted melanocytes by SSG and NCES.[6] In both the surgeries, the re-pigmentation was uniform in color and texture to normal skin at the end of 16 weeks of follow-up.

The idea of SSG can be conceptualized by Meek[7,8] that the multiple pieces of a large graft provide more active edges for pigment regeneration similar to postage stamp skin grafting. It is known that keratinocytes migrate from the graft edges to re-epithelialize the wound. Thus, smaller graft pieces have greater potential for regeneration at a lesser duration of time.

The need of donor tissue required for grafting is same in both surgeries, that is, donor: recipient area ratio of 1:10. Both procedures have distinct advantages because it can be performed with minimal scarring. Both the surgical modalities gave aesthetically acceptable and similar results of excellent re-pigmentation. Since re-pigmentation was noticed early in the process, patients developed confidence in the surgeries and showed good compliance to the treatment.

In the study performed by Krishnan and Kar,[3] they noticed pigmentation beginning after a period of 2–3 weeks following SSG and all patients achieved >90% re-pigmentation by 5th month post-surgery. This was similar to our study; we noticed specks of pigmentation by 2–3 weeks and since we had a shorter follow-up period, most of our patients (66.66%) achieved excellent re-pigmentation by 4 months after SSG.

We noticed excellent response in 60% of the patients with NCES similar to Pandya *et al.*,[9] who reported that an excellent response was seen in 52.17% of the cases with NCES. According to Olsson and Juhlin[10] and van Geel *et al.*,[11] a significant re-pigmentation was observed in 70% of the patients with NCES, similar to our study.

Except for few complications such as secondary infection, color mismatch with hyperpigmentation, peri-graft halo, and contact dermatitis due to adhesive bandage, none of our patients showed complications such as hypertrophic scar formation, bleeding, cobblestoning, variegated appearance, and hypopigmentation. Hyperpigmentation in the recipient site may be related to overstimulation of melanocytes by growth factors or cytokines during the healing. Gupta *et al.*[12] suggested that post-operative radiation therapy should be stopped to reduce hyperpigmentation, until sufficient color matching is obtained. Scars or keloid formations have not been reported at the donor site in any patients.

But as far as the cost is taken into consideration, Group A is very economical considering the need of specialized equipments such as an incubator, centrifuge machine, and costly reagents for Group B. Also, Group B was more time-consuming due to multiple intermediate processes involving incubation when compared with Group A, which was very feasible and less time-consuming. This study reminds the use of older, forgotten techniques such as SSG in resource-poor settings, yet having an equally effective result when compared with newer techniques such as NCES in treating stable vitiligo to achieve desirable re-pigmentation.

Previous studies have shown that suction blister grafting or split thickness grafting has achieved maximum re-pigmentation,[13] whereas we have seen excellent re-pigmentation in both the techniques employed here. Further studies are needed to confirm this. The limitation of the study was smaller sample of the study groups and shorter follow-up period. It can be recommended to study these techniques in larger number of patients over a longer period of time.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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