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DERMABRATION FOLLOWED BY TOPICAL 5% 5-FLUOROURACIL AND AUTOLOGOUS MELANOCYTE TRANSPLANTATION IN STABLE VITILIGO

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ABSTRACT

Context: Vitiligo is an acquired melanocytopenia of unknown etiology. Recent studies have shown that vitiligo areas contain dormant melanocytes which can be activated by exogenous and endogenous stimulation. Topical 5% 5-fluorouracil after dermabrasion and autologous melanocyte transplantation are two different methods of surgical management in stable vitiligo lesions refractory to medical treatment.

Aim: To compare the efficacy of topical 5% 5-fluorouracil after dermabrasion and autologous melanocyte transplantation in repigmentation of stable vitiligo lesions.

Settings and Design: At Tertiary care center, Skin opd, GNDH Amritsar, A Randomised Prospective interventional trial.

Methods: Forty stable vitiligo lesions in 20 cases of localized stable vitiligo patients not responding to medical treatment were selected and randomised into two groups. Group A lesions (n=20) were subjected to dermabrasion followed by 5% 5-FU application and Group B lesions (n=20) were subjected to dermabrasion followed by melanocyte transplantation by autologous noncultured epidermal suspension (NCES). Follow up was done at 4, 8, 12 weeks of procedure to assess the repigmentation achieved.

Statistical analysis used: Data were analyzed statistically by applying student test (t) (unpaired) &Wilcox an Signed Rank test.

Result: Patients included in the study belonged to different strata of society. Their age varied from 18-40 years and the male and female ratio was 1:3. At the end of the study, more than 75% repigmentation in 3/20 (15%) lesions in group A and 10/20 (50%) lesions in group B was achieved. There was no complication with any method. Statistically, the difference in the repigmentation by two methods was significant.

Conclusion: Topical application of 5% FU is easy to perform, does not require much expertise and is relatively inexpensive. Though noncultured autologous grafting is a novel surgical method of cellular transplantation it requires trained personnel and laboratory facilities.

INTRODUCTION

Vitiligo is apigmentary disorder of theskin and/or mucous membranes characterized by solitary or multiple achromic macules and patches resulting from loss of functioning epidermal melanocytes and sometimes hair follicle melanocytes. It is of great social and cosmetic importance, particularly in dark races[1]. It affects 1- 2% of the world's population without any racial, sexual, or regional differences in prevalence[2]. Although treatment of vitiligo has improved in recent times, it is still unsatisfactory all the medical therapies such as topical and systemic corticosteroids, immunomodulators and phototherapy offer some results but repigmentation is often incomplete. This has led to the evolution of various surgical modalities to treat recalcitrant stable lesions. Various surgical modalities for vitiligo available now include autologous suction blister grafting, [3]minipunch grafting[4], follicular unit grafting[5], smash grafting, cultured epidermal suspension.[6] Recent studies have shown the existence of metabolically inactive melanocytes with decreased dendrite size in areas showing vitiligo, which with appropriate stimulation might lead to skin repigmentation[7]. Some authors have reported the use of dermabrasion and

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topical 5% 5-fluorouracil after dermabrasion in the management of such localized stable vitiligo[8].

Aims and Objectives

To compare the efficacy of topical 5% 5-Fluorouracil application after dermabrasion versus melanocytic transplantation using non-cultured epidermal grafting.

MATERIAL AND METHOD

Forty lesions in twenty cases of localized stable vitiligo, recalcitrant to medical treatment, after histological confirmation were selected at random from outdoor patients at the Department of Dermatology, Venereology and Leprosy of the Guru Nanak Dev Hospital attached to the Government Medical College, Amritsar for the present study. Written informed consent of the cases was taken. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Inclusion criteria

Stable patients over ten years of age and not responding to amedical line of management were included in the study. The disease was considered stable when there was no eruption of fresh lesions or extension of pre-existing vitiligo lesions for last twelve months.

Exclusion criteria

Patients with active/ progressive vitiligo, keloid and bleeding diathesis, infection at the recipient site, pregnancy, and lesions occurring over palms and soles or patients with ahistory of other autoimmune diseases were excluded from the study.

Preoperative protocol

Detailed history, clinical examination regarding thesite, size and number of lesions, and relevant laboratory investigations like haemogram and coagulogram, HIV, Hepatitis B and C virus serology were recorded. Forty lesions in twenty patients were further divided into two groups i.e. Group A (20 lesions subjected to topical 5% 5-FU application after dermabrasion) and Group B (20 lesions of stable vitiligo were subjected to dermabrasion followed by subsequent melanocyte transplantation by autologous noncultured epidermal suspension obtained from healthy normochromic skin zone.

Group A.

The selected lesion was anesthetized with local application of eutectic mixture of local anesthetics (EMLA) patch sixty minutes preoperatively. Electrical or mechanical dermabrader of appropriate size was used to abrade the lesions up to the junction of mid and deep papillary dermis. The lesions were further deep dermabraded manually for better depth control up to the junction of theupper and mid-reticular dermis. The perimeter of the lesions was then feathered gently into the surrounding one cm of normal skin. Haemostasis was achieved by compression with a normal saline soaked piece of gauze on the abraded area. This was followed by a topical 5% 5-fluorouracil occlusive dressing daily for seven days and complications if any were recorded. This was followed by antibiotic-impregnatedgauzedressing every 3rd day up to 2weeks and then weekly up to one month to prevent secondary infection until epithelisation was completed. Postoperatively oral antibiotics and non-steroidalanti-inflammatory drugs were given for ten days.

After 48 hours, arecord of post-operative complication like pain, edema, exudation, crusting, secondary infection, or any allergic reaction was made. Two weeks later, complications like secondary infection, crusting, or anallergic reaction if any were recorded.

Group B

Donorsite preparation and graftharvestation

Normally pigmented skin of anterolateral aspect of thethigh was selected for donor grafts. Preoperatively, the donor site was anesthetized with the application of eutectic mixture of local anesthetics (EMLA) for 60 minutes. After surgical preparation, the split skin graft was harvested (equal to one-tenth of avitiligenous area approximately) with the cutting edge of razor blade advanced tangentially through the upper papillary dermis. The emphasis was laid on the thinness of the graft rather than getting it in one piece (unlike Thiersh graft). The graft was then transferred to a petri dish containing saline. After achieving hemostasis, pressure dressing was applied to the donor areas.

Preparation of non-Cultured Melanocyte Graft suspension

The skin graft harvested was cut into small pieces and transferred to a petri dish containing Trypsin (0.25%) and ethylenediaminetetraacetic acid (EDTA 0.02%) solution (Sigma Aldrich). The graft was incubated at 37^0C for 50 minutes with the epidermal side upwards. After incubation, the trypsin-EDTA solution was pipetted out and the graft was washed in phosphate buffered saline (ready-made sachets from Sigma Aldrich, reconstituted in distilled water) to neutralize the trypsin. The dermis was separated from the epidermis, and the dermal side of the epidermal layer was scraped using a blunt forceps to release cells from the basal layer of the epidermis into the saline. The solid waste of the tissue was removed and the suspension thus obtained was centrifuged at 1,000 rpm for 5 minutes. The supernatant was then discarded and the pellet containing melanocytes, and basal keratinocytes was diluted in patient's own serum. This suspension was then loaded into a tuberculin syringe attached to an 18-G needle.

Recipientsite (Vitiligenousarea) Preparation

After achieving adequate anesthesia and surgical preparation, the vitiligenous sites were abraded down to thedermo-epidermal junction with manual or electric dermabrader until pinpoint bleeding was observed. The dermabrasion was extended one cm beyond the margins of the lesions. After achieving adequate hemostasis, the denuded areas were covered with gauze moistened with phosphate-buffered saline. On the recipient site, the melanocyte suspension within the tuberculin syringe was spread uniformly onto the dermabraded area. The site was then left open for 15-20 minutes to allow for drying of the exudates so that the grafts remain adherent to the vitiligenous site rather than the overlying dressing. The recipient area was covered with a collagen dressing (Kollagen) and sterile gauze moistened with phosphate-buffered saline. The patients were immobilized for six hours to allow cells to adhere to the recipient site rather than to the dressing. Further, the patients were instructed to avoid vigorous activities and perform only restricted movements for next ten days. Postoperatively, patients were put on oral antibiotics, analgesics, and anti-inflammatory drugs for ten days when the dressing was removed and all surgical sites were examined and left without protection thereafter. Patients were advised to

avoid scrubbing the area for one more week but were allowed to gently bathe. No adjuvant treatment was given except for sunlight exposure after two weeks of surgery.

Postoperative follow-up (fig 1,2,3)

The patients were assessed postoperatively at four, eight and twelve weeks in order to assess the progress of repigmentation with the surrounding normal skin. At the end of the study, the percentage of repigmentation was graded in both the groups as (Table no.1)

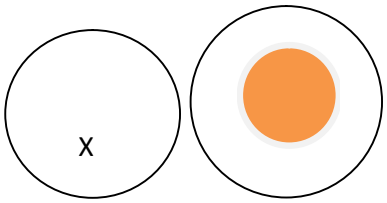
Table 1

Grade of repigmentation	Percentageof repigmentation
Grade 0	no pigmentation
Grade I (minimal)	<25%
Grade II (mild)	26-50%
Grade III (moderate)	51-75%
Grade IV (marked)	76-100%

Repigmentation of all the recipient areas was assessed as:

% of repigmentation= Y/X x 100

Where, X = Area of vitiligo Y = Area of repigmentation (shaded area)



Comparison of the grades of repigmentation of both groups was performed to assess the relative efficacy of the procedure. Patients were also assessed for complications such as bleeding, secondary infection, scarring, hyperpigmentation, hypopigmentation, keloid formation, hypertrophic scar formation and Koebner's phenomenon.

Statistical methods

Data wereanalyzed statistically by applying student test (t) (unpaired) &Wilcox a Signed Rank test.

RESULTS

All the patients completed the study of twelve weeks and were included in the final analysis. Baseline characteristics of the patients were recorded. All 20 cases in our study had a history of prior treatments: corticosteroids (oral or topical), PUVASOL (oral or topical), placentrex gel, topical irritants, levamisole, indigenous and homeopathic medicine. All patients had not attempted any treatment for three months prior to enrolment in the current study. The socio-demographic profile of patients is as follows (Table 2).

Table 2 The socio demographic profile of patients

Age incidence (in years)	12-20	4 (20%)
	21-30	12 (60%)
	31-40	3 (15%)
	41-50	0 (0%)
	>50	1(5%)
Male: female ratio	2:3	
Family history	Positive in 2 (10%)	
Duration of disease (in years)	>5	12 (60%)
	3-5	1 (5%)
	1-3	7 (35%)
Duration of stability (in years)	1-2	12 (60.0%)
	2-4	1 (5%)
	>4	7 (35%)
Type of vitiligo	Localized segmental	16 (80%)
	Localized focal	4 (20%)

When the status of repigmentation of two groups was analyzed at the end of twelve weeks(Table 3), more than 75% of repigmentation (grade IV) was noticed only in 2(10%) lesions of Group A (topical 5% 5-FU group). On the other hand10 (50%) lesions of Group B (autologous melanocytes transplantation group) showed grade 1V pigmentation. 50-75% of repigmentation (grade III) was observed in 9 (45%) lesions in Group A while it was in 6 (30%) lesions in Group B. Repigmentation between 26-50% (grade II) was achieved in 6 (30%) lesions in Group A and in 3 (15%) lesion of group B.2 (10%)lesions of group A and 1(5%) lesion of group B showed grade I repigmentation. While 1(5%) lesion of Group A showed no repigmentation. The results were not significant statistically (p>0.05).

Table 3 Results of repigmentation at 12 weeks.

	Results of repigmentation (%age of repigmentation)	Number of patients (%)		Z value	P value	Significance
		Group A	Group B			
12th wk follow up	Grade 0	1(5%)	0(0%)	-1.942	0.52	Not significant
	Grade I (<25%)	2 (10%)	1(5%)			
	Grade II (25-50%)	6 (30%)	3 (15%)			
	Grade III (50-75%)	9 (45%)	6(30%)			
	Grade IV (>75%)	2(10%)	10 (50%)			
	Total	20 (100%)	20 (100%)			

DISCUSSION

Vitiligo is a major psycho-social problem,especially in dark-skinned individuals. The treatment of vitiligo has undergone evolutionary changes down the ages. During the last few years, several surgical techniques have been developed to replenish the melanocytes population in depigmented skin lesions. Chances of the hypertrophic scarring, post-operative hypo or hyperpigmentation, polka dot appearances are quite high with tissue grafting techniques, while cultured techniques of melanocytes transplantation require special lab facilities, equipment, and expertise. So the present study investigated the comparative efficacy of topical 5% 5-FU application after dermabrasion with that of autologous melanocytes transplantation by NCES.

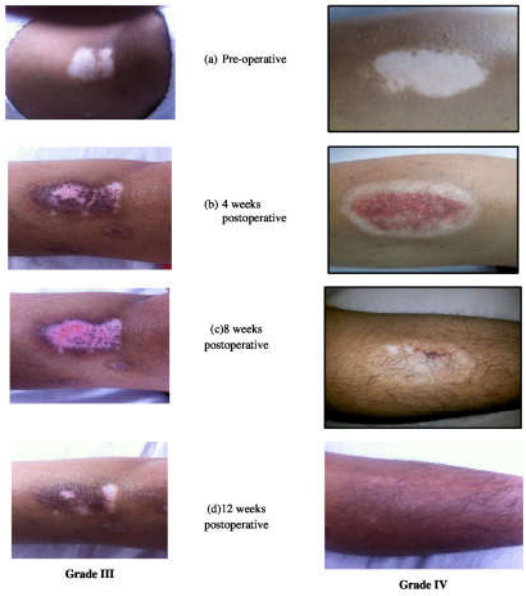
Time taken for re-epithelisation of the dermabraded patch treated with 5%5-FU was 3-4 weeks as 5-FU causes necrosis of the skin hence longer healing time[8]. All patches showed pseudomembrane formation with surrounding erythema for postoperative 7days which had to be removed mechanically before next dressing.

In the present study, we observed early pigmentation at two weeks, which continued improvement up to and beyond three months. In group A, usually, repigmentation started as small brown perifollicular macules, which then enlarged and coalesced. However, in some patches repigmentation appeared diffusely right from the beginning. The mechanism of the pigmentation was probably by overstimulation of the follicular melanocytes, which subsequently migrated to the surface during re-epithelisation, resulting in hyperpigmentation[9]. Hyperpigmentation is a known side-effect of 5-fluorouracil, observed during the treatment of skin tumors and psoriasis[10]. However, in our study, in group B all patients demonstrated a “perimarginal/perigraft” type of repigmentation. Pigment spread was initially seen circumferentially around the graftsand later became “diffuse”. This suggests that repigmentation was due to transplanted melanocytes by NCES.

Autologous melanocytes transplantation grafting was found to be a better technique than topical 5% 5FU application after dermabrasion as per the degree and time took for repigmentation. A good number of lesions i.e. 10 (50%) lesions in Group B were able to achieve >75% repigmentation and only 2(10%) lesions in group A achieved >75% of the pigmentation at the end of twelve weeks.

The results from our group A cohort were comparable to results reported byTsuji T, Hamada T on 28 patients with stable vitiligo showing the efficacy of the dermabrasion

Figure 1: SHOWING PRE-OPERATIVE AND POST OPERATIVE REPIGMENTATION IN GROUP A AND B



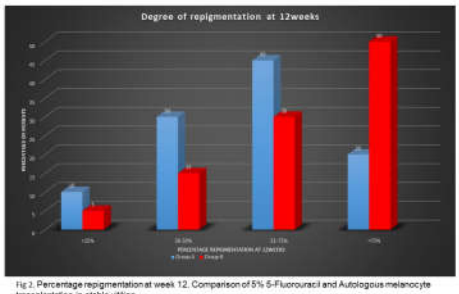


Fig 2. Percentage repigmentation at week 12. Comparison of 5% 5-Fluorouracil and Autologous melanocyte transplantation in stable vitiligo.

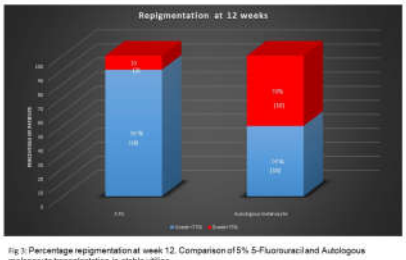


Fig 3: Percentage repigmentation at week 12. Comparison of 5% 5-Fluorouracil and Autologous melanocyte transplantation in stable vitiligo.

followed by 5% 5-fluorouracil technique to be 64%′.While in a study conducted on 30 patients of stable vitiligo by Shweta Sethi *et al*, the efficacy was upto the tune of 73.33% after six months of followup[11]. Our results were in concordance with these studies. The results from our Group B cohort were comparable to those reported byVan Gel *et al*, who reported up to 72% of repigmentation within 3-12 months. Similarly, Olsson *et al* (1993) reported 85% success rate after application of basal cell layer epidermal cell suspension rich in melanocytes. The treated area in their study ranged from 2 to 10 times the donor area, which was comparable to our study[12]. Repigmentation of more than 90% was observed in 88.8% patients in the study conducted by Kanika Sahni *et al* (2011) in which autologous melanocyte transplantation was performed using patients' own serum after 16 weeks of surgery. Except for hypertrophic scar and secondary infection in one case each, none of our other cases showed any complications such as milia formation, perigraft halo, hypertrophic scar formation, bleeding, variegated appearance or hypopigmentation. Both the techniques were found to be safe and effective for achieving repigmentation of recalcitrant stable vitiligo patches. Since the donor area required for graft was small ($1/10^{th}$ of the recipient area), small donor sites were able to achieve repigmentation of large recipient areas. We did not observe any of the complications that are frequently encountered when other methods are employed (cobblestoning, variegated appearance, beaded edges,and scarring etc.).As repigmentation is slow to progress and may continue beyond twelve months following the transplantationprocedure, further studies with longer follow-up and a larger patient cohort are eagerly anticipated.

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