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Repigmentation of Stable Vitiligo after Punch Minigrafting Followed by Excimer Laser: A Prospective Study

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ABSTRACT

Punch grafting is a well-established technique for the treatment of recalcitrant stable vitiligo. Post-operative exposure to Narrow-band Ultraviolet-B (NB-UVB) radiation was reported to augment the effect of punch grafting with better and rapid cosmetic results than monotherapy. This prospective study was done to evaluate the results of combination therapy with punch minigrafting followed by 308 nanometer (nm) excimer laser for the management of stable focal or segmental vitiligo. Thirty patients with refractory stable focal or segmental vitiligo were treated with minigrafting followed by postgrafting 308 nm excimer laser three times weekly for 24 sessions. The patients were followed up every three months up to one year post-operative for evaluation of the results. All thirty patients showed repigmentation and overall results were graded as excellent in 16 patients (53.33%), good in 12 patients (40%) and fair in 2 patients after one year of postoperative follow-up. Two patients developed depigmentation at the donor site, whereas the transplanted areas retained the pigment in all patients. Percentage of excellent response was significantly higher in patients younger than 30 years compared to patients older than 30 years ($p \leq 0.05$). Excellent response was also higher in patients with segmental vitiligo compared to patients with focal vitiligo but the difference was not statistically significant ($p > 0.05$). In conclusion, combination treatment with punch minigrafting and postsurgical exposure to 308 nm excimer laser in patients with stable focal or segmental vitiligo can lead to fast, cosmetically satisfying results especially in younger age groups.

Key words: Punch minigrafting, excimer laser, segmental vitiligo, focal vitiligo, repigmentation

INTRODUCTION

Vitiligo is a common acquired depigmentary skin disorder occurring in about 1% of the world's population, regardless of age, sex and skin color. It results from melanocyte destruction with loss of melanin which causes sharply demarcated depigmented skin macules. The basic pathogenesis remains unknown (Mosher *et al*., 1993).

A number of therapeutic options for repigmentation of vitiligo are available. Treatments, such as topical steroids, Psoralen Plus Ultraviolet A (PUVA) and narrowband (311 nm) UVB (NB-UVB) are currently the most widely used but yield unsatisfactory results in all or most of cases (Taneja, 2002).

Surgical therapies can be used alone or in conjunction with medical therapy to achieve repigmentation in cases refractory to medical treatment, provided that the disease is stable. Surgical treatment options for vitiligo offer the potential for rapid and more desirable amounts of

repigmentation. The different modalities of surgical techniques include organ-cultured fetal skin allografting, epidermal culture grafting, melanocyte culture grafting, autologous noncultured melanocyte-keratinocyte cell transplantation, epidermal grafting by the suction blister technique, thin Thiersch split skin grafting or miniature punch grafting. These methods depend on transplant of melanocytes from areas of normal skin to the vitiliginous patches (Falabella, 1989; Van Geel *et al.*, 2001; Ozdemir *et al.*, 2002; Yaar and Gilchrest, 2001; Rusfianti and Wirohadidjodjo, 2006).

The 308 nm excimer laser emits a monochromatic light of 308 nm and induces photobiological effects similar to NB-UVB, thus stimulating adjacent melanocytes in the outer root sheath of hair follicles and on the margins of lesions to migrate and repopulate the vitiliginous areas. Several studies have shown the response of vitiligo patches to excimer laser and United States (US) Food and Drug Administration (FDA) has approved it for the treatment of vitiligo and psoriasis (Homan *et al.*, 2011; Nicolaidou *et al.*, 2009; Hofer *et al.*, 2006; Casacci *et al.*, 2007; Al-Mutairi *et al.*, 2010). Excimer laser permits the selective treatment of only lesional skin in a small number of treatment sessions over a short period of time and ensures no unnecessary radiation of healthy skin. Thus the patient receives less radiation, reducing the risk of skin aging and carcinogenesis. Furthermore, the unsightly tanning of all perilesional skin is avoided (Casacci *et al.*, 2007).

Combination of grafting and other nonsurgical modalities such as PUVA (Skouge *et al.*, 1992; Barman *et al.*, 2004) and narrow-band UVB (NB-UVB) (Lahiri *et al.*, 2006) have been used to achieve further repigmentation with better and faster results than monotherapy. Similarly, fewer studies reported that punch grafting or split-skin-thickness grafting followed by excimer laser was found to be effective in inducing repigmentation in vitiligo patients (Homan *et al.*, 2011; Al-Mutairi *et al.*, 2010). In most of these studies, the results were evaluated only in terms of extent of pigmentation. Complications and color match were evaluated separately. This approach, however, may not give a fair idea about the results.

This study was done to evaluate the results of combined treatment of stable focal and segmental vitiligo with punch minigrafting followed by postoperative excimer laser.

MATERIALS AND METHODS

Patients: Thirty patients with focal stable or segmental vitiligo were enrolled in this study. The patients were seen during the period from January 2007 to October 2009. In patients with stable focal vitiligo, only one site was selected for the study. Stable vitiligo was defined as no progression of existing lesions or no appearance of new lesions during the previous 12 months, absence of the Koebner phenomenon and a positive minigrafting test which was done according to Falabella *et al.* (1995). A minigraft test was performed by implanting two minigrafts (2 mm) in the lesions to be grafted. Patients with 1 to 2 mm spread of pigment beyond the graft margins after 3 months were selected for final transplantation.

Exclusion criteria included a large vitiliginous patch, history of hypertrophic scarring and/or keloid, koebnerization and bleeding diathesis. Certain areas such as body folds, fingers, toes, palms, soles, lips, eyelids, nipples and areola, elbows, knees and genitals were considered difficult to treat sites and were excluded, due to the difficulty in immobilizing the treated area. Also, pregnant women, patients receiving immunosuppressive therapy and patients with obsessive or compulsive attitudes toward their pigmentary defect, especially when they are excessively concerned about their aesthetic alterations, were excluded from the study.

All participants provided signed informed consent. Information about each patient's name, age, sex, occupation and contact details were noted on a form. A detailed medical history, general examination and systemic evaluation were performed in all the patients to exclude any other concomitant dermatological or medical disorders. All patients had a baseline photograph taken. A baseline investigation, including complete blood count, differential count and coagulation profile, was performed on all patients.

Preparation of donor site: The site used was the lateral aspect of thigh or the lateral-upper area of the arm. After shaving off the hairs and proper cleansing with povidone iodine and 70% ethanol, the area was anaesthetized with 1% xylocaine with epinephrine. Two millimeter punches were used to prepare the donor chambers. The chambers were made at a distance of 3-4 mm from each other (Fig. 1). The depth of the donor grafts should not include the subcutaneous fat. If it is present, it was trimmed off. The number of the minigrafts was decided according to the size of the recipient vitiliginous area. Figure 1 shows donor site on the lateral upper arm immediately after taking nine 2 mm punch grafts in one of our patients with stable focal vitiligo on the right leg. The donor grafts were immersed in a petri dish containing normal saline, till preparation of the recipient site. The defects created at the donor sites were then covered with normal dressing for 10 days.

Preparation of recipient site and postoperative care: The recipient site was prepared and anaesthetized as done at the donor site. Similar number of punch grafts was removed from the recipient site using the same size of punches. The tissues removed from the recipient site were taken 1 mm deeper than that taken from the donor site to reduce the possibility of cobble-stone appearance. Hemostasis was achieved by pressing a saline-soaked gauze piece over the area. Donor grafts were then placed at the holes created at the recipient site which was done at a distance of 8-10 mm (Fig. 2). Care was taken to ensure that the graft is not folded and the tissue is not crushed or placed upside down. The needle of the syringe or the tip of the scissors was used for the proper placement of grafts in the recipient chambers. The grafts were then fixed at the recipient site using a fixing spray (Opsite®,UK) by applying the spray to the surface of the grafts at a distance of 15 cm and then left for 5 min before dressing. Figure 2 shows seven donor minigrafts

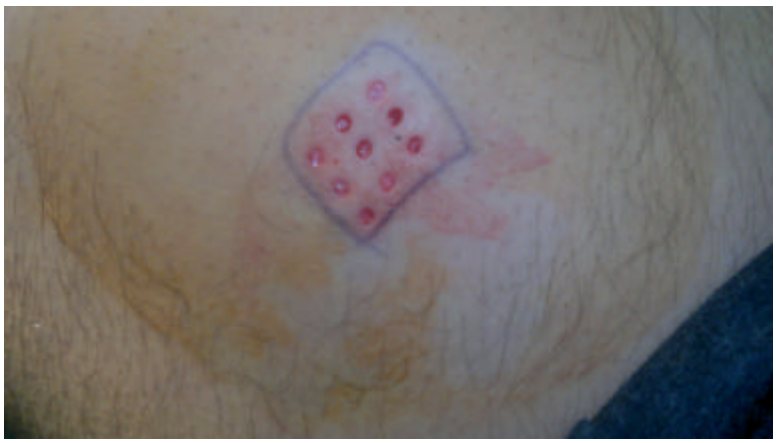

Fig. 1: Patient at donor site immediately after taking the minigrafts

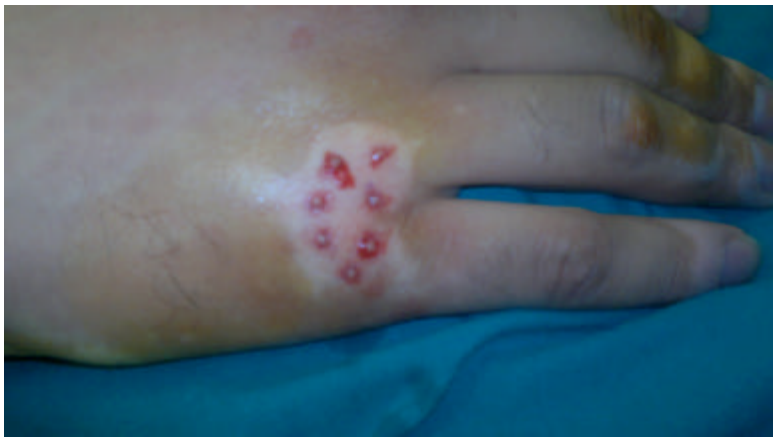

Fig. 2: Patient with focal vitiligo immediately after PMG procedure

immediately after it was placed at the holes created at the recipient site on the right hand of one of our patients. The holes were done at a distance of 8-10 mm on the vitiliginous area.

After placing the punch grafts in the acceptor site, both the acceptor and donor sites were covered with steri-strip and bio-occlusive micropore. Immobilization of the recipient area was then done using a pressure bandage. Dressings were opened after 24 h to look for any dislodgement of grafts. Patients were advised to avoid excessive movement of the grafted area. Systemic antibiotics were not given routinely except in diabetic patients with large number of grafts. Two weeks after surgery, the dressing was removed and patients were asked to apply topical antibiotic cream twice daily for another one week.

Excimer laser: A monochromatic 308 nm xenon chloride (XeCl) excimer laser (Talos, WaveLight®, Germany) was used. The laser was operated with pulse repetition frequency of 200 hertz (Hz) and impulse energy of 6.5 mJ. The pulse width was 60 nsec. Laser light was delivered through an articulated mirror arm to a handpiece with spot diameters of 15, 20 and 25 mm that was changed according to the size of the treated lesions. Excimer laser was administered three times weekly but never on 2 consecutive days, for an average of 8 weeks. Treatment was started with 100 mJ^{-2} centimeter ($mJ\ cm^{-2}$) for body lesions and 50 $mJ\ cm^{-2}$ for the face and neck. This was increased by 50 $mJ\ cm^{-2}$ per sitting until erythema appeared. If erythema remained less than 48 h, the dose was kept the same. If erythema persisted more than 48 h, the dose was reduced to the last well-tolerated one. Whenever burning or blistering developed, a treatment was skipped. The eyes were protected with UV-protective goggles. Only the application of emollients was allowed during the study.

Response assessment: Final assessment of the response was done one year after the transplantation procedure which is a sufficient time for the emergence of the final picture of the surgical procedures. The scoring system in this study was similar to that suggested by Gupta *et al.* (2002). They have developed a scoring system with holistic approach considering the extent of pigmentation, color match and the complications of the donor and the recipient areas, all taken together. In this scoring system, the score for individual criteria was multiplied with a factor, the

value of which was decided on the basis of relative importance of each criterion. The extent of pigmentation and color match was multiplied with a positive factor (×5 and ×3, respectively), while the complications of both the donor and the recipient areas were multiplied with a minus factor (×-2 and ×-3, respectively). The scoring was done by two independent observers who are regularly involved in the surgical correction of vitiligo. The final results of the evaluation by both investigators were identical in all the patients. Patients were also asked to judge the results of punch grafting as excellent, good, fair or poor. Furthermore, they were asked if they would recommend punch grafting to other patients with vitiligo.

Statistical analysis: The data were analysed using the statistical package SPSS version 15.0. The data were described using the mean for quantitative data and percent for qualitative data. Statistical analysis was performed using chi-square (χ^2) test. The significant level was set at $p<0.05$.

RESULTS

This study included 30 patients (16 female and 14 male) with focal stable or segmental vitiligo. All the patients were treated with 2 mm punch grafting in combination with excimer laser. Eleven patients had segmental vitiligo and 19 with focal vitiligo. Eight of the treated lesions were on the head and neck, three on the hands, three on the foot, three on the trunk, six on the legs, six on the arms and one on the thigh. Three individuals with focal vitiligo vulgaris had hyperthyroidism and one had hypothyroidism. In the segmental group, none of the individuals had any endocrinal abnormalities.

The patients' characteristics are shown in Table 1. All the patients had received various therapies in the past, including topical steroids, PUVA, NB-UVB, topical calcineurin inhibitors and excimer laser with minimal or partial response. Some patients received combination of therapies. None of the patients had received surgical treatment. The period for which the disease was clinically inactive ranged from one up to 10 years (mean of 2.54 years).

Investigators' global assessment for evaluation of the final response was done one year after surgery using the scoring proposed by Gupta *et al.* (2002). Sixteen patients (53.33%) were given a score of 17 to 21(excellent response) (Fig. 3), 12 patients (40%) given a score of 12 to 16(good response) and 2 patients (6.66%) given a score of 7 to 11 (fair response). So, 93.33% of patients were assessed to have an outcome ranging from good to excellent (Table 1). Figure 3a shows a 20-year-old male with segmental vitiligo on the left upper leg before treatment (recipient site). Figure 3b shows the same patient 12 months after punch minigrafting (PMG) and excimer laser with excellent results regarding both pigmentation and color matching, with no evidence of any complications either at donor or recipient sites.

Seven of 11 patients with segmental vitiligo (63.36%) showed excellent response according to investigators' global assessment (Table 2). Of the 19 patients with focal vitiligo, 9 patients (47.36%) showed excellent response. The comparison between both groups was not statistically significant (Table 2). Of 14 patients having age of 30 years or below, 11 of them (78.57%) showed excellent response, while of 16 patients aged more than 30 years, only 5 of them (31.25%) showed excellent response (Table 2). This difference was statistically significant with $p<0.05$ (χ^2 test) (Table 2).

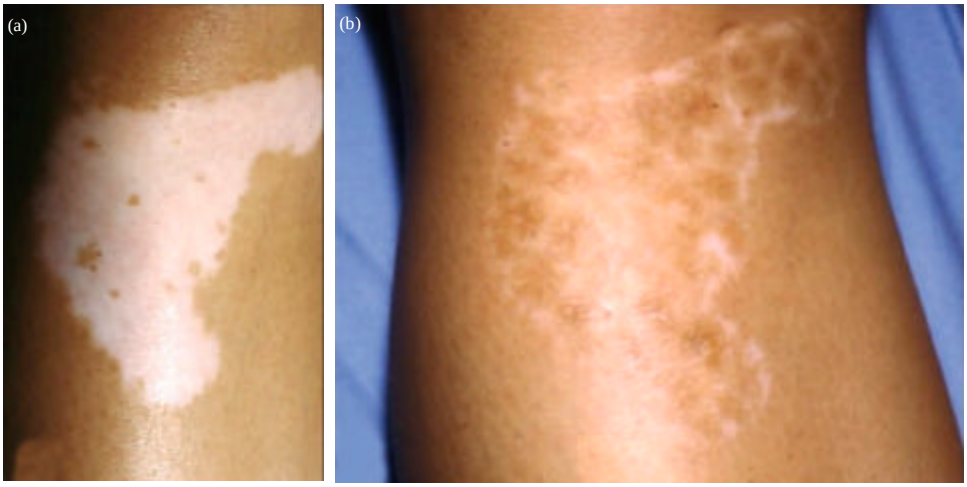

Fig. 3(a-b): (a) A 20-year-old male with segmental vitiligo before treatment (recipient site) and (b) Same patient 12 months after PMG and excimer laser showing excellent results

Table 1: Patient characteristics and response to treatment according to investigators' global assessment

	Excellent response	Good response	Fair response	All patients
Patient				
Number	16	12	2	30
Percentage	53.33	40	6.66	100
Age (year)				
Mean	27.64	3.08	37.5	31.86
Range	18-50	22-49	36-39	18-50
Sex				
Male	6	7	1	14
Female	10	5	1	16
Type of vitiligo				
Segmental	7	4	0	11
Focal	9	8	2	19
Duration (years)				
Mean	8.11	9.3	10.5	9.7
Range	3-15	8-17	9-12	3-17
Number of grafts				
Mean	34	31.6	16	35.33
Range	16-50	20-50	12-20	12-50
Area (cm^2)				
Mean	29.4	29.58	13	3.06
Range	12-45	16-45	10-16	10-45

Patient's global assessment were as follow: 15 as excellent (50%), 12 as good (40%) and 3 as fair results (10%). So, 90% of patients assessed their outcome ranging from good to excellent (these results were not shown in a table).

The complications observed during the study were minimal. It occurred in 5 patients (16.66%).Two patients developed depigmentation at the donor site, whereas the transplanted areas

Table 2: Effect of age group and type of vitiligo on the percentage of excellent response analyzed by chi-square (χ^2) test

	Excellent response		
Variables	No.	%	p-value
Age			
<30 years (n =14)	1	78.57	0.0095
>30 years (n =16)	5	31.25	
Type of vitiligo			
Segmental (n = 11)	7	63. 63	
Focal (n = 19)	9	47.36	0.4

*Values are significant at p≤0.05

retained the pigment. Two patients developed cobble-stoning. One patient developed infection followed by keloid formation at the donor site. None of the patients developed depigmentation of the transplanted skin during the follow-up period till one year post-operative.

DISCUSSION

Currently, the modalities of vitiligo treatment, especially dermatosurgery, have increased slowly but steadily. There are several techniques, ranging from simple to complicated and sophisticated approaches (Rusfianti and Wirohadidjodjo, 2006). Punch grafting is a widely used method in treating vitiligo, as it is relatively inexpensive and easy to perform (Falabella and Barona, 2009). However, after punch grafting, the pigment spread takes its own time that may be several months. Ultraviolet radiation following punch grafting may stimulate the migration of melanocytes from the grafts into the vitiliginous skin, thereby increasing the rate of repigmentation. So, combination treatment with grafting and postsurgical PUVA or NB-UVB therapy has been used to augment and speed the rate of pigmentation (Barman *et al*., 2004; Homan *et al*., 2011; Lahiri *et al*., 2006).

The 308 nm excimer laser emits a monochromatic light of 308 nm and induces photobiological effects similar to NB-UVB but with fewer side effects. So, this study was done to study the results of combined treatment of stable focal and segmental vitiligo with punch minigrafting followed by postoperative excimer three times weekly for 24 settings.

In the present study, the results were assessed using a novel scoring system suggested by Gupta *et al*. (2002), for evaluation of results of autologous transplantation methods in vitiligo. They considered that comparison of different studies of vitiligo surgery carried out at different centers may be difficult in the absence of uniform evaluation criteria. In most of the published studies, the results were evaluated in terms of extent of pigmentation. Complications and color match were evaluated separately. This approach, however, may not give a fair idea about the results. Gupta *et al*. (2002) have developed a scoring system considering the extent of pigmentation, color match and the complications of both the donor and the recipient areas, all taken together. They applied this scoring system on twelve patients who underwent thin Thiersch split skin grafting (TSTG) (2 patients), Suction Blister Grafting (SBG) (3 patients) and Punch Minigrafting (PMG) (7 patients). The results among 7 patients who underwent PMG showed that 5 of 7 patients (71.42%) had good to excellent outcome. In contrast, the results of this study showed that 93.33% of patients were assessed to have an outcome ranging from good to excellent, after the same period of follow-up (1 year). This difference may be explained by the additional effect of postoperative excimer laser which was used by us to augment pigment spread after minigrafting.

In a prospective study by Lahiri *et al*. (2006) which included 66 individuals with stable, refractory vitiligo, repigmentation with punch grafting followed by NB-UVB was evaluated. The patients were followed up for a maximum period of 18 months after grafting. They found that

repigmentation was achieved in only 57 (86.36%) cases, in spite of longer period of follow up after surgery. This was in contrast to the present study which showed that repigmentation was achieved in all patients (100%). So, postoperative enhancement of pigment spread using excimer laser appears to be superior to that of NB-UVB.

Homan *et al.* (2011), found that punch grafting followed by excimer laser or NB-UVB induced a comparable grade of repigmentation with no statistically significant difference between both modalities after 3 months follow up. They concluded that choice between excimer laser and NB-UVB cannot solely be based on repigmentation but rather on other factors, such as patients' preferences, cumulative dose, availability, ease of administration and costs. However, given the lower UV dose of excimer laser, they recommend its use in vulnerable populations, such as in small children and patients with sun-damaged skin with a history of long-term UVB treatment.

Al-Mutairi *et al.* (2010), treated 17 patients with stable focal or segmental vitiligo with split-skin-thickness grafting followed by 32 sessions of 308 nm excimer laser. All seventeen patients showed repigmentation and overall results were graded as excellent in 12 patients (70.58%) and good in the other five (29.4%) when evaluated one year after surgery patients. The percentage of excellent pigmentation in the present study (53.33%) was lower than the results of Al-Mutairi *et al.* (2010) and this may be attributed to different technique of grafting and due to different method of evaluation.

In this study, 63.36% of patients with segmental vitiligo (7/11) showed excellent response in contrast to 47.36% of patients with focal vitiligo (9/19) who showed the same response. However, this difference was not statistically significant due to small number of patients. To our knowledge, there are no controlled trials yet done to compare the difference in treatment response among patients with segmental and focal vitiligo, using combined surgical therapy and excimer laser. Generally, patients with segmental vitiligo are considered good candidates for surgical treatment. In a recent study by Kato *et al.* (2011), the results suggested that 1-mm minigrafts were effective for treating patients with vitiligo with better results occurred in patients with segmental and limited subtypes. Although medical treatment is often helpful in segmental vitiligo, complete repigmentation is almost always difficult to obtain. Thus, surgical treatment is necessary for complete repigmentation in many cases of segmental vitiligo (Falabella and Barona, 2009).

In the present study, excellent response was achieved in 78.57% (11/14) of patients aged 30 year or below, while of 16 patients aged more than 30 years, only 5 of them (5/16, 31.25%) showed excellent response. This difference was statistically significant. These results were in agreement with other studies. Kato *et al.* (2011), found that repigmentation covered a broader area and occurred more quickly in patients under 15 years of age than in those over 20 years of age. In a recent study by Feetham *et al.* (2012) who used different post-operative treatments following punch minigrafting, they concluded that Patients younger than 20 years achieved the greatest average improvement in repigmentation whereas those aged 60 and older showed the least improvement.

CONCLUSION

Punch grafting is a useful treatment modality for many patients with stable vitiligo who are not responding to other treatment options, with most patients showing some degree of improvement. It is simple, easy to perform with minimal complications. Combination treatment with autologous punch minigrafting and postsurgical exposure to 308 nm excimer laser in patients with stable focal or segmental vitiligo can lead to fast, cosmetically satisfying results for both physicians and patients. As a treatment variable, segmental vitiligo and vitiligo in patients younger than 30 years achieved the greatest average improvement in repigmentation.

This study may provide an evidence that monotherapy of stable recalcitrant vitiligo with punch grafting should be changed to a combination regimen with excimer laser to get the benefit of faster and more excellent repigmentation which can dramatically improve the quality of life of the patients. Further prospective studies on larger number of patients should be done to determine the effect of other variables, as skin color of patient, site to be treated and punch size used, with longer period of follow-up to determine the stability of pigmentation achieved by this combination regimen.

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