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Combined Treatment of Post-burn Leucoderma with Autologous Minigrafting and Topical Khellin-natural Sunlight among Egyptian Patients

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ABSTRACT

Deep burns often result in hypo-pigmentation, referred to as leucoderma. It is often distressing especially in black-skinned individuals. Several tissue-grafting methods have been reported to treat post-burn leucoderma with different treatment outcomes. Post-operative photo-chemotherapy was also reported to augment the results of grafting techniques. This prospective study was designed to evaluate the efficacy of combined treatment of post-burn leucoderma with autologous punch minigrafting followed by topical khellin and exposure to natural sunlight. Patients with post-burn leucoderma were treated with autologous minigraft. This was followed by post-grafting application of topical khellin and exposure to natural sunlight, either on three weekly bases or daily, for 6 months. Follow up was done for extent of the re-pigmentation, color matching and appearance of complications for additional 6 months. Final grading of re-pigmentation was as follow: 75-100% (excellent response), 50-75% (good response) and less than 50% re-pigmentation was considered as a poor response. Twenty one patients completed the study. Among them, 18 cases (85.7%) showed spread of pigmentation beyond graft borders which is more than that occurred after the minigraft test for individual patient and they were considered as “responders” to treatment. The remaining 3 cases (14.3%) did not show evidence of pigment spread more than that occurred after the minigraft test, after one year follow-up and were considered as “non-responder”. Eight patients (38.1%) showed excellent response, 6 patients (28.6%) showed good response while 4 patients (19%) showed poor outcome. Significant better response was recorded in covered parts compared to exposed parts. No significant differences were recorded between the response to daily versus 3 times weekly exposure to Khellin/natural sun light therapy. Thirteen patients (61.9%) were satisfied with the regimen. Combined treatment of post-burn leucoderma with autologous punch minigrafting and postsurgical application of topical khellin and exposure to natural sunlight is an easy to perform, cost-effective and satisfying to the patients in terms of re-pigmentation and color matching.

Key words: Burn, leucoderma, minigraft, phototherapy

INTRODUCTION

Dyspigmentation, in the form of either hyperpigmentation or hypopigmentation, is often a serious psychological problem for the self-image of patients (Halder and Richards, 2004). This is especially the case after a burn injury. The most common alteration in skin color occurs as the result of changes in the epidermal melanin or the underlying vascular bed (Burm *et al.*, 2007). Deep

burns often result in hypo-pigmentation, referred to as leucoderma which has a similar psychosocial impact on the patients as that of vitiligo (Mulekar *et al.*, 2011). It is especially distressing in black-skinned individuals (Degheidy *et al.*, 2003). Depigmented skin after a burn injury has been reported to contain little melanin pigment in the basal cells and marked thickening of all skin layers (Burm *et al.*, 2007).

A variety of surgical treatment options were used for leucoderma. However, many of the procedures have the potential to cause pigmentary changes and the patient may have a more severe problem after surgical intervention (Tyack *et al.*, 1997). The surgical goals for effective treatment of patients with post-burn dyspigmentation disorders are to remove scar tissue, establish even coloration from abnormal vascular structures and injured melanocytes and to produce healthy melanocytes (Halder and Nootheti, 2003).

Several techniques have been described for the treatment of post burn leukoderma with variable outcome. Small lesions can be peeled off and replaced with an epidermal graft or a conventional sheet skin graft. Such lesions are often extensive and a large donor site is needed (Kahn and Cohen, 1996). Other techniques have been described for the treatment of post burn leukoderma, such as split-thickness skin grafting (Erol and Atabay, 1990) chip skin grafting (Harashina and Iso, 1985) sheet grafting (Taki *et al.*, 1985) and cultured epithelial autografts (Stoner and Wood, 2000) in addition to cosmetic camouflage and tattooing (Guyuron and Vaughan, 1995). Falabella (1986) have treated several achromic defects by minigrafting, achieving noticeable improvement and patient satisfaction.

Post-operative phototherapy was reported to augment the results of grafting techniques. 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 re-pigmentation with better and faster results than monotherapy. Khellin, a furanochromone with a chemical structure resembling that of the psoralen family, is activated by UVA and UVB (Morliere *et al.*, 1988). Khellin has been used to treat vitiligo successfully (Ortel *et al.*, 1988; Riccio *et al.*, 1992) as its phototherapeutic properties are similar to those of the psoralens but it has the advantage of lower phototoxic and mutagenic effects (Schimmer, 1997).

The aim of this prospective study was to evaluate the efficacy and safety of combined treatment of post-burn depigmentation with autologous punch minigrafting and topical khellin paint followed by exposure to natural sunlight.

MATERIALS AND METHODS

Patients with post-burn leucoderma attending the out patients clinic of Mansoura University Hospital were the subjects of this study that was conducted between March 2012 and April 2013.

Inclusion criteria were:

- Leucoderma secondary to physical burns
- Leucoderma limited to one anatomical region
- The duration of lesions is six months or more
- **Positive minigraft test:** Minigraft test was done according to that described by Falabella *et al.* (1995). It was performed by implanting 3-4 minigrafts (2 mm) in the recipient site. Patients with 1-2 mm spread of pigment beyond the graft margins within 3 months were termed as “positive test” and were selected for final transplantation

Exclusion criteria were:

- Lesions affecting more than one anatomic site
- Lesions at sites which are difficult for immobilization (e.g., knuckles, elbows)
- Patients' age less than 18 years, or pregnant women
- Immuno-compromised or uncontrolled medical disorders e.g., diabetes
- Presence or history of hypertrophic scarring and/or keloid
- Patients with history of bleeding diathesis, or on anticoagulant therapy
- Patients with obsessive or compulsive attitudes toward their pigmentary defect

All participants provided signed informed consent and the study was approved by the local medical ethical committee. 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 photograph taken before intervention and also serially during follow-up. A baseline investigation, including complete blood count, differential count and coagulation profile, was performed for all patients.

Preparation of the donor sites: The sites selected as donors were 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 mm punches were used to prepare the donor chambers. The chambers were made at a distance of 3-4 mm from each other. 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 lesional skin. The donor grafts then immersed in a Petri dish containing normal saline, till preparation of the recipient site.

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. 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 about 1 cm. Care was taken to ensure that the graft are not folded and the tissue is not crushed or placed upside down. The needle of a syringe or the tip of scissors was used for the placement of grafts in the recipient holes. The grafts were then fixed at the recipient site using a spray (Opsite®, UK) by applying it to the surface of the grafts and left for 2 min before dressing.

After placing the punch grafts at the acceptor sites it was covered with Steri-Strip and bio-occlusive micropore. Immobilization of the recipient area was then done using a pressure bandage. Patients were advised to avoid excessive movement of the grafted area. Two weeks after surgery, the dressing was removed and patients were asked to apply topical antibiotic cream twice daily for another one week.

Phototherapy: Three weeks after surgery, patients were instructed to apply topical khellin 2% paint on the lesions followed by exposure to sunlight thirty minutes later. Patients were instructed to be exposed to sun before 10 a.m. or after 4 p.m. The lesions were exposed to escalating doses of sunlight starting from 5 min with 5 min increments each time, until maximum dose of 30 min, or

until appearance of evident erythema at leucoderma sites. Patients were divided randomly into two groups according to the frequency of phototherapy application whether daily or three times per week. All patients were advised to avoid further sun exposure on the days of phototherapy. The duration of post-operative phototherapy was 6 months. This was followed by additional 6 months follow-up.

RESPONSE ASSESSMENT

The final assessment of the response by the investigators was done at the end of one year following surgery, regarding re-pigmentation, appearance of complications and color matching. 75-100% re-pigmentation was considered an excellent response. 50-75% re-pigmentation was considered a good response. Less than 50% re-pigmentation was considered a poor response. The patient was considered non-responder if he did not showed pigment spread more than that occurred after the minigraft test after one year. Patients' general satisfaction with therapy was recorded by asking the patients to answer -by Yes or No- the following Global Efficacy Question (GEQ) at the end of whole treatment and follow up period "did you find the treatment you received satisfactory or not?".

Statistical analysis: Recruited data were subjected first to test for normal distribution (KS test). Chi square was used for categorical variants. Analysis was done using MedCalc Statistical Software version 12.7.2 (MedCalc Software bvba, Ostend, Belgium; <http://www.medcalc.org>; 2013).

RESULTS

Twenty one patients with post-burn leucoderma completed the study. All patients were Egyptian including 13 females and 8 males with a mean age of 33.24 ± 10.22 years (Fig. 1 and 2). The patients' characteristics are shown in Table 1.

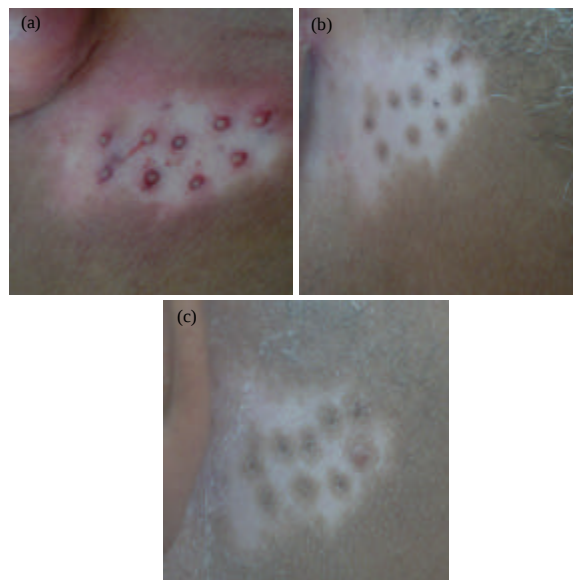


Fig. 1(a-c): (a) Male patient with leukoderma on post-auricular area intra-operative after placement of minigrafts, (b) Same patient 3 months after treatment and (c) Same patient at the end of follow-up period showing good response with cobble-stoning appearance

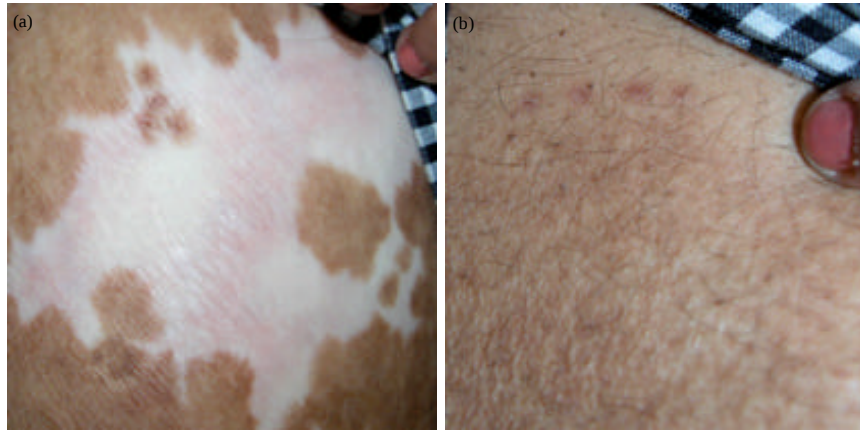


Fig. 2(a-b): (a) Female patient with leukoderma on the thigh with positive minigraft test before final minigrafting and (b) Same patient at the end of follow-up period showing excellent response

Table 1: Patient characteristics and response to treatment

Case No.	Age (years)	Sex	Site	Size of area (cm ²)	No. of grafts	Phototherapy	Response assessment by investigators	Patients' satisfaction by GEQ
1	27	Female	Thigh	50	52	Three/week	Excellent	Yes
2	48	Male	Hand	30	32	Three/week	Excellent	Yes
3	30	Male	Foot	9	10	Daily	Poor	No
4	33	Female	Chest	40	43	Daily	Excellent	Yes
5	45	Male	Hand	15	17	Three/week	No response	No
6	18	Female	Leg	20	21	Three/week	Excellent	Yes
7	33	Male	Forearm	20	22	Daily	No response	No
8	28	Female	Hand	30	33	Three/week	Poor	No
9	37	Female	Forearm	25	26	Daily	Poor	No
10	22	Male	Retroauricular	9	10	Three/week	Good	Yes
11	36	Female	Leg	10	11	Daily	Good	Yes
12	29	Female	Forearm	15	15	Three/week	Excellent	Yes
13	18	Female	Chest	12	13	Daily	Excellent	Yes
14	34	Male	Hand	35	38	Daily	Good	Yes
15	50	Female	Foot	15	15	Three/week	No response	No
16	38	Female	Hand	18	20	Daily	Good	Yes
17	24	Female	Chest	42	45	Three/week	Good	Yes
18	52	Female	Foot	45	48	Daily	Poor	No
19	19	Male	Hand	18	20	Daily	Good	No
20	35	Female	Thigh	30	33	Daily	Excellent	Yes
21	42	Male	Leg	42	44	Three/week	Excellent	Yes

Among the whole 21 patients, three patients (14.3%) were found to be non-responders to treatment while 18 patients (85.7%) were considered as responders. Eight patients (38.1%) showed excellent response, 6 patients (28.6%) showed good response while 4 patients (19%) showed poor outcome.

Table 2: Differences in response in covered versus exposed skin

Parameters	Exposed (%)	Covered (%)	Total (No-%)	X ²	p
No response	3 (14.3)	0 (0.0)	3 (14.3)	8.920	0.0304
Poor response	3 (14.3)	1 (4.8)	4 (19.0)		
Good response	2 (9.5)	4 (19.1)	6 (28.6)		
Excellent response	1 (4.8)	7 (33.3)	8 (38.1)		
Total (No. %)	9 (42.9)	12 (57.1)	21 (100.0)		

Table 3: Differences in response in daily versus three times week⁻¹ phototherapy

	Phototherapy daily (%)	Phototherapy 3 times/week (%)	Total (No. %)	X ²	p
No. response	1 (4.8)	2 (9.5)	3 (14.3)	2.458	0.4829
Poor response	3 (14.3)	1 (4.8)	4 (19.0)		
Good response	4 (19.0)	2 (9.5)	6 (28.6)		
Excellent response	3 (14.3)	5 (23.8)	8 (38.1)		
Total (No. %)	11 (52.4)	10 (47.6)	21 (100.0)		

None of the patients developed any complications at the donor skin site. At the recipient site however, 4 patients (19%) showed cobble-stoning appearance. In all patients who showed any degree of re-pigmentation, the color was compatible with that of normal surrounding skin. All patients who showed excellent results (8 cases), besides 5 patients with good results, (total 13 cases or 61.9%), were satisfied with this mode of therapy as they stated by answering the GEQ.

In terms of site of lesions, statistically significant higher response rate were recorded in the covered sites compared to that recorded for exposed parts (Table 2).

In terms of frequency of phototherapy, no significant difference in response was recorded between those with daily phototherapy and those who received phototherapy 3 times week⁻¹ (Table 3).

DISCUSSION AND CONCLUSION

In this study, almost two thirds of cases responded to minigraft augmented by khellin and natural sun exposure by good to excellent response. Patients' satisfaction with therapy reached nearly similar proportions. These results could generally point to the effectiveness of this treatment protocol of post-burn leucoderma which is known to represent a real therapeutic challenge.

As the growth behavior and the differentiation of melanocytes is markedly affected by the presence of keratinocytes (De luca *et al.*, 1988). We then decided to treat this kind of depigmentation by transplantation of melanocyte-keratinocyte as a unit by punch minigrafting. We had selected the minigrafting technique as a method for melanocyte transplantation because it is simple, easy to perform and cost-effective. Minigrafting test which was first described by Falabella *et al.* (1995) in a group of vitiligo patients, before the final transplantation was first done in our patients to detect which patients are best candidates for this maneuver and to avoid unnecessary procedures and eliminates further esthetic problems if the donor and recipient sites are subjected to unsuccessful surgical maneuvers.

Phototherapy induces stimulation of melanocyte migration from the hair follicle reservoir. Melanocytes then spreads centrifugally from the infundibulum to the basal cell layer and recolonizes the epidermis with active and functional melanocytes (Parrish *et al.*, 1976; Ortonne *et al.*, 1980). We had used post-operative topical khellin and sunlight exposure in a trial to augment the results of surgical treatment. In the present study, we found statistically significant difference regarding the extent of repigmentation between lesions on exposed parts, namely the

hands and feet and lesions on non-exposed parts. This higher response to Khellin phototherapy in covered parts of the skin was also reported before (Orecchia and Perfetti, 1992; Valkova *et al.*, 2004). When we compared the difference in efficacy between 3 times weekly and daily application of khellin, we found that the difference was statistically non-significant. So it seems wise to use phototherapy only 3 times weekly to avoid adverse reactions of exposure to a photosensitizer and to ensure better patient's compliance.

Review of literature actually showed relative paucity of researches of treatment of post-burn leucoderma. In the early trial of Lahiri and Sengupta (1997), sixty Indian cases of stable and refractory depigmented skin conditions which included only 8 patients with chemical leucoderma and post-burn depigmentation, was treated by punch minigrafting followed by oral 8-MOP followed by sunlight exposure. The follow-up period was 18 months, 70-100% repigmentation was achieved in 31 patients, 50-70% in 21 patients, 30-50% in 5 patients and 0-30% in 3 patients. So, 52 out of 60 patients (86.66%) showed 50-100% repigmentation. The apparent high results obtained by the authors compared to our findings could be explained by the high percentage of depigmented skin lesions caused by other diseases, rather than leucoderma, also the different mode of phototherapy they adapted, namely oral phototherapy. Another important variation that could also play a role in variable results obtained is the different ethnicity and skin phototype.

In another trial, Mulekar *et al.* (2011) treated 10 patients with post-burn leucoderma with a cell suspension formed of non-cultured Melanocyte-Keratinocyte Transplantation (MKTP) taken from a donor skin sample and transplanted on to a dermabraded recipient area. This was followed by 18 sessions of excimer laser starting 1 month post-operatively. Of the 10 patients treated, 3 were lost to follow-up. The remaining 7 patients showed repigmentation ranging from 90 to 100% with good color matching. Compared to Mulekar *et al.* (2011), our study included a higher number of cases, a much less costly phototherapy compared to excimer laser and a simple minigraft technique and in spite of that, our treatment protocol reached reasonable success rate but it was more time-consuming. The simplicity and low cost of the technique used for grafting and phototherapy could be very valuable especially for treatment of patients in developing countries like Egypt.

In a recent trial, Iman *et al.* (2013) tried to treat post burn leucoderma in 28 Persian patients by dermabrasion followed by either intraepidermal injection of autologous epidermal cell suspension (10 cases) or spraying a suspension of these cells over the dermabraded area (18 cases). The authors did not try to augment the results of grafting by phototherapy. The authors did not record significant differences between both methods in terms of repigmentation and the results of both techniques in general were found to be unsatisfactory for both patients and clinicians. Compared to our study, we adopted a simple and less costly minigraft technique that was supported by phototherapy and we obtained satisfactory results in general.

The limitations encountered in this study were mainly the number of cases that would be better increased to have more powerful results. The second limitation was depending on natural sunlight for phototherapy. Although not a significant problem in Egypt, or even could be considered an advantage due to its economic value, this may represent a problem in other countries that lack sunny weather most of the year. Finally the assessment of the response to treatment by the investigators that was subjective rather than objective. Although lack of an objective method for the assessment of response may represents a real challenge for the investigators, we tried to minimize this effect by concurrent checking of the response to treatment as evaluated by the patients themselves through answering GEQ.

In conclusion, punch minigrafting followed by Khellin/natural sun light exposure could be generally considered as a safe and effective therapeutic option for treatment of post-burn leucoderma, particularly over covered skin areas.

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