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Original Research Article

Efficacy of Miniature Punch Grafting Followed By Puvasol Therapy in Refractory Stable Vitiligo

Anu Gupta[1], Vippan Goyal[2], Chintu Chaudhary[3], Dhruvendra Lal[4]
[1]Assistant Professor, Department of Dermatology, AIMSR, Bathinda, Punjab, India
[2]Associate Professor, Department of Dermatology, AIMSR, Bathinda, Punjab, India
[3]Assistant Professor, Department of Community Medicine, AIMSR, Bathinda, Punjab, India
[4]P.G. 2nd year, Dept. of Community Medicine, AIMSR, Bathinda, Punjab, India

***Corresponding author**
Vippan Goyal
Email: vippangoyal@rediffmail.com

Abstract: Vitiligo affects approximately 2% of the worldwide population regardless of race, ethnic background or gender. When the medical methods are not successful, surgical procedures may be considered with the aim of placing a new source of pigment cells to reinitiate melanogenesis within the affected areas. This study was conducted to evaluate the efficacy of combining punch grafting followed by PUVASOL therapy in vitiligo patients. After punch grafting patients are given PUVA/PUVASOL exposure. The present study was conducted in the malwa region from 2014-2016. Thirty patients of chronic stable vitiligo not responding to medical treatment comes on OPD basis were included in study. Appropriate statistical methods were used to analyze the data. Patients subjected to Miniature Punch Grafting showed more than 80% acceptance in all selected age groups and among both the sex. There was an inverse relationship between number of grafts inserted and rejection rate. Mean pigmentation attained after 4 weeks of PUVASOL was 78.67%, which progressively increased and maximum repigmentation was seen at 12 weeks i.e. 90.13%. So there was significant repigmentation after 12 weeks after PUVASOL. Mini punch grafting with PUVASOL is very effective for attaining excellent re-pigmentation. Post-operative PUVASOL therapy has been fou. L1nd to expedite this re-pigmentation.
Keywords: stable, vitiligo, miniature, punch grafting, puvasol, repigmentation

INTRODUCTION

Vitiligo affects approximately 2% of the worldwide population regardless of race, ethnic background or gender. About 10% of cases are localized and 90% are generalized. There are a number of medical therapies that may restore, improve or at least decrease the depigmentation. These are topical corticosteroids, calcipotriol, topical or systemic methoxypsoralen, and oral psoralen plus ultraviolet A radiation (PUVA), ultraviolet B radiation, pseudocatalase plus calcium plus UVB, vitamin supplementation, human placental extract, systemic corticosteroids, other immunomodulators, and topical L-Phenylalanine in combination with UVA (PAUVA)[1,2]. However, such treatments usually induce incomplete repigmentation and occasionally the outcome is poor [2-4].

When the medical methods are not successful, surgical procedures may be considered with the aim of placing a new source of pigment cells to reinitiate melanogenesis within the affected areas. Several surgical procedures for the treatment of intractable lesions have been reported to be effective, including thin Thiersch grafts suction-blistered epidermis [1, 3], mini grafting [4, 5] and injection of non-cultured and various cultured cell grafting techniques. Only patients in whom the vitiligo is stable (i.e. no progression within 4 to 6 months) are considered good candidates for grafting.

On the other hand PUVA, either systemic or topical, increases the number of melanocytes and synthesis of melanin [2]. Topical outdoor PUVA is a popular and efficacious therapeutic option for patients with limited vitiligo [6]. The mechanism of PUVA rests on the synergistic interaction of the two components (8 — methoxypsoralen and UVA light) in the skin [7].

This study was conducted to evaluate the efficacy of combining punch grafting followed by PUVASOL therapy in vitiligo patients. This procedure is adapted only when the disease is stable for a sufficient period of time. In punch grafting small (1-2 mm) round, full thickness grafts are harvested with a trephine from a pigmented site and transplanted into an area of vitiligo. It causes restoration of melanocytes in the depigmented skin. Such repigmented skin then regains its normal immune / inflammatory function [8].

After punch grafting patients are given PUVA/PUVASOL exposure. It stimulates tyrosinase activity in melanocytes and may stimulate other components of skin, such as keratinocytes to release inflammatory mediators. Some of those may act as basic fibroblast growth factor (b-FGF) and may enhance further proliferation of melanocytes. Giving PUVASOL therapy after punch grafting will help in perifollicular repigmentation. It has been found that during adequate systemic methoxysalen therapy, melanocytes are stimulated, migrate from the hair follicle reservoir, and spread centrifugally from the infundibulum into the basal cell layer, recolonizing the epidermis with functional pigmentary cells [9, 10].

AIMS AND OBJECTIVE

This study was conducted to evaluate the efficacy of combining punch grafting followed by PUVASOL therapy in vitiligo patients. After punch grafting patients are given PUVA/PUVASOL exposure.

MATERIAL & METHODS

The present study was conducted in the malwa region from 2014- 2016. Thirty patients of chronic stable vitiligo who were not responding to medical treatment and attending the outpatient department of dermatology were included in study. Patients were of stable vitiligo where there were no new lesions and no progression seen in the previous lesions for the past one year. After doing punch grafting PUVASOL therapy was given and patients were followed for a period of 12 weeks i.e. 3 months.

Patients with unstable vitiligo, Children < 12 years of age , pregnant and lactating women, patients with history of intolerance to PUVA/PUVASOL, patients with past history or family history of skin cancers, patients with renal disease / liver disease , patients having any chronic debilitating diseases and tendency to develop hypertrophic scars or keloids were excluded from the study.

Preliminary investigations which were carried out were complete blood counts, urine routine, liver function tests, renal function tests and ophthalmoscopic examination. Informed consent of the patient was taken. Both donor and recipient areas were prepared surgically by cleaning them thoroughly with savlon, followed by betadine and spirit. At the recipient area 2.5 mm punches were taken out at a distance of 5-10 mm. The punched out tissue was discarded. Donor area was buttock or front of thigh. From donor area 3 mm punches were taken and kept in a petridish containing normal saline. The donor area was dressed with antiseptic dressing. The 3 mm punches from the donor site were placed in 2.5 mm crators created at the recipient site. The edges were ironed out and grafted area was covered with non-sticking framycetin dressing and then pressure dressing was given using dynaplast. The patients were also advised a course of antibiotic therapy and analgesics for a week and allowed to go home immediately after dressing with the instructions to avoid any vigorous activity which could displace the dressing. Patients were then called after 48 hrs to change the dressing and further follow up was after one week when patients were examined and complications if any were noted.

After 15 days of successful punch grafting, patients were given PUVASOL therapy, where we gave Tab. 8-methoxypsoralen in the dosage of 0.6 mg/kg/day twice a week and then the patients were asked to expose the affected area to natural sunlight after 2 hours. While on PUVASOL patients were instructed to protect their eyes from direct sunlight with the help of UV opaque goggles on the day of PUVASOL. Initially sun exposure was for 5 minutes and 5 minute increment was given after every three sittings subject to a maximum of 30 minutes. Patients were followed at 2 weekly intervals for total of 12 weeks. On every follow up visit, the grafted area was examined for evidence of new pigment development (i.e. repigmentation). Close up clinical photographs were taken. The results obtained were recorded in performa.

At the end of the study, the results obtained were compiled and subjected to appropriate statistical procedure to arrive at valid conclusions.

RESULTS AND OBSERVATION

Thirty patients of chronic stable vitiligo who fulfilled the inclusion criteria were included in the study. The mean age group of the study population was 23.93 years. Majority of them were females and from urban areas (Table 1). Most of the patients had surface area involvement in the range of 0.3-0.5% with a mean of 0.49 of total body surface area. The duration of the disease varied from 1 to 16 years. Majority of the patients (i.e. 53.33%) had disease duration between 6-10 years. Most of the patients had segmental form of the disease (i.e.67%) with (13%) having generalized and (20%) focal form of the disease (Table 2).

Table 1: Socio-Demographic Characteristics of the Study Group

AGE GROUP	NO OF PATIENTS	%AGE
11 — 20	9	30.00
21-30	15	50.00
31-40	2	6.67
41-50	4	13.33
MEAN	23.93	
SEX		
MALE	10	33.33
FEMALE	20	66.67
AREA OF RESIDENCE		
RURAL	8	26.7%%
URBAN	22	73.3%

Table 2: Description of the Vitiligo

DISTRIBUTION ACCORDING TO PERCENTAGE BODY SURFACE AREA BSA (%)	NO.	PERCENTAGE
0.1	4	13.3
0.1-0.3	6	20
0.3-0.5	12	40
0.5-0.7	0	0
0.7-1	8	26.7
MEAN	0.49	
SD	0.34	
DISTRIBUTION ACCORDING TO DURATION (YRS)		
1-5	10	33.33
6-10	16	53.33
11-16	4	13.33
DISTRIBUTION OF SUBJECTS ACCORDING TO TYPE OF VITILIGO		
GENERALIZED	4	13.00
FOCAL	6	20.00
SEGMENTAL	20	67.00

The age of patients with graft inserted was varied from 12 to 62 years (Table 3). Percentage acceptance in different age groups varied from 81.36 to 87.26%. The difference in the graft acceptance rate in different age groups was not statistically significant. Percentage acceptance in males was 92.10 while in females was 93.53. However the difference in graft acceptance between males and females was not statistically significant (Table 4). The mean of grafts accepted was 30.67 with SD of 13.66 and mean of grafts rejected was 5.00 with SD of 2.36 (Table 5).

Table 3: Parameters Related To Graft Inserted

Age in years	NO. OF PATIENTS WITH GRAFT INSERTED	PERCENTAGE
11-20	8	26.67
21-30	2	6.67
31-40	8	26.67
41-50	8	26.67
>50	4	13.33
Total	30	

Table 4: Grafts acceptance in relation to sex:

SEX	
MALE	92.10
FEMALE	93.53
P VALUE	>0.01

Table 5: Distribution of Subjects According To No. Of Grafts Rejected

GRAFT REJECTED	NO OF PATIENTS	PERCENTAGE
2-3	8	26.67
4-5	12	40
6-7	4	13.33
8-10	6	20
MEAN	5.133	
SD	2.403	

Most common side effect observed was cobble stoning which was observed in 60% of the patients. Other side effects which were observed were hematoma formation and displacement of the graft (Table 7). Mean pigmentation attained after 4 weeks of PUVASOL was 78.67%, which progressively increased and maximum repigmentation was seen at 12 weeks i.e. 90.13%. So there was significant repigmentation after 12 weeks after PUVASOL. Most common side effect with PUVASOL was erythema seen in 40% of the patients while itching was seen in 20%. 40% didn't experience any side effect of PUVASOL. However none of the patients discontinued treatment due to side effects of PUVASOL (Table 8).

Table 6: Grafts inserted, rejected and percent rejection among subjects

NO. OF GRAFTS INSERTED			NO. OF GRAFTS REJECTED		PERCENTAGE REJECTION
NO.	MEAN	SD	MEAN	SD	
11-20	17.5	3.79	4.00	0.82	23.75
21-30	25	-	5	-	20.00
31-40	34	1.41	3.75	2.87	11.08
41-50	46.75	1.71	5.75	2.06	12.33
>50	58.5	4.95	8	2.83	13.52

Table 7: Distribution of subjects according to side effects

SIDE EFFECT	NO OF PATEINTS	PERCENTAGE
DISPLACEMENT OF GRAFT	10	33
COBBLE STONING	18	60
HAEMATOMA	2	7

Table 8: Trends in pigmentation achieved

TIME (WEEKS)	MEAN (%)	SD
4	28.67	10.26
6	47	9.6
8	66	9.3
10	81.67	6.73
12	90.13	3.89

F Ratio: 137.72 p value < 0.01 CD: 6.04

DISCUSSION

Vitiligo is a common pigmentary disorder leading to great cosmetic embarrassment, psychological distress and a cause of disruption of the social relationship. The outcome of this disorder is often unpredictable and uncertain. There are many cases of vitiligo who either fail to respond or only partially respond to the medical line of treatment indicating that melanocyte reservoir is no more available for repigmentation in these areas [11]. These non responding cases of stable vitiligo can be treated with autologous skin grafting. Autologous skin grafting can be done in various ways such as epidermal culture grafting[12], pure melanocyte culture grafting[14], epidermal grafting by suction blister technique[13], thin Thiersch's split skin grafting[15], or thin split skin miniature punch grafting. The later method was first advocated by Falabella to treat small to medium sized localized patches of focal and segmental vitiligo [4, 5].

The age range in our study was between 12-50 years. Majority of the patients were in the age group of 21-30 years with a mean age being 23.9 years. In a study done by Malakar and Dhar [22], majority of the

cases were in the age of 10-30 years with a mean age of 20 years. So age group included in our study was almost similar to other studies. Vitiligo affects both sexes equally. However many studies have shown female preponderance of vitiligo [16,17]. In present study also majority i.e. 66% of patients were females. In fact, this may be a reflection that females are more concerned about cosmetic looks and are more stressed due to social and matrimonial problems due to the disease. Most of the patients in our study (i.e. 73.35%) were residents of urban area and large majorities (86.7%) were educated. This could be due to the fact that urban educated patients are more concerned regarding the cosmetic disfigurement resulting from vitiligo.

Most of our patients had segmental vitiligo (i.e. 67%), followed by focal vitiligo (20%) and generalized vitiligo (i.e.13%). This is in concordance to other studies [7, 18] that also included cases with focal and segmental vitiligo. The duration of vitiligo ranged from 1-16 years with a mean of 7.17 years (i.e. 53.33%). 8 patients had disease duration between 6 - 10 years, years, 5 patients (33.33%) between 1-5 years and rest 2 patients disease duration between 1 -16 years. Also the surface area involvement was in our study in range of 0.3-0.5% of total body surface area with a mean of 0.49.

The number of grafts inserted per person in the study varied between 12-62 with a mean of 35.67 grafts and in majority of patients 30-50 grafts were put. Mean percentage of acceptance of graft was 84.29%. There was no statistically significant difference in the graft acceptance rate in two sexes and in different age groups. Percentage graft rejection was 15.71% and it was found that as the number of grafts inserted increased, percentage rejection decreased. Percentage graft rejection in another study conducted by Rathi [19] due to various reasons like movement, secondary infection was around 10%. The most important side effect observed was cobble stoning seen in 60% of the patients while displacement of graft with hematoma formation (33%) and hematoma formation alone was seen in (7%) of the patients. This is in concordance with the study done by Savant [20] who also observed cobble stoning as the commonest side effect seen in 50% of the patients. In a study done by Bajaj [18], cobble stoning was again observed in 22%. It was seen in 10 patients (total 32 cases) in the initial stages but with the passage of time it was rectified in majority of the patients. Rathi et al [19] also observed cobble stoning in 22.5% with sinking pits in 10%. No case of sinking pits was observed in the present study. This could be due to relatively small study group. The displacement of graft(s) observed in the study was due to improper immobilization by the patients. Savant [20] observed three cases of contact allergic dermatitis to framycetin but no case showed framycetin allergy in present study.

Postoperative PUVASOL was given and the commonest side effect observed was erythema (40%), itching (20%) and another 40% didn't experience any side effect due to PUVASOL. None of the patients however required discontinuation of therapy due to side effects. Trends in repigmentation were observed for up to 12 weeks. Mean repigmentation attained after 4 weeks of PUVASOL was 28.67% which progressively increased and maximum repigmentation was seen at 12 weeks (i.e. 90.13%). There was significant improvement in the repigmentation achieved with the passage of time and postoperative PUVASOL therapy has been found to expediate repigmentation. In another study, Falabella [5] developed his own technique using minipunch to treat localized vitiligo. He used grafts of 2-4 mm in diameter taken from donor site to graft them at the chamber of same size at the recipient site; by spacing them 3-4 mm apart and further secured those by using Monsel's solution and segmental and focal vitiligo were selected for autologous miniature punch grafting. 15 patients received treatment by mini grafting and remarkable repigmentation was obtained in 13 cases with 90-100% improvement, in one case 70% and another one 50% improvement. However no sealing solution was used in this study to secure the grafts as in Falabella's technique since simple pressure was enough to secure them. In a prospective study of 1000 patients of stable and recalcitrant vitiligo the results of miniature punch grafting was evaluated by Malakar and Dhar [23]. In 656(74.55%) patients, 90-100% repigmentation was achieved, in 93 (10.57%) patients there was no spread of pigment, while in 21 (2.39%) depigmentation of the graft was noticed. In another study 33patients were selected by Jha et al [21] for punch grafting. They modified the method which was described by Falabella and Behl by using one punch per cm^2 using 3 mm punch device. There was complete repigmentation in 10 patients and in 23 patients pigmentation was spreading slowly. No recurrence or scar was noticed at donor or recipient site in any of the patients. Rate of re pigmentation observed in the present study is similar to other study.

CONCLUSION

The study on patients of vitiligo who were subjected to Miniature Punch Grafting showed more than 80% acceptance in all selected age groups and among both the sex. There was an inverse relationship between number of grafts inserted and rejection rate. Most common side effect seen was cobble stoning (60%) with few patients showing graft displacement and hematoma formation too. Mean pigmentation achieved after 12 weeks of PUVASOL was above 90% and there were some adverse effects with PUVASOL however none of the patients discontinued the treatment

due to the side effects. Mini punch grafting with PUVASOL is very effective for attaining excellent re-pigmentation. Post-operative PUVASOL therapy has been found to expedite this re-pigmentation.

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