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Dermatology

Evaluation of Efficacy of Follicular Unit Extraction as a Therapeutic Option for the Treatment of Stable Vitiligo Patches Along With Reduction in Noticeability of Same Patches

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Original Research Article

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Abstract: Vitiligo is an acquired, hypomelanotic disorder characterized by circumscribed depigmented macules in the skinresultingfromloss of functional melanocytesfrom the cutaneous epidermis. A prospective study was done to analyze follicular unit extraction (FUE) as a therapeutic option for vitiligo and to assess repigmentation post follicular unit extraction in stable vitiligo patches. Patients withActive vitiligo lesions,Koebner phenomenon,Keloidal tendency and Bleeding disorders were not included in the study.
Study Design: Prospective Observational Study.
Keywords: FUE, Therapeutic, Vitiligo, Repigmentation Post Follicular Unit.

INTRODUCTION

As noticed clinically,with any treatment for vitiligo, the repigmentation begins at the orifice of hair follicles. The existence of undifferentiated stem cells in the hair follicle in follicular units, can serve as a source of melanocytes for repigmentation is the rationale behind transplanting hair follicles.

In 1959, Staricco RG showed melanocytes in the external root sheath that suggested immature pigment cells[1]. Ortonne *et al*. in 1979 proposed a melanocyte reservoir in the hair follicles [2] Grichnik *et al*. in 1996 demonstrated immature dendritic, tyrosinase negative and c-kit positive pigment cells mainly concentrated around the follicular ostium, and to a lesser extent in the rete pegs and the outer root sheath[3] thus making pigmented hair follicle unit a melanocyte reservoir.

Mammalian stem cells are divided in two categories: Tissue specific and Non tissue specific[4-6].Tissue-specific stem cells are usually found in a specialized environment within specific organs called the niche that provide important signals to guide their function[7].

Nishimura *et al*. in 2002 identified melanocyte stem cells in the lower permanent portion of Dct-lacZ transgenic mice hair follicles, which become activated at early age as they are tightly coupled to the hair regeneration cycle[8]. They are located in the lower part of the hair follicle bulge, just below the hair follicle stems cells[9].

The bulge region is the site of relative immune privilege, protecting the hair follicle epithelial stem cell reservoir from auto aggressive immune attacks[10].

A population of cells with molecular characteristics of stem cells and transient amplifying cells related to keratinocytes has been demonstrated at the tips of deep rete ridges in the interfollicular skin of breast, palms and soles[11-13].

Hair follicle units those at the edge of vitiligolesions may also contribute to repigmentation, as hair follicle melanocytes may be present in vitiligo areas even many years after depigmentation takes place[14-16].

The presence of pigmented hairs in vitiliginous skin is a good prognostic sign for vitiligo recovery speaking in favor of an intact melanocyte reservoir[17]

As leukotrichia represents melanocyte reservoir exhaustion ,repigmentation of depigmented skin in vitiligo by medical therapies is very unlikely and melanocyte transplantation may be the best therapy for depigmented skin[19]

Modern hair transplantation was introduced in the 1950s by Dr. Orentreich [20] Then the concept of mini and micrografting was initiated [21,22] and later in 1990s the Follicular Unit Hair Transplantation (FUT) [23] took over.

Bernstein and Rassman[24] invented the FOX procedure, also known as FUE (Follicular Unit Extraction) that extracts the follicle individually without causing any significant scarring.

MATERIALS & METHODS

A prospective observational study, conducted from November 2015 to September 2017,involving 30 stable lesions of vitiligo in thirty subjects.

Inclusion Criteria

- Patients with localized **"Stable Vitiligo"**(stability defined as per theIADVL Task Force criteria. (A patient reporting no new lesions, no progression of existing lesions, and absence of Koebner phenomenon during the past 1 year).
- Patients were diagnosed to have vitiligo,clinically based on presence of depigmented patch, history, duration, progression of disease, and appearance of chalky white on wood's lamp examination.

Exclusion Criteria

- Active vitiligo lesions,
- Koebnerphenomenon,
- Keloidaltendency,
- Bleeding disorders.

Procedure

After written informed consent, a detailed history about the site of onset, duration, progression, stability of the disease, and history of medication was taken. Then patients were thoroughly examined for the number of lesions, size, site, and type of vitiligo.

Photographs were taken before the procedure and then periodically to compare the pigmentation. All the patients were investigated for complete hemogram, bleeding time -clotting time & HIV testing was done. Lignocaine sensitivity was tested before the surgery.

After tumescent anesthesia, the donor follicles were extracted using motorized 0.9mm sharppunch. The donor area was occipital scalpe.The hair follicles so extracted were stored in cold normal saline.The hair follicles were transplanted, using 18-G needle to create slits,by Jewelers forcep, as follicular units. After grafting occlusive dressing was applied for 14 days.

Assessment

The patients were followed up for a period of 6 months, at an interval of every 2 weeks or till repigmentation was first observed , then monthly up to 2 months & then in two months intervals upto 6 months. The results were assessed at the end of 6 months.

The repigmentation was evaluated qualitatively on the basis of Visual Analogue System(VAS)scoreon the basis of photographs taken on every follow up, by a third observer who was blinded and belonged to medical fraternity only.

0-24% — poor, 25-49% — fair, 50-74% — good,75-100% — excellent response

The second opinion was patient's assessment on vitiligo noticeability of the lesion at the end of six months, post procedure by a validated scale known as VitiligoNoticeabilty Scale(VNS) [1,6,2] which was as follows:

More noticeable (1)
As noticeable (2)
Success criteria: VNS Score of 4 or 5
Slightly less noticeable (3)
A lot less noticeable (4)
No longer noticeable (5)

Based on the two above parameters the study conductor labeled each individual case on the Global treatment success criteria as:

Treatment success was defined as 'yes' on global assessment with a VNS score of 4 or 5, and 75 % or >75% repigmentation[1,6,2].

STATISTICAL ANALYSIS

The information collected was recorded in a master chart. Data analysis was done using IBM SPSS Statistics v22 statistical software.

OBSERVATION AND RESULTS

Table-1: Distribution of patients according to age

Age	Number	Percentage
<=20 years	5	16.7
21-40 years	22	73.3
41-60 years	2	6.7
>60 years	1	3.3
Total	30	100.0

Majority of the patients were in the age group 21-40 years.

Table-2: Distribution of patients according to gender

Gender	Number	Percentage
Female	5	16.7
Male	25	83.3
Total	30	100.0

There were 5 (16.7%) females and 25 (83.3%) males

Table-3:Distribution of patients according to VNS

VNS	Number	Percentage
1	0	0.0
2	6	20.0
3	4	13.3
4	17	56.6
5	3	10.0
Total	30	100.0

Majority of the patients were in the VNS grade 4.

Table-4: Distribution of patients according to GTS

GTS	**Number**	**Percentage**
No	10	33.3
Yes	20	66.7
Total	30	100.0

Majority of the patients had GTS "Yes".

Table-4: Distribution of patients according to repigmentation grading (VAS)

Repigmentation Grading (VAS)	Number	Percentage
Poor	3	10.0
Fair	7	23.3
Good	15	50.0
Excellent	5	16.7
Total	30	100.0

3 (10.0%) patients had poor repigmentation grading, 7 (23.3%) patients had fair repigmentation grading, 15 (50.0%) patients had good repigmentation grading and 5 (16.7%) patients had excellent repigmentation grading.

Table-5: Comparison of repigmentation between various time intervals

Time Interval	Incidence	't' value	P value
Follow-up at 1 month	19.67 ± 9.19	-11.73, df=29	0.000*
Follow-up at 2 months	44.33 ± 16.23		
Follow-up at 1 month	19.67 ± 9.19	-16.39, df=29	0.000*
Follow-up at 4 months	65.50 ± 19.49		
Follow-up at 1 month	19.67 ± 9.19	-16.47, df=29	0.000*
Follow-up at 6 months	77.00 ± 22.42		
Follow-up at 2 months	44.33 ± 16.23	-11.73, df=29	0.000*
Follow-up at 4 months	65.50 ± 19.49		
Follow-up at 4 months	65.50 ± 19.49	-8.59, df=29	0.000*
Follow-up at 6 months	77.00 ± 22.42		

Paired 't'test

There was statistically significant improvement in the repigmentation as the time intervals increases (P<0.05).

The patients in whom GTS was successful i.e. "yes" had better repigmentation grades in comparison to the patients in whom GTS was unsuccessful i.e. "no". There is a statistically significant association seen between GTS and the repigmentation grading (P<0.05).

Table-6: Association between GTS and Repigmentation Grading

GTS	Grade				
	Poor	Fair	Good	Excellent	Total
No	3 100.0%	6 85.7%	1 6.7%	0 0.0%	10 33.3%
Yes	0 0.0%	1 14.3%	14 93.3%	5 100.0%	20 66.7%
Total	3 100.0%	7 100.0%	15 100.0%	5 100.0%	30 100.0%

Pearson Chi-Square = 21.943, DF = 3, P-Value = 0.000, Significant

Table-7: Association between Poor or hyper pigmentation and GTS

Factors responsible for poor pigmentation	GTS		
	No	Yes	Total
Bony prominence	2 20.0%	0 0.0%	2 6.7%
Greying / old age	1 10.0%	0 0.0%	1 3.3%
Hyper-pigmentation	0 0.0%	2 10.0%	2 6.7%
Loss of follicles	3 30.0%	0 0.0%	3 10.0%
None	4 40.0%	18 90.0%	22 73.3%
Total	10 100.0%	20 100.0%	30 100.0%4

Pearson Chi-Square = 41.359, DF = 12, P-Value = 0.004

In majority of the patients who had a successful GTS were not having any factors responsible for poor pigmentation. Mostly the factors responsible for poor pigmentation were seen in the patients who had unsuccessful GTS i.e. "no".

Table-8: Comparison of mean difference of repigmentation between 1 month and 2 months in relation to stability duration

Stability Duration	No.	Mean ± SD	F value	P value
12-24 months	19	23.42 ± 12.37	1.95	0.161, NS
24-36 months	6	19.17 ± 12.42		
36-48 months	5	33.00 ± 8.37		

One-way ANOVA test applied

Stability duration is defined as the number of months the patient reported that their vitiligo size has not increased and has remained stable.

The mean difference in the 12-24 months stability duration was 23.42 ± 12.37, in the 24-36 months it was 19.17 ± 12.42 and in 36-48 months it was 33.00 ± 8.37.

The difference was found to be statistically not significant (P>0.05), showing that the difference in the repigmentation between 1 month and 2 months is comparable between the stability duration.

Table-9: To find out the pair wise differences, post hoc Tukey was applied

Pair	't' value	P value
24-36 months to 12-24 months	-0.77	0.727, NS
36-48 months to 12-24 months	1.61	0.261, NS
36-48 months to 24-36 months	1.92	0.151, NS

* Significant

There was no statistically significant difference seen in the pairs (P>0.05), showing that the difference of repigmentation was comparable between the pairs.

The pair wise comparison was done between 24-36 months to 12-24 months; 36-48 months to 12-24 months and 36-48 months to 24-36 months.

There was no statistically significant difference seen in the pairs (P>0.05), showing that the difference of repigmentation was comparable between the pairs.

There is no statistically significant association seen between stability duration and the repigmentation grading (P<0.05), showing that repigmentation is independent of the stability duration.

Table-10: Association between stability duration and Repigmentation Grading

Stability Duration	Grade				
	Poor	Fair	Good	Excellent	Total
12-24 months	2 66.7%	6 85.7%	7 46.7%	4 80.0%	19 63.3%
24-36 months	1 33.3%	1 14.3%	3 20.0%	1 20.0%	6 20.0%
36-48 months	0 0.0%	0 0.0%	5 33.3%	0 0.0%	5 16.7%
Total	3 100.0%	7 100.0%	15 100.0%	5 100.0%	30 100.0%

Pearson Chi-Square = 6.817, DF = 6, ----------

Table-11: Association between Stability Duration and GTS

Stability Duration	GTS		
	No	Yes	Total
12-24 months	7 70.0%	12 60.0%	19 63.3%
24-36 months	3 30.0%	3 15.0%	6 20.0%
36-48 months	0 0.0%	5 25.0%	5 16.7%
Total	10 100.0%	20 100.0%	30 100.0%4

Pearson Chi-Square = 3.355, DF = 2, P-Value = 0.187, Not significant

There is statistically no significant association seen between stability duration and GTS (P<0.05), showing that the GTS is independent of the stability duration.

Table-12: Association between stability duration and VNS grading

Stability Duration	VNS Grade					
	1	2	3	4	5	Total
12-24 months	0 0.0%	4 66.7%	3 75.0%	10 58.8%	2 66.7%	19 63.3%
24-36 months	0 0.0%	2 33.3%	1 25.0%	2 11.8%	1 33.3%	6 20.0%
36-48 months	0 0.0%	0 0.0%	0 0.0%	5 29.4%	0 0.0%	5 16.7%
Total	0 0.0%	6 100%	4 100%	17 100%	3 100%	30 100%

Pearson Chi-Square = 5.406, DF = 6, ----------

There is no statistically significant association seen between stability duration and the VNS grading (P<0.05), showing that VNS grading is independent of the stability duration.

Table-13: Association between VNS grading & factors for poor pigmentation

VNS grading	Factors responsible for poor pigmentation					
	Bony prominence	Greying / old age	Hyper-pigmentation	Loss of follicles	None	Total
1	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
2	2 100%	1 100%	0 0.0%	3 100%	0 0.0%	6 20.0%
3	0 0.0%	0 0.0%	0 0.0%	0 0.0%	4 18.2%	4 13.3%
4	0 0.0%	0 0.0%	0 0.0%	0 0.0%	17 77.3%	17 56.7%
5	0 0.0%	0 0.0%	2 100%	0 0.0%	1 4.5%	3 10.0%
Total	2 100%	1 100%	2 100%	3 100%	22 100%	30 100%

Pearson Chi-Square = 49.091, DF = 12, ----------

There is a statistically significant association seen between factors responsible for poor pigmentation and the VNS grading (P<0.05), showing that VNS grading is dependent on the factors responsible for poor pigmentation.

There is statistically significant association seen between VNS grading and GTS (P<0.05), showing that the GTS is dependent on the VNS grading.

Table-14: Association between VNS Grading and GTS

VNS Grading	GTS		
	No	Yes	Total
1	0 0.0%	0 0.0%	0 0.0%
2	6 60.0%	0 0.0%	6 20.0%
3	4 40.0%	0 0.0%	4 13.3%
4	0 0.0%	17 85.0%	17 56.7%
5	0 0.0%	3 15.0%	3 10.0%
Total	10 100.0%	20 100.0%	30 100.0%4

Pearson Chi-Square = 30.000, DF = 3, P-Value = 0.000, Significant

There is a statistically significant association seen between VNS grading and the repigmentation grading (P<0.05), showing that the repigmentation grade is dependent on the VNS grading.

DISCUSSION

The mean age of patients of stable vitiligo was 29.5 years.Maximum patients were between 21 and 40 years of age.This study factor cannot be compared due to small sample size.

The male to female ratio was 5:1, male (83.3% &16.7 %). In a similar study conducted by Parul Thakur *et al*. [26] the male: female ratio was 3:2. This could be due to less females patients giving the consent to trim their hair from the occipital regions & taking hair on recipient site cosmetically unacceptable.

Table-15: Association between VNS Grading and Repigmentation Grading

VNS Grading	Repigmentation Grade				
	Poor	Fair	Good	Excellent	Total
1	0 0.0%	0 0.0%	0 0.0%	0 0.0%	0 0.0%
2	3 100%	3 42.9%	0 0.0%	0 0.0%	6 20.0%
3	0 0.0%	3 42.9%	1 6.7%	0 0.0%	4 13.3%
4	0 0.0%	1 14.3%	13 86.7%	3 60.0%	17 56.7%
5	0 0.0%	0 0.0%	1 6.7%	2 40.0%	3 10.0%
Total	3 100.0%	7 100.0%	15 100.0%	5 100.0%	30 100.0%

Pearson Chi-Square = 33.549, DF = 9, P-Value = ----------

19 out of 30 patients had stability between 12-24 yrs (63.3%). Our study further validated the guidelines for the patient selection in vitiligo surgeries [27] as there was no significant difference (p>0.05) in the repigmentation observed in respect to longer stability in our study.

The study aimed at reducing the bias in results by taking two different opinions excluding the study conductor.

20 out of 30 patients (67.7%) were labeled Global treatment success YES and 10(33.3%) as NO. Statistical comparison of repigmentation at various time intervals showed significant improvement (p<0.05).

Excellent repigmentation (75 or >75 %) was seen in 67% of the patients, good in 23 % and fair in 10 % patients. Study conducted by Parul Thakur *et al*. [26] showing excellent repigmentation in 61.9%, good in 25.4% and poor in 12.7%.

FUE conducted by Kumaresan [28] showed excellent repigmentation after 4-8 weeks with no recurrences. Malakar *et al*. reported success in all the five lesions with FUE [29].

Chouhan *et al*. [30] showed complete repigmentation at 12 weeks after body hair transplantation in vitiligo. Vitiligo noticeability scale (VNS) which probably, was not used in the studies done so far, was used in our study.

The above scale is a validated scale for reduction in vitiligo noticeability[25] and our study further acknowledges it. No major complications were seen in this procedure.

Two patients out of twenty patients (10%) had hyperpigmentation over the operated lesion. This can be attributed to the higher density of the follicles transplanted.

Six out of 10 patients with GTS as NO, had bad prognostic factors like grey hair[1], bony prominence site[2]and loss of hair due to poor post-operative care [3].

On doing a statistical analysis of these factors with percentrepigmentation, VNS & GTS, significant association (p=0.00)) was seen between them.

Four out of 10 patients who also had a GTS as NO, showed no such bad prognostic factors. These patients should be followed up for another six months as the repigmentation might be slow.

CONCLUSION

High density of melanocytes in hair provides a larger melanocyte and stem cell reservoir. The occiput being the donor site gives negligible scarring compared to other procedures which usually leave visible scar .Further this method can be applied to a small area of vitiligo. This procedure is the best modality inleucotrichia.

FUE does not require special equipment or a sophisticated operation theatre setting making it quite affordable option, less time consuming & comparatively easier.

Hairy areas can be treated effectively. Using FUE rather than FUT simplifies the procedure and reduces the chances of complications making.Complications of this procedure are minimal with good color match.

However, this procedure is best suitable for small lesions involving the hairy areas making it cosmetically unacceptable at non hairy or visible body sites.

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