A Study of Noncultured Extracted Hair Follicle Outer Root Sheath Cell Suspension for Transplantation in Vitiligo

Aarti N Shah, Ritu K Marfatia, Siddhartha S Saikia

Department of Dermatology, NHL Medical College, V.S. General Hospital, Ahmedabad, Gujarat, India

ABSTRACT

Context: Vitiligo surgeries have come a long way from tissue grafts to cultured and non-cultured cell transplantation. Extracted hair follicle outer root sheath cell transplantation (EHF ORS) suspension is more enriched with melanocyte. In a hair bulb, there is one melanocyte for every five keratinocytes which is much higher than the epidermal melanin unit. Aims: To analyse the effectiveness of cultured EHF ORS and to perform objective evaluation based on clinical improvement & photographic evidence. To observe any untoward events or side effects. Settings and Design: The study was open and uncontrolled. All the patients were screened at preliminary visit. Reviews were done every two weeks. The endpoint selected was six months post procedure. Materials and Methods: Twenty five patients of stable Vitiligo were included in the study and follicular unit were harvested by Follicular Unit Extraction method. Outer root sheath cells were extracted by trypsinization. The solution was transplanted over dermabraded recipient site. Pressure dressing was given. Patients were followed up regularly. Statistical Analysis Used: Descriptive Statistics, Chi-Square. Results: Mean ± SD repigmentation was 80.15% ± 22.9% with excellent repigmentation (90-100%) in 60% of patients. Conclusions: This method is safe, effective, and simpler than the other methods involving cell culturing and requiring a laboratory set-up but selection of patients is crucial for the success of the outcome.

Key words: Follicular Unit Extraction, melanocytes stem cells, noncultured extracted hair follicle outer root sheath suspension, vitiligo

INTRODUCTION

Vitiligo is a common pigmentary disorder of great cosmetic concern, social embarrassment, and psychological distress, especially among the dark skinned individuals like Indians. Surgical treatment is indicated in stable disease not responding to medical treatment. There are various surgical modalities available for vitiligo, which are based on the idea of restoring melanocytes on the recipient site. They are as follows:

Tissue grafting:
- Suction blister epidermal grafting
- Thin and ultrathin split-thickness skin grafting
- Mini punch grafting

Cellular grafts:
- Cultured pure melanocyte transplantation
- Co-cultured melanocyte – keratinocyte suspension cell transplantation
- Cultured epidermis
- Noncultured basal cell layer enriched epidermal cell suspension transplantation

Context

The role of the hair follicle in the pigmentation of the vitiliginous lesion has been well proven in the landmark paper by Cui et al. who observed that some others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Shah AN, Marfatia RK, Saikia SS. A study of noncultured extracted hair follicle outer root sheath cell suspension for transplantation in vitiligo. Int J Trichol 2016;8:67-72.
dihydroxyphenylalanine (DOPA-negative) “inactive” melanocytes (proposed to be melanocyte stem cells) were found to reside in the outer root sheath (ORS) and bulge areas of the hair follicle. These melanocytes did not produce melanin under normal conditions but became active to produce melanin when stimulated either by ultraviolet radiation or by dermabrasion. It was also observed that in vitiliginous lesions, there was destruction of only the active (DOPA-positive) melanocytes, whereas the inactive melanocytes in the ORS of the hair follicle were undamaged. These melanocytes were proposed to be responsible for repigmentation in vitiligo by dividing and migrating upward along the surface of the hair follicle to the nearby epidermis where they continued to move radially and lead to repigmentation of surrounding skin. Vanscheidt and Hunziker used single cell suspension of “plucked” hair follicles in the treatment of vitiligo where they found almost complete (>90%) repigmentation in three of five patients with vitiligo, around 50% repigmentation in one patient, and <10% repigmentation in one patient. However, the cell yield was less in the case of plucked hair follicles. Cell suspension prepared from hair follicles obtained by Follicular Unit Extraction (FUE) method contains more CD200+ cells (a marker for HFSCs, i.e. hair follicle stem cells) when compared to plucked hair so as a refinement of this technique. Mohanty et al. performed follicular cell suspension in 14 patients of vitiligo using FUE method and achieved >75% pigmentation in nine patients.

There are very few studies on noncultured extracted hair follicle (EHF)-ORS cell suspension for transplantation in vitiligo. Keeping these factors in mind, we decided to undertake this study to determine the efficacy and safety of this therapeutic approach in our patients.

Aims and objectives

- To assess the effectiveness of noncultured EHF-ORS cell suspension in stable vitiligo
- To perform objective and subjective evaluation based on clinical improvement and photographic evidence
- To observe any untoward events or side effects
- To assess the stability of EHF-ORS induced repigmentation.

MATERIALS AND METHODS

Study design

The study was open and uncontrolled. All the patients were screened at the preliminary visit. Reviews were done every 2 weeks. The endpoint selected was 6 months postprocedure.

Patients

A total of 25 patients with vitiligo were enrolled from the outpatient Department of Dermatology during the period of July 2012 to July 2014. Approval of Hospital Ethical Committee was taken. The patients were selected as per the inclusion criteria.

Selection of the patient

Inclusion criteria

1. Age of patient more than 18 years
2. Patients who have not taken any form of treatment in the past 1-month
3. Patients willing for the procedure
4. Patients with vitiligo stable for 1 year.

Exclusion criteria

1. Patients with a history of bleeding disorders
2. Patients on anticoagulant medications (aspirin, warfarin, and heparin)
3. Patients with active infection at the local site
4. Patients with keloidal tendency
5. Patients with a history of psoriasis or lichen planus because of risk of Koebner phenomenon
6. Patients with a history of recurrent herpes labialis
7. Hepatic, renal disease, epilepsy, or any major medical illness
8. Patients positive for HIV and HBsAg
9. Patients with unrealistic expectations
10. Patients not giving consent
11. Pregnancy
12. Age <18 years
13. Patients unable to come for 6 months follow-up.

At the first visit, a pro forma was filled in noting the detailed history and a thorough dermatological examination was carried out taking note of the number of depigmented lesions and approximate percentage of body surface area involved using the rule of nine.

Clinical photographs were taken.

Hematological and biochemical investigations were carried out in all the patients.

For each patient, the localization of the lesions was divided in six different body sites: Face, neck, trunk, upper limb, hands, and lower limbs including foot.
Informed written consent was obtained from each and every patient after having carefully explained to them the nature of the treatment including details of benefits, possible side effects, and other modalities of treatment available.

A prophylactic course of tablet cefadroxil (500 mg) twice daily was started 1 day before the procedure.

The technique

The technique employed was the autologous EHF-ORS cell suspension (noncultured) technique, which shares a common principle of selective replenishment of melanocytes at the recipient stable Vitiligo patches.[9] Donor area (for hair follicle tissue harvest)

In all patients, FUE was done from the occipital area. Hairs were trimmed to a length of approximately 2 mm. The area was cleaned with povidone-iodine and 70% ethanol and draped. Local anesthesia was given with 2% lignocaine, which was infiltrated in the skin, encircling the area chosen for FUE. To obtain follicular units, a 1 mm punch was rotated to the mid-dermis in the direction of the hair follicle. Care was taken not to go into the subcutaneous plane to avoid transection of the hair follicle.[9] Then, the follicular unit was pulled out gently using hair-follicle-holding forceps by holding the skin surrounding the hair shaft. Depending on the area to be transplanted, approximately 20–25 pigmented follicles were extracted per subject and collected in collection medium containing Dulbecco’s Modified Eagle Medium (DMEM), pH 7.2, supplemented with penicillin, streptomycin, and amphotericin B (Figure 1b-d). Donor area was not dressed, rather it was kept open and topical mupirocin 2% cream was applied. Mupirocin 2% cream was continued for 7 days after removal of the first dressing. After 1 week, oral analgesic (ibuprofen 200 mg twice a day × 7 days) and oral antibiotic (cefadroxil 500 mg twice a day × 7 days) were prescribed to the patient. The dressing was removed at the first follow-up visit after 1 week.

The recipient area was abraded manually until tiny pinpoint bleeding spots were seen, which implied that the dermoeidermal junction had been reached (Figure 2a). The suspension was poured evenly from the pipette to the denuded surface, which was then covered with a collagen dressing, NeuSkin® (Eucare Pharmaceuticals, Chennai, Tamil Nadu, India). This was then covered with Jelonet® (Smith and Nephew, Maharashtra, India). The dressing was kept in place by a Dynaplast® (Johnson and Johnson, Maharashtra, India) dressing. The patient was allowed to go home 30 min later. Dressing kept in place for total 7 days, oral antibiotic (cefadroxil 500 mg twice a day × 7 days) and oral analgesic (ibuprofen 200 mg twice a day × 7 days) were prescribed to the patient. The dressing was removed at the first follow-up visit after 1 week.

Posttransplant treatment

Topical mupirocin 2% cream was given for local application for seven days after removal of the first dressing.

The number of cells in the suspension were counted using hemocytometer. The viability of the cell suspension was checked by the trypan blue dye exclusion method.

Recipent area

All precautions were taken to minimize any risk of transmission of infectious agents via aerosol production. All OT staff was wearing mask and goggles during the procedure.

The recipient area was cleaned, painted, and draped with povidone-iodine, 70% ethanol, and washed thoroughly with normal saline. It was anesthetized by infiltrating 2% lignocaine.

Transplantation of suspension

The recipient area was abraded manually until tiny pinpoint bleeding spots were seen, which implied that the dermoeidermal junction had been reached (Figure 2a). The suspension was poured evenly from the pipette to the denuded surface, which was then covered with a collagen dressing, NeuSkin® (Eucare Pharmaceuticals, Chennai, Tamil Nadu, India). This was then covered with Jelonet® (Smith and Nephew, Maharashtra, India). The dressing was kept in place by a Dynaplast® (Johnson and Johnson, Maharashtra, India) dressing. The patient was allowed to go home 30 min later. Dressing kept in place for total 7 days, oral antibiotic (cefadroxil 500 mg twice a day × 7 days) and oral analgesic (ibuprofen 200 mg twice a day × 7 days) were prescribed to the patient. The dressing was removed at the first follow-up visit after 1 week.

Posttransplant treatment

Topical mupirocin 2% cream was given for local application for seven days after removal of the first dressing. After
15 days, patients were advised to apply topical mometasone furoate (0.1%) cream in the morning and topical tacrolimus (0.03%) ointment at night till four months postprocedure and then followed up for another 2 months.

Evaluation

Response was graded as:[17]
- Grade 1 (poor) - <50% repigmentation
- Grade 2 (fair) - 50–74% repigmentation
- Grade 3 (good) - 75–89% repigmentation
- Grade 4 (excellent) - 90–100% repigmentation.

Moreover, the repigmentation pattern was noted as “diffuse,” “perifollicular,” or “dotted.”

A note was also made on the color matching of the repigmented skin as “lighter than,” “similar,” or “darker than” normal skin.

The clinical outcome was documented every 2 weeks by standardized photographs for up to 6 months [Figures 3, 4]. The repigmentation was assessed subjectively by comparing pretreatment and posttreatment photographs.

RESULTS

Total twenty patients completed the study. Five patients were lost to follow-up. To determine the statistical aspects of the results, the following observations were made and analyzed.

Demographic and disease characteristics

Age group

Of the 20 patients, 15 (75%) of patients were in the age group of 18–25 years. This finding is comparable with age range observed by Lahiri and Sengupta where the majority of the patients were in the age group of 16–25 years.[18]

Mean ± standard deviation (SD) age of patients 24.45 ± 6.07 years ranging from 18 to 43 years. The mean...
Shah, et al.: A study of noncultured extracted hair follicle outer root sheath cell suspension for transplantation in vitiligo

age is comparable to the study by Mohanty et al. where the mean ± SD age was 22.8 ± 4.8 years. And also to the study by Singh et al. where mean ± SD age was 23.33 ± 4.894.

Sex distribution

Of the 20 patients, 15 (75%) were females and five (25%) were males. The female: male ratio was 3:1. This shows a definite female preponderance signifying major cosmetic concerns among females seeking more treatment. The findings regarding sex distribution are in accordance with the observation of Mohanty et al.

Duration of vitiligo

The duration of vitiligo ranged from 2 to 20 years with majority (50%) having a duration between 6 and 10 years. The mean ± SD duration of vitiligo was 7.7 ± 4.25 years.

This observation was similar to the study by Singh et al. where mean ± SD duration was 5.13 ± 1.727.

Family history

There was a positive family history of vitiligo in 30% patients. Vitiligo is said to have a positive family history is 30–40% of the patients.

Marital status

Majority of the patients (85%) were unmarried. This implicates that vitiligo is still a social stigma and patients seeking the treatment are majorly eligible bachelors.

Associated conditions

Anemia was the most common comorbidity seen in 15% patients followed by diabetes mellitus in 5% of patients. This is in accordance with the study by Singh et al. where they observed associated autoimmune diseases in 13.3% of patients (2 of 15 patients).

These comorbidities were corrected before the procedure.

Site of involvement

Since many patients had more than one body sites involved, the total is more than 20.

Majority of the patients (65%) had involvement of lower limbs, followed by face and neck (30%), trunk (15%), hands (10%), and upper limbs (5%).

The Chi-square is 26.903 and $P = 0.008$ and hence statistically significant.

Type of vitiligo

Forty percent of patients were of vitiligo vulgaris type, followed by acral (25%), focal (15%), segmental (15%), and lip tip variant (5%). Singh et al. included 13.33% patients of focal vitiligo and 40% patients of segmental vitiligo.

The mean ± SD of the number of hair follicle units taken was 30.25 ± 5.76 in the present study.

The mean ± SD numbers of cells transplanted are 3718.25 ± 803.5. This is different from the study by Mohanty et al. This difference is due to interindividual variation in the method of taking graft for FUE.

The mean proportion of viable cells in the suspension was 98%. This is in contrast to the study by Mohanty et al. where the proportion of viable cells was 91%.

The mean area transplanted was 37.3 cm².

Number of patients in different grades of vitiligo

Excellent repigmentation (90–100%) was achieved in 60% of patients. Only one patient with lip tip vitiligo showed <50% repigmentation.

The mean ± SD pigmentation was 80.15 ± 22.9%.

Comparison of mean pigmentation

The difference in mean pigmentation was because Mohanty et al. selected three patients with disease stability of <1 year and viability of the cells was also less. Hence, in spite of the less number of cells transplanted in the present study, mean pigmentation observed was more.

Comparison of mean repigmentation in segmental versus vulgaris type

The mean ± SD repigmentation was more in the segmental group (88.33 ± 7.63%) compared to vulgaris (80.5 ± 18.06%).

Side effects

No side effects were observed at the donor site.

Recipient area side effects

Erythema was the most common side effect (90%) followed by hyperpigmentation (75%), pruritus (25%),
xerosis (10%), and infection in one patient. Erythema was asymptomatic and resolved spontaneously in 1-week after removal of dressing.

Color match

The repigmentation was darker in 75% patients and similar to normal skin in 25% patients.

The repigmentation was diffuse in all patients and not follicular. This suggests that repigmentation was because of the transplantation and not due to dermabrasion.

At the first follow-up, soon after the removal of dressing, the treated area appeared bright pink. Repigmentation was first seen 2 to 3 weeks after the procedure and was completed in up to 6 months. It was almost of a uniform color. In a few cases, there was initial hyperpigmentation that subsequently faded to match normal skin color.

Stability at 6 months follow-up

All the patients were stable, and there was no recurrence at 6 months follow-up.

CONCLUSION

• This study which was done over a span of 2 years revealed that noncultured EHF-ORS cell suspension for transplantation in vitiligo could serve as a novel, minimally invasive, scarless technique. It gives a good yield of melanocytes, melanocyte stem cells, and other stem cells (keratinocyte stem cells and mesenchymal stem cells in the surrounding dermis and dermal papilla). It results in an excellent pigmentation with no visible residual scarring of the donor area and good color match of the recipient
• Preparation of ORS cell suspension is technically less challenging than preparation of epidermal cell suspension
• Extracted hair follicular ORS cell suspension transplantation is effective in repigmentation of vitiligo stable for a year or more.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Parsad D, Dogra S, Kanwar AJ. Quality of life in patients with vitiligo. Health Qual Life Outcomes 2003;1:58.
2. Falabella R. History and chronology of development of surgical therapies for vitiligo. In: Gupta S, Olsson MS, Kanwar AJ, Ortonne JP, editors. Surgical Management of Vitiligo. 1st ed. Massachusetts, USA: Blackwell Publishing; 2007. p. 41-8.
3. Khunger N, Kathuria SD, Ramesh V. Tissue grafts in vitiligo surgery – Past, present, and future. Indian J Dermatol 2009;54:150-8.
4. Falabella R. Epidermal grafting. An original technique and its application in achromic and granulating areas. Arch Dermatol 1971;104:592-600.
5. Falabella R. Repigmentation of leukoderma by minigrafts of normally pigmented, autologous skin. J Dermatol Surg Oncol 1978;4:916-9.
6. Falabella R, Escobar G, Borrero L. Transplantation of in vitro-cultured epidermis bearing melanocytes for repigmenting vitiligo. J Am Acad Dermatol 1989;21 (2 Pt 1):257-64.
7. Gauthier Y, Surleve-Bazeille JE. Autologous grafting with noncultured melanocytes: A simplified method for treatment of depigmented lesions. J Am Acad Dermatol 1992;26 (2 Pt 1):191-4.
8. Cui J, Shen LY, Wang GC. Role of hair follicles in the repigmentation of vitiligo. J Invest Dermatol 1991;97:410-6.
9. Kumar A, Mohanty S, Sahni K, Kumar R, Gupta S. Extracted hair follicle outer root sheath cell suspension for pigment cell restoration in vitiligo. J Cutan Aesthet Surg 2013;6:121-5.
10. Staricco RG. Melanocytic melanomas in the outer sheath of the human hair follicle. J Invest Dermatol 1959;33:295-7.
11. Staricco RG, Miller-Milinska A. Activation of the amelanotic melanocytes in the outer root sheath of the hair follicle following ultraviolet rays exposure. J Invest Dermatol 1962;39:163-4.
12. Staricco R. Mechanism of migration of melanocytes from the hair follicle into the epidermis following dermabrasion. J Invest Dermatol 1961;36:99-104.
13. Malakar S, Dhar S. Repigmentation of vitiligo patches by transplantation of hair follicles. Int J J Dermatol 1999;38:237-8.
14. Vanscheidt W, Hunziker T. Repigmentation by outer-root-sheath-derived melanocytes: Proof of concept in vitiligo and leucoderma. Dermatology 2009;218:342-3.
15. Kumar A, Gupta S, Mohanty S, Bhargava B, Airan B. Stem cell niche is partially lost during follicular plucking: A preliminary pilot study. Int J Trichology 2013;5:97-100.
16. Mohanty S, Kumar A, Dhawan J, Sreenivas V, Gupta S. Noncultured extracted hair follicle outer root sheath cell suspension for transplantation in vitiligo. Br J Dermatol 2011;164:1241-6.
17. Singh C, Parsad D, Kanwar AJ, Dogra S, Kumar R. Comparison between autologous noncultured extracted hair follicle outer root sheath cell suspension and autologous noncultured epidermal cell suspension in the treatment of stable vitiligo: A randomized study. Br J Dermatol 2013;169:287-93.
18. Lahiri K, Sengupta SR. Treatment of stable and recalcitrant depigmented skin conditions by autologous punch grafting. Indian J Dermatol Venereol Leprol 1997;63:11-4.
19. Anstey AV. Disorders of Skin Colour. In: Burns T, Breathnach S, editors. Rook’s Textbook of Dermatology; 8th ed. West Sussex, UK-Wiley-Blackwell publication; 2010. p. 58.46.