

Efficacy of Autologous Smashed Follicular Dermal Graft and Epidermal Cell Suspension in the Treatment of Chronic Nonhealing Trophic Ulcers in Hansen's Patients

Abstract

Context: Plantar ulcers occur in patients with leprosy not because of the disease but because of its neuropathic effects on the skin on the feet. Neuropathy increases the risk of trauma to patients' feet, leading to the development of ulcers. With the introduction of new therapeutic regimens, leprosy can now be cured. However, complications of the disease, such as sensory loss, muscle palsy, absorption of extremities, and recurrent ulcers, still lead to substantial morbidity. This short article reviews the current management of leprosy plantar ulceration in such (nonhealing chronic ulcers) patients. **Aims:** To evaluate the efficacy of autologous smashed follicular dermal graft and epidermal cell suspension in the treatment of chronic nonhealing trophic ulcers in Hansen's patients. **Materials and Methods:** A total of 23 chronic nonhealing trophic ulcers were treated with autologous smashed follicular dermal graft and epidermal cell suspension. Ulcers were assessed based on the rate of ulcer size reduction every week till 12 weeks and then once a month till the sixth month. **Statistical Analysis:** Analysis was done using SPSS 26 software. Independent *t*-test was used and a *P* value of <0.05 was considered statistically significant. **Results:** Amongst the Hansen's patients who were released from treatment, all 23 (100%) ulcers had healed within the study period of six months, and all 23 (100%) ulcers had healed within 8 weeks. **Conclusion:** With this modified technique of combining autologous smashed follicular dermal graft with epidermal cell suspension, trophic ulcers heal faster with good results of reepithelialization of the ulcer bed.

Keywords: Autologous smashed follicular dermal graft, epidermal cell suspension, leprosy, ulcer

Introduction

Leprosy, a chronic infectious disease caused by the *Mycobacterium leprae*, continues to be one of the public health challenges for countries like India, which accounts for more than half of the world's new leprosy cases.^[1] Dysfunction of peripheral nerves resulting in skin anesthesia is one of the salient clinical manifestations of leprosy, which further leads to disabilities, including ulcers.^[2] Managing chronic trophic ulcers is challenging. When all the conservative and medical methods fail, surgical management of ulcers may be the only ray of hope.

Autologous noncultured epidermal cell suspension (ECS) has already gained popularity in stable vitiligo. Like cultured keratinocyte allograft, autologous ECS stimulates the growth factors and extracellular matrix proteins.^[3] Thus, when used in ulcers, it may promote migration

or multiplication of acceptor keratinocytes and formation of extracellular matrix proteins. To prepare cultured keratinocytes for grafting, a minimum of 3–4 weeks is required,^[4] whereas ECS is easily trypsinized and economically prepared in few hours.^[3]

Hair follicles (HFs) act as ulcer promoters.^[5] The restoration of epithelium after the injury takes place by migration of epithelial cells from the old epithelium adjoining an ulcer or by centrifugal migration from any HFs remaining within the ulcer.^[6] Thus, follicular unit grafting into ulcer beds is feasible and represents a promising therapeutic alternative for managing nonhealing chronic leg ulcers.^[7]

Materials and Methods

After obtaining approval from the Institutional Ethics Committee and

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informed written consent from each subject, male patients between 18 and 60 years of age, with nonhealing trophic ulcers secondary to Hansen's disease, who have already been released from treatment, were included in the study. Trophic ulcers more than 6-week duration and not more than 5 cm × 5 cm were included. Patients with bleeding disorders, anemia and other hematological disorders, uncontrolled diabetes, malignant ulcers, and venous ulcers were excluded. The ulcer size in terms of length, breadth, and depth was measured. Complete evaluation with relevant basic investigations was done. Primary infection, if any, was treated using antibiotics and/or surgical debridement as and when required before including them in the study.

Procedure

Step 1—Harvesting ECS [Figure 1]

The anterolateral aspect of the thigh was chosen as a donor site. The parts were prepared and anesthetized using topical anesthesia, i.e., a mixture of Lidocaine 25 mg and Prilocaine 25 mg under occlusion was applied for 1 h. Ultrasplit thickness graft measuring 5 × 5 cm² was then harvested from the donor site using a razor blade holding dermatome. The graft harvested was placed in 10 ml trypsin-EDTA (trypsin-ethylenediaminetetraacetic acid) solution with the epidermis facing upward in a petri dish and incubated at 37°C for 50 min. After incubation, the graft was transferred to Dulbecco's Modified Eagle's Medium in another petri dish. Using blunt forceps and agitation, the epidermis was separated from the dermis. The cell suspension obtained was then transferred to a 15-ml centrifuge tube.

Step 2—Harvesting dermal grafts [Figure 2]

After taking graft for ECS, follicular dermal grafts were harvested using punches of size 2.5 mm from the same donor site, as for the ECS procedure, spaced at least 5 mm from each other, ensuring that each graft consists of a HF. The number of grafts required were to be approximately two grafts per cm² of ulcer area. The grafts were then transferred to a petri dish containing normal saline. Dermal grafts were minced with surgical blade number 15 to form smashed dermal grafts. The smashed dermal graft was transferred into the same 15-ml centrifuge tube that contained ECS from step one.

Step 3—Dressing of donor area

Firm compression with dressing paraffin gauze dressing was applied over the donor area.

Step 4—Application of dermal graft and ECS to ulcer bed [Figure 3]

The mixture of smashed dermal grafts and ECS harvested above in the 15-ml centrifuge tube was centrifuged at 3000 rpm for 10 min. The pellet thus obtained was



Figure 1: Graft is transferred to a petri dish containing trypsin-EDTA and then incubated. The incubated specimen is then subjected vigorous agitation to separate epidermis and dermis



Figure 2: (a) Harvesting follicular dermal grafts with biopsy punch. (b) Follicular grafts obtained after smashing



Figure 3: (a) Pellet obtained after centrifugation. (b) Application of smashed follicular dermal graft and ECS mixture to the ulcer bed

spread evenly over the ulcer bed. The ulcer bed was then covered with paraffin gauze and Johnson and Johnson 3M Dynaplast. In case of ulcers in the vicinity of joints, such as the ankle, immobilization was required with a below-knee plaster of Paris slab.

Postprocedure instructions and follow-up

All subjects were instructed to take complete rest and to avoid all vigorous physical activities for 7 days. They were prescribed oral antibacterial agents for 5 days. Below knee plaster of Paris cast, if any, was removed after 7 days postsurgery. Dressing on both donor and recipient was removed, and regular follow-up was done with regular paraffin gauze and saline dressing every week till 12 weeks and then once a month till the sixth month or complete healing, whichever was earlier. Baseline and at every follow-up visit, wound area was measured by the formula Length × Width × 0.7854 (an ellipse is closer to a wound shape than a square or rectangle), and volume measured by Length × Width × Depth × 0.7854.^[8] A percentage reduction of 100% in size of ulcer was defined as complete healing and anything less than that was considered as incomplete healing.

Percentage of change in volume and area of the ulcer^[7]

$$= \frac{(\text{Baseline measurement} - \text{Assessment day measurement})}{100} \times 100\%$$

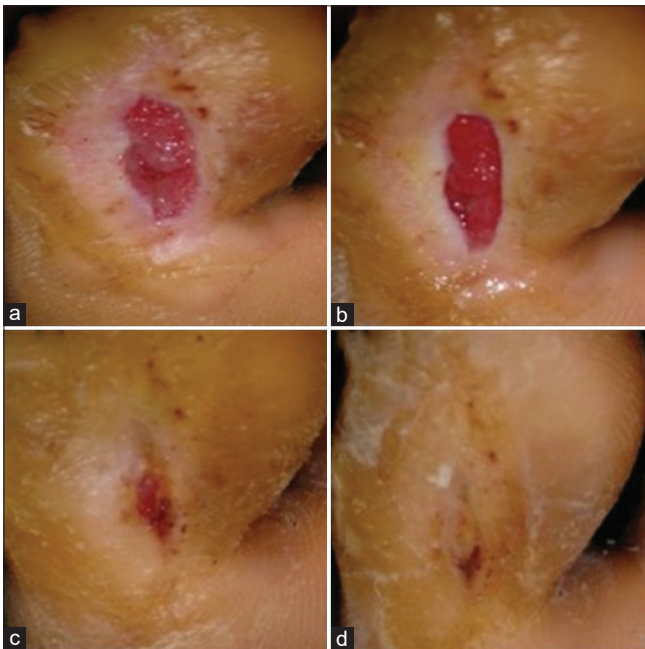


Figure 4: Case 1. (a) Before procedure. (b) 1 week postprocedure. (c) 3 weeks postprocedure. (d) 5 weeks post procedure

Results

Among the 23 ulcers studied, the average duration of ulcers before inclusion in the study was 8.68 months. Out of the 23 ulcers, 8 (34.78%) ulcers were situated on the base of great toe suggesting that the majority of trophic ulcers in the study were found on the base of great toe. Even though the ball of the foot is the most commonly affected site in trophic ulcers, it was found to be the least affected site in our study. The mean area of ulcers with respect to baseline was 6.78 ± 2.33 cm² after 1 week of treatment, which reduced to 1.04 ± 0.94 cm² in 4 weeks and 0.01 ± 0.03 cm² in 7 weeks, which later healed completely in 8 weeks [Table 1], [Figures 4 and 5]. The mean volume of ulcers with respect to baseline was 6.70 ± 4.68 cm³ after 1 week of treatment, which reduced to 0.30 ± 0.45 cm³ in 4 weeks and 0.01 ± 0.00 cm³ in 7 weeks, which later healed completely in 8 weeks. Out of 23 trophic ulcers, 3 (13%) ulcers had healed within 5 weeks, 8 (34.8%) ulcers in 6 weeks, 10 (43.5%) ulcers in 7 weeks, and 2 (8.7%) ulcers in 8 weeks [Table 2].



Figure 5: Case 2 (a) Before procedure. (b) 1 week post procedure. (c) 3 weeks post procedure. (d) 5 weeks post procedure

Table 1: Mean area and volume of ulcers at different study points

Time of follow up	Mean area of ulcer (Mean±SD) (cm ²)	P	Mean volume of ulcer (Mean±SD) (cm ³)	P
Baseline	8.06±2.77	0.302	6.65±7.16	0.107
2 nd week	3.48±2.04	0.024**	4.16±2.79	0.048**
4 th week	1.04±0.94	<0.001**	0.30±0.45	<0.001**
6 th week	0.09±0.12	<0.001**	0.03±0.02	<0.001**
8 th week	0.00±0.00	–	0.00±0.00	–

** P value<0.05=Significant

Discussion

The technique involving ECS was invented to enable the possibility of treating larger wounds with a small piece of donor skin.^[9] In a pilot study on the effect of autologous ECS in chronic nonhealing wounds by Shukla *et al.*,^[3] 6 (40%) out of 15 patients had completely healed at 12 weeks, 1 patient (7%) at 16 weeks, and 2 patients (13%) at 20 weeks postprocedure. In a study by De Angelis *et al.*,^[10] on the use of a noncultured autologous ECS in chronic ulcers, complete ulcer healing, defined as 100% reepithelialization, was observed between 40 and 60 days in 14 patients (70%). At day 60 postprocedure, 80% reepithelialization was present in 5 patients (25%), whereas 1 patient with concomitant psoriasis had 50% reepithelialization.

In a study on follicular unit grafting in chronic nonhealing leg ulcers by Budamakuntla *et al.*,^[7] a total of 15 patients with 17 ulcers were treated with follicular unit graft. The

baseline mean area of the ulcer was 6.72 cm² and baseline volume was 2.87 cm³. The final area of the ulcer at the end of 18 weeks after the procedure was 3.84 cm² and the final volume was 1.21 cm³, which was statistically significant. In a randomized controlled study on HF containing punch grafts in chronic ulcers by Martínez *et al.*,^[11] ulcer healing measured as the average percentage reduction, 18 weeks postintervention was significantly increased ($P = 0.002$) in the HF group with a 75.15% ulcer area reduction compared with 33.07% in the control group (nonhairy grafts).

Nagaraju *et al.*^[12] in his study noted that in the technique of autologous smashed follicular dermal graft with ECS in 12 cases of chronic nonhealing ulcers, 41.6% of the ulcers healed within 4 weeks postprocedure, and 100% ulcers had completely healed by the 8th week. Ulcers healed both from the periphery as well as in a centrifugal manner, which gradually merged to cover the ulcer bed completely. Hypertrophic granulation tissue was seen in two of the ulcers, which subsided spontaneously within a week with regular normal saline dressing. The donor site in all cases showed complete healing by 7 days postprocedure with mild pigmentation. No other adverse effect was noted in any case.

The results from all the above studies [Table 3] infer that noncultured ECS is one of the better surgical modalities in treating chronic nonhealing ulcers as it seems to be an effective, simple, economical, and time-saving method and larger area can be treated with smaller donor area. Similarly, hair follicular dermal grafting is a minimally

Table 2: Time of complete healing of the trophic ulcers

Time of Healing	Number of Ulcers	Percentage (%)
Healed		
5 th week	3	13
6 th week	8	34.8
7 th week	10	43.5
8 th week	2	8.7
Not healed	0	0
Total	23	100

Table 3: Comparison of our data with other studies

Parameters	Other Studies	Our Study (Smashed Follicular Dermal Graft + ECS)
Mean area of ulcer at baseline	<i>Budamakuntla et al.</i> ^[7] : (Follicular dermal graft) The mean area of ulcers at baseline was 6.72 cm ² . <i>Shukla et al.</i> ^[3] : (ECS) The mean area of ulcers at baseline was 80.96 cm ² .	The mean area of ulcers at baseline in our study was 8.06 cm ² .
Time for complete healing	<i>Shukla et al.</i> ^[3] : (ECS) 6 (40%) out of 15 patients had completely healed at 12 weeks, 1 patient (7%) at 16 weeks, and 2 patients (13%) at 20 weeks after treatment. <i>De Angelis et al.</i> ^[10] : (ECS) 100% reepithelialization was observed between 40 and 60 days in 14 (70%) out of 20 patients. At day 60 postprocedure, 80% reepithelialization was present in 5 patients (25%), whereas 1 patient with concomitant psoriasis had 50% reepithelialization. <i>Nagaraju et al.</i> ^[12] : (Smashed follicular dermal graft + ECS) At the end of 4 weeks, mean percentage of improvement in ulcer area was 98.33% and that in ulcer volume was 99.26%.	In our study, out of 23 ulcers, 3 (13%) ulcers had healed within 5 weeks, 8 (34.8%) ulcers in 6 weeks, 10 (43.5%) ulcers in 7 weeks, and 2 (8.7%) ulcers in 8 weeks. The mean percentage of change in the area of the ulcers with respect to baseline showed 34.13% improvement after 1 week of treatment, which improved to 87.26% in 4 weeks and 99.88% in 7 weeks, which later completely healed with 100% improvement in 8 weeks. All 23 (100%) ulcers had healed within the study period of 6 months.
Secondary procedures	<i>Shukla et al.</i> ^[3] : (ECS) 1 patient required skin grafting, and 3 patients were lost to follow-up.	In our study, none of the patients required secondary procedures and none were lost to follow-up.

invasive surgical procedure that appears to be effective as a therapeutic tool for chronic leg ulcers. Hence, epithelial cells from ECS, HFs, and collagen from dermis may enhance the ulcer healing process and reduce the duration of ulcer healing.

Conclusion

The rapid healing of ulcers improves the patient's quality of life by reducing the morbidity. Hence, a search for viable alternative treatment methods is always on the lookout. With improvising the surgical graft technique, there is certainly a great improvement in the treatment of chronic nonhealing ulcers. The use of autologous smashed follicular dermal graft with ECS is a simple technique, manageable even on an outpatient basis that quickly reduces and hastens the reepithelialization of ulcer. Thus we conclude that, it is possible to achieve a faster rate of granulation tissue and a faster recovery of the ulcer from this procedure.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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