

Autologous non-cultured keratinocyte cell suspension in non-healing diabetic ulcers: A preliminary study

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ABSTRACT

Background: Diabetic ulcers are a common morbidity associated with poorly controlled glycemic status. Most patients often have neuropathy and vasculopathy as the etiology behind such ulcers. These factors not only lead to poor wound healing but also nonhealing ulcers. Nonhealing ulcers pose therapeutic challenges as they are usually not amenable to be treated with simple wound care and hence require newer modalities to effectively cure this condition that leads to a plethora of poor health outcomes. This study was conducted to see the effect of autologous noncultured keratinocyte cell suspension in chronic nonhealing diabetic ulcers that failed to normal wound care. **Material and Methods:** It was an observational pilot study. A total of 05 patients with nonhealing ulcers, attending the tertiary care teaching hospital in North India, were included in the study. Inclusion criteria was type 2 DM with more than 5 years duration of diabetes mellitus and glycated hemoglobin (HbA1c) <9 g%. History was taken in detail; name, age, sex, address, duration of disease and various other treatments taken from outside were noted; and size of ulcer was recorded as per Proforma. **Results:** A total of 5 patients were enrolled in the study, three (60%) and two (40%) patients were male and female, respectively. Three (60%) patients were habitual for tobacco use. Mean age of patients was 45 ± 6.51 years. Mean duration of ulcers was 4.8 ± 1.48 months. Area of ulcers ranged from 31.2 to 122.2 cm². Majority of cases, three (60%) of diabetic foot ulcers, were improved at 9–12 weeks and remaining two cases with large size of ulcer were healed at 13–16 weeks and 17–20 weeks, respectively. Majority (80%) of patients revealed >50% reduction in the size of ulcer within 2 weeks. **Conclusion:** Noncultured keratinocytes are useful in healing of nonhealing diabetic ulcer.

Keywords: Autologous, cell suspension, keratinocyte, noncultured, nonhealing diabetic ulcers

Introduction

Nonhealing ulcers are a cause of high morbidity and reduced quality of life among patients suffering from these ulcers.^[1] The main reasons for nonhealing ulcers are peripheral arterial disease, diabetes mellitus, stasis, and trauma. Diabetes mellitus affects limbs through multiple mechanisms principally through vascular and neurological affliction.^[2] As diabetes mellitus causes ulcers

through various mechanisms, its treatment is not only difficult but also challenging at times. The chronicity of ulcers leads to not only functional loss but also the poor health-related quality of life.^[3] Also, diabetic foot ulcers had a higher mortality rate and there are several factors which increase the risk of mortality in diabetic foot ulcer patients such as age, CKD, and low albumin levels.^[4] An earlier study revealed 45.7% ulcer healing, 36.2% amputations, 9.5% died before healing of ulcer and 8.5% patients were lost to follow-up.^[5] HbA1c and BMI were found useful in early identification of diabetic foot ulcers and, hence, could prevent the amputations of lower extremity. Therefore, glycemic control and improvement in knowledge of health care personnel are necessary steps to diagnose foot ulcers in the early stages.^[6]

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Received: 15-04-2020

Revised: 10-06-2020

Accepted: 02-07-2020

Published: 30-09-2020

Access this article online

Quick Response Code:



Website:
www.jfmprc.com

DOI:
10.4103/jfmprc.jfmprc_627_20

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How to cite this article: Barnwal S, Kant R, Yadav P. Autologous non-cultured keratinocyte cell suspension in non-healing diabetic ulcers: A preliminary study. J Family Med Prim Care 2020;9:4686-91.

Even after treatment, many nonhealing diabetic foot ulcers persist for months/years and required advanced wound care strategy for complete healing.^[7] A previous experimental study using autologous noncultured and cultured keratinocytes in a porcine model suggested that wounds transplanted with keratinocyte suspension showed rapid healing.^[8] This pioneering work was undertaken to study the effect of autologous noncultured keratinocyte cell suspension in chronic nonhealing diabetic ulcer.

Aim

To assess the efficacy of autologous noncultured keratinocyte cell suspension in chronic nonhealing diabetic ulcer.

Objectives

1. To identify the main reasons for chronic nonhealing ulcers in diabetic patients
2. To assess the morphological features of chronic nonhealing ulcers in the diabetic population.

Material and Methods

Study design

An observational pilot study.

Study settings

Data was collected in the department of Dermatology at Institute of Medical Sciences, Banaras Hindu University. A total of 05 patients with nonhealing ulcers, attending wound clinic were included in the study. History and physical examination were done in detail, treatment taken from outside was noted, and size of ulcer was recorded as per Proforma. Ethical permission was also obtained from IEC, Institute of Medical Sciences, Banaras Hindu University. Informed consent was taken from each patient before performing the procedure. Patients with healthy granulation tissue at the base of nonhealing ulcer were included and patients with a sign of systemic or local malignancy were excluded. Patients admitted in the ward, proper antibiotic and wound care was done till the swab culture became sterile from the ulcer.

Inclusion criteria

- Nonhealing ulcers
- Type 2 DM with more than 5 years duration of diabetes mellitus
- HbA1c <9%.

Exclusion Criteria

- HbA1c >9%
- Severe anemia
- Presence of gangrene in affected limb
- Nonpalpable pulses in lower limb
- End-stage renal disease
- Coronary artery bypass graft.

Procedure

i. Taking of skin graft:

Maintaining sterile condition, part preparation was done with Savlon and spirit, after shaving the hair of the donor site. After that local anesthesia was given with 2% xylocaine, thin shaving graft was taken from medial aspect of thigh and kept in screw-capped bottle containing trypsin [0.25%], glucose phosphate buffer solution

ii. Preparation of cell suspension:-

Screw capped bottle was transported to the department of microbiology where tissue was incubated overnight at 4°C. Tissue graft was rinsed thoroughly in normal saline and thin film of 0.8% solution of sodium citrate in normal saline was prepared and tissue graft was further soaked for a few minutes. The dermis was separated from the epidermis by using a tweezer. The resultant coarse suspension was then taken by mean of glass pipette to a small glass tube. Vigorous agitation with the pipette was done to separate the epidermal cells. Epidermal cell suspension thus prepared was used for inoculation

iii. Inoculation procedure:

For maintaining sterile conditions, patients were shifted to the procedure room, and adequate autologous noncultured epidermal cell suspension was inoculated in the prepared ulcer bed by using a syringe and distributed as evenly as possible and dressed with Jelonet followed by a pressure pad. Patients were advised to do a minimum possible activity. A systemic antibiotic was given and primary inspection of the operation field was carried out 7 days postoperatively. The patients were called after 2 weeks, and follow-up of the patients was carried out monthly till patients improved. The observations obtained were tabulated and statistical analysis was done.

Results

A total of 05 patients were enrolled in the study; their clinical characteristics are as given in Table 1. Mean age of patients was 45 ± 6.51 years. Three (60%) and two (40%) patients were male and female, respectively. Majority of patients, three (60%), were habitual for tobacco use. Table 2 shows clinical characteristics of the ulcers as assessed by the standard description of the ulcers like, size, floor, margin, edge, discharge, and surrounding structures. Area of ulcers ranged from 31.2 to 122.2 cm². Only 1 patient had unhealthy floor. Table 3 shows brief details of laboratory parameters. All patients had HbA1c >7% and Hb level more than 10 g/dL. TLC counts ranged from 4200 to 8000 10⁹/L. Table 4 shows mean \pm SD of continuous variables.

Mean duration of ulcers was 4.8 ± 1.48 weeks and mean size of ulcers was 66.33 ± 37.37 cm².

Minimum duration of healing of ulcer was 10.50 weeks. Table 5 shows the follow-up details of patients. Majority of cases, three (60%), diabetic foot ulcers were improved within 9–12 weeks and the remaining two cases were improved at

13–16 weeks and 17–20 weeks, respectively. A patient with a size of ulcer 31.2 cm² had improvement at 9–12 weeks with a reduction in size of ulcer (82%) within 8 weeks [Images 1–4]. Another patient with 122.2 cm² size of ulcer had responded with 93% healing at 13–16 weeks. Majority (80%) of patients revealed >50% reduction in the size of ulcer within 2 weeks. Table 6 shows significant positive correlation ($r = 0.920$, $p = 0.027$) between habituation of tobacco and size of diabetic foot ulcer.

Discussion

The current study was undertaken to assess the effect of autologous noncultured keratinocyte cell suspension in chronic nonhealing diabetic ulcer. The results of the present study described that all cases were healed completely with autologous noncultured keratinocyte cell suspension and in

the majority (60%) of cases, significant reduction in sizes of nonhealing ulcers was noted within 9–12 weeks. In a previous systematic review and meta analysis of studies reporting collagen wound dressings used in the treatment of nonhealing diabetic foot ulcers, found only 58% of wounds, the ulcers treated with collagen wound dressings were completely healed, but the size of ulcers was not taken into consideration in this study.^[9] Further, a study revealed median time period for complete ulcer healing was 12.71 weeks (Confidence interval = 10.00–16.67 weeks) for diabetic nonhealing ulcers with a median duration of 3 months but the majority of patients were not healed completely.^[10] However, in our study, all patients were completely healed and majority (60%) of cases of diabetic foot ulcers were improved in <12 weeks with a median duration of nonhealing ulcers of 5 months. In a previous study, a decision model for the treatment of diabetic foot ulcers was prepared by combining published clinical outcome. In this, patients with nonhealing ulcers with a duration of >6 months with the treatment of a collagen-containing dressing along with conventional care are expected to enhance the chance of healing from 8% to 53% nearly at 17 weeks.^[11] In comparison to the present study, 80% patients revealed a reduction in the size of ulcers from 53.6% to 62.7% within 2 weeks. In another measure of nonhealing ulcer, the topical oxygen delivery revealed a significant effect on wound size as mean ulcer size had reduced by 51% in 8 weeks.^[12] But our study observed early improvement in healing of ulcers as compare to this measure. Because we found that in the majority of cases, three (60%), diabetic foot ulcers were completely improved within 9–12 weeks and these

Table 1: The distribution of cases with age, sex, and their habituation status

Cases	Age in years Mean±SD 45±6.51	Sex (M=Male, F=Female)	Habituation
Case 1	35 (20%)	M	None
Case 2	43 (20%)	M	Tobacco
Case 3	49 (20%)	F	Tobacco
Case 4	46 (20%)	F	None
Case 5	52 (20%)	M	Alcohol/Tobacco

Table 2: Clinical characteristics of the ulcers

Ulcer characteristics	Case 1	Case 2	Case 3	Case 4	Case 5
Duration (Months)	5	7	5	4	3
Size (L=Length, B=Breadth)	6.5 × 4.8	7.5 × 6.29	9.6 × 9	7.1 × 6.3	12 × 10.18
Area	31.2	47.6	86.8	45	122.2
Floor (granulation tissue)	Healthy	Healthy	Unhealthy	Healthy	Healthy
Discharge	Serosanguinous	Serous	serosanguinous	Serous	Serous
Margin	Smooth	Smooth	Smooth	Smooth	Smooth
Edge	Sloping	Sloping	Sloping	Punched out	Sloping
Wagner grade	1	1	2	2	1
Tenderness	None	None	Present	None	None
Surrounding Structures	Healthy	Healthy	Healthy	Healthy	Healthy

Table 3: The laboratory parameters of the enrolled cases

Parameter	Case 1	Case 2	Case 3	Case 4	Case 5
Hemoglobin (Hb)	11.4	12	11.8	10.6	11.6
Total leukocyte count (TLC 10 ⁹ /L)	5800	6500	8000	4600	4200
Differential leukocyte count (DLC /mm ³)	N54L40M4E2	N56L42M2	N65L35	N61L38M1	N59L41
Erythrocyte sedimentation rate (ESR mm in 1 h)	18	24	16	27	10
Total protein (g/dL)	6.8	7	5.8	6.6	6.3
Albumin (g/dL)	3.8	4	2.7	3.6	3.3
HbA1c (%)	8.1	7.7	8.8	7.2	7.7
Blood sugar (F) (mg/dL)	116	123	120	104	103
Blood sugar PP (mg/dL)	166	145	184	157	134
Urea (mg/dL)	32	24	28	30	27
Creatinine (mg/dL)	1.2	1.16	1.4	1.26	1.10



Image 1: Pretreatment

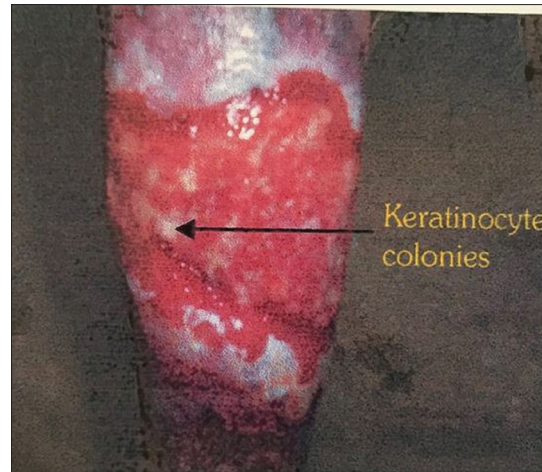


Image 2: Keratinocyte colonies after 7 days of follow-up



Image 3: Follow-up after 5–8 weeks



Image 4: Follow-up after 9–12 weeks

Table 4: Mean ± SD of continuous variables

Variables	n	Minimum	Maximum	Mean±SD
Hb	5	10.6	12	11.48±.54
Duration of ulcers (months)	5	3	7	4.8±1.48
Size of ulcers (cm ²)	5	31.2	122.16	66.33±37.37
ESR (in 1 h)	5	10	27	19±6.70
Total Protein (g%)	5	5.8	7	6.5±.469
Albumin (g%)	5	2.7	4	3.48±.506
HbA1c (%)	5	7.2	8.8	7.9±.595
TLC (per)	5	4200	8000	5820±1527
FBS (mg%)	5	103	123	113.20±9.2
PPBS (mg%)	5	134	184	157±19.25
Urea (mg%)	5	24	32	28.20±3.033
Creatinine (mg%)	5	1.10	1.40	1.224±0.11
Duration of healing (weeks)	5	10.50	18.50	12.9±3.5

cases showed a reduction in the size of ulcer (53.6% to 62.7%) within 2 weeks. In another study, 30 patients were included for the study; 17 were treated with a collagen dressing material and 13 patients were treated with foam dressing and grade of wounds

were with 1 or 2 (Wagner grade). Results revealed the response rate as 82.4%, and 38.5% patients, respectively, which is quite lower than the present study.^[13] In another study, with the use of a collagen wound dressing, conducted with 6 patients had 7 diabetic foot ulcers. But sizes of wounds were not mentioned; the only duration of wounds was taken into consideration and it was ranged from 2 to 18 months. They revealed findings that three patients with lesser duration of ulcers had a reduction in ulcer size, even one patient showed an increase in ulcer area and one patient died of an unrelated cause.^[14] But in comparison to this study, the present study found 100% improvement in nonhealing diabetic ulcers with a lesser duration of healing. In addition, in the results of the present study, it was also observed that habituation of tobacco was strongly correlated with the duration of diabetic foot ulcer. In concurrence with the present study, two meta-analyses independently noted that^[15,16] smoking was found to be associated with nonhealing of diabetic foot ulcers. As, smoking is only one form of tobacco consumption; thus, our study is different in respect to this finding as we found tobacco habituation in any form, which was significantly correlated with the size of nonhealing ulcers. As reduction in the size of diabetic

Table 5: Follow-up characteristics of ulcers

Cases	Size of ulcer (in cm ²) and percent of ulcer during follow-up in weeks							
	1st visit	0-4			5-8	9-12	13-16	17-20
		0-1	1-2	2-4				
1	31.2	26.2 (16%)	12.6 (62.7%)	--	5.5 (82%)	Improved		
2	47.6	40.2 [15.54%]	22 [54%]		9 [81%]	Improved		
3	86.8	74.3 [15%]	42.1 [53.6%]		23.4 [76%]	9.3 [93%]	Improved	
4	45.0	40.4 [10%]	20.5 [54%]		8.8 [79.6%]	Improved		
5	122.2	110.0 [10%]	85.4 [30.2%]		54.0 [56%]	29.6 [76%]	9.0 [93%]	Improved
Median Size of ulcer (cm ²)	47.17	40.40	22		9			

Table 6: Correlation between habituation and size of diabetic foot ulcer

	Size of diabetic foot ulcer	
	Correlation coefficient	P
Habituation	0.920	0.027

Pearson Correlation, P-value <0.05*

ulcers may reduce the chance of amputation^[17] and adversely affecting the health-related quality of life of diabetic patients with diabetic foot ulcers.^[3] Diabetic patients often have chronic diabetic foot ulcer leading to amputation or loss of work, and timely intervention with noncultured epidermal cell suspension leads to rapid healing and can prevent amputation.^[18,19] This study was also a novel for the management of nonhealing diabetic foot ulcers as we have used autologous noncultured keratinocyte cell suspension which was never attempted previously. As it was a preliminary study with limited sample size, future research should be suggested with larger sample size.

Conclusions

The findings conclude with significant effects of autologous noncultured keratinocyte cell suspension on healing diabetic foot ulcers. We found improvement in chronic diabetic ulcers and positive significant correlation of tobacco consumption habit also with the size of diabetic foot ulcers. Chronic diabetic foot ulcer leads to amputation or loss of work but timely intervention with noncultured epidermal cell suspension leads to rapid healing and can prevent amputation/work absenteeism. Primary care physician can timely decide upon this intervention.

Key points

Chronic nonhealing diabetic foot ulcers are amenable to treatment and cure by noncultured epidermal cell suspension. Poor prognosis associated with the worth outcome is smoking.

Declaration of patient consent

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for 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.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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