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Research Article

Effectiveness of amniotic membrane dressing versus conventional dressing in non-healing lower limb ulcers

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Abstract

Context (Background): Non-healing ulcers pose a challenge for a dressing material which will provide an ideal covering with promotion of healing by preventing infection, loss of fluids and the same time adherent to the underlying tissues. The search for such a material of wound dressing led us to human amniotic membrane which promises well with the expectations.

Objectives: This study was designed to assess the effectiveness of amniotic membrane dressing over normal saline dressing in the treatment of non-healing ulcer in terms of promotion of epithelialization, biodegradation of the membrane, prevention of wound infection, prevention of exudation at the site.

Methodology: The prospective and comparative study done during the period of December 2009 to February 2011 in a tertiary care center. The study group included 200 patients with chronic lower limb ulcers, who consented for the study. 85% glycerol preserved seronegative amniotic membranes were used for dressing in 100 randomly selected non-healing ulcer patients (test group) and normal saline (conventional) dressing were done in rest 100 patients (control group). They were visually analyzed at intervals of 7, 14 and 21 days for epithelialization, infection, exudation and biodegradation and response evaluated by scoring criteria.

Results: The observation infers that in 88% of the cases there was complete epithelialization in test group compared to 54% complete epithelialization in control group. There was no exudation by 7 days and membrane peeled off on its own and disappeared at ulcer sites by 21 days in 86 patients treated with amniotic membrane dressing. Infection was observed in 12 of the cases in test group compared to 96% of cases in control group.

Interpretation and conclusion: The results prove that amniotic membrane is an effective dressing in chronic ulcers and acts as an efficient interposition graft to prevent adhesions at ulcer sites and reduce secondary infections in comparison to conventional dressing.

Keywords: amnion; non-healing ulcers: glycerol preservation; biologic dressing, normal saline.

1. Introduction

Chronic skin ulcers, including diabetic ulcers, venous ulcers and pressure ulcers remain a great challenge in the clinic. This has led surgeons to look out for biological materials in treating chronic ulcers. Many researchers have used amniotic membrane dressings in the past in treatment of these ulcers. Amniotic membrane, used as skin graft offers many features of ideal wound coverage like protect wound from secondary infection, provide and maintain a moist environment, prevent loss of fluids, promote healing, be elastic and non-antigenic, adhere well to wound[1]. It's an immunologically inert graft as it do not express HLA-A, B, C and DR or β2 micro globulin[2]. In addition the membrane produces various growth factors including basic fibroblast growth factor, transforming growth factors, angiogenic factors, all contributing to faster granulation and better reepithelialisation[3,4].

This study was designed to evaluate the effectiveness of amniotic membrane dressing in treating chronic ulcers of lower limb in comparison to routine saline water dressings. The efficacy of human amniotic membrane in the re-epithelialization of various ulcers in terms of epithelialization, biodegradability, prevention of infection and exudation from the sites was analysed in this study. In the present study, amniotic membrane is glycerol preserved unlike many previous studies where either air dried, deep freezed[5] or silver impregnated[6]membranes are employed for the dressings.

2. Methodology

A total of two hundred patients reporting to the department of general surgery of tertiary care centre, with non-healing lower limb ulcers were the selected for the study with their informed consent between the period of December 2009 and February2011 after obtaining the necessary clearance from the ethical committee. Only patients with minimum size of ulcer of 5 x 5cms were selected. Patients with tubercular ulcer, malignant ulcers and burns were excluded. Based on the first hospital visit, alternate patients were assigned into amniotic membrane dressings and regular dressing groups and were treated accordingly. Initial clinical evaluation of the patients was done including detailed history of the ulcer, onset, progression and factors responsible for chronicity. All patients underwent detailed laboratory investigation including complete hemogram, ESR, blood sugars – fasting and postprandial, liver and kidney function tests. All patients were admitted and a detailed history with previous treatment modalities and clinical examination with appropriate investigations like x-ray of required area, culture sensitivity of ulcer, edge biopsy, was carried out. Details of ulcer measurements (greatest size and depth), location, edges, floor, debridement and underlying structures were noted in each patient.

Methodology of collection of data: The selected patients have undergone screening for a period of one to two weeks, to stabilize the wound and institute appropriate medical and surgical line of treatment like diabetic control, control of infection by initiating appropriate antibiotic based on culture sensitivity report, surgical debridement, correction of anemia and correction of other medical illness. Once the ulcer showed healthy granulation tissue, patients were subjected to study. Prior to study a repeat culture swab taken from ulcer for microbiological evaluation. *E. coli*, *Pseudomonas*, *Klebshiela* etc.,

if present are treated with appropriate antibiotics according to culture sensitivity report and patients were subjected to study when culture showed no growth. After the initial screening period the eligible patients were treated with either amniotic membrane or routine dressing according to first hospital visit.

The human amniotic membrane necessary for the study were obtained from seronegative mothers who underwent caesarean section at the department of obstetrics and gynecology with their informed consent. The amniotic membrane was separated from the chorion of placenta under sterile aseptic conditions. The amniotic membrane was cleared of all gross tissue attachments and blood clots by washings in copious amounts of tap water. The membrane was then placed in large bottles containing 85% glycerol and stored at room temperatures for 24 hours and then stored at 4^0C in the refrigerator until use.[5,6] The membranes were tested for bacterial count and culture sensitivity done prior application to ulcer area. At the time of application the membrane was reconstituted by soaking it in normal saline for a period of 10 minutes. It was then spread over the surface of the ulcer and non occlusive dressing was placed over the surface. The dressing was left in situ for 4 days and was observed for any exudation. Redressing thereafter was done once in 3 days and evaluated on 7^{th}, 14^{th}, 21^{st} day.

Amniotic membrane dressing were compared with normal saline dressing in terms with promotion of epithelialization, biodegradation of membrane, prevention of infection and prevention of exudation at the site. The promotion of epithelialization was evaluated by considering the size of ulcer. The week in which complete epithelialization occurs is numbered as 1, 2 or 3 accordingly. Even if there was a small breach in epithelium at the end of 3^{rd} week, it was considered as no epithelialization and denoted as X. As ulcer epithelialized and gets adhered to wound there was gradual missing of membrane totally, the week in which this occurred was numbered as 1, 2 or 3 accordingly. If membrane showed necrosis and sloughing of membrane with no epithelialization was denoted as 'NA – Not applicable'. Foul smelling muco-purulent discharge, any change in color of membrane and the surrounding erythema with localsigns of inflammation indicating infection. Presence of infection scored as 1. If above signs were not present it was considered as No Infection and given a score of 0. Observed for soakage of dressing – if 2 layered gauze dressing was soaked it was considered as exudation present-1, scoring criteria. If there was no soakage –considered No Exudation and given 0 during scoring. Scoring criteria given in table 1.

Table-1: Table of scoring criteria used in present study

Epithelialization	
1	Epithelialized at the 7 days
2	Epithelialized at the 14 days.
3	Epithelialized at the 21 days.
X	No epithelialization
Infection	
0	Absent
1	Present
Exudation	
0	Absent
1	Present
Biodegradation	
1	Missing at the end of 7 days
2	Missing at the end of 14 days
3	Missing at the end of 21 days
X	Not applicable

2.1 Statistical analysis

All values are tabulated and expressed as percentage. The statistical significance was evaluated by using student 't' test and chi-square test. p value of <0.05 was taken as statistically significant.

3. Results

Mean age of the patients recruited to the study was 53.5 and 50.2 years in the amniotic dressing group and conventional study group respectively. Table 2 shows tabulation of the ulcer type and details of pathogenic factors involved.

Table 2: Tabulation of cases selected for conventional and amniotic membrane dressing and the etiological considerations of ulcers

	Conventional dressing group (N=100)	Amniotic membrane group (N=100)
Mean age (in years)	53.5	50.2
Sex – males and females	68 & 32	74 & 26
Type of ulcer		
Vascular		
Chronic venous insufficiency	58	46
Arterial	18	23
Lymphatic	01	0
Metabolic		
Diabetic	22	26
Others		
Contaminated surgical wound	01	02
Traumatic pressure ulcer	0	01
Neurogenic ulcers	0	02

3.1 Epithelialization

Complete epithelialization was noted in 88% of cases of amniotic membrane dressing group. 18 cases after first week, 45 cases after second week (Fig- 1 & 2) and 25 cases showed complete epithelialization after third week (Fig-3 & 4). In 12 cases of the test group, amniotic membrane dressing epithelialization failed. In these cases, there was profuse granulation at the end of one week and by the end of 2 weeks it was exuberant and infected. Biopsy was taken in all cases and pus was microbiologically analyzed. The biopsy was positive for squamous cell carcinoma in one case of long standing venous ulcer, which showed negative for skin malignancy in earlier biopsy taken before the start of study. Other 2 cases of failure of amniotic membrane were neurogenic ulcer present on heel of foot. Remaining cases were of uncontrolled diabetes mellitus which got infected at first week of amniotic membrane dressing and lead to failure of epithelialization of ulcer.

Conventional dressing group showed epithelialization at second and third week in 23 and 31 cases respectively. In rest 46 cases in control group, showed no epithelialization due to poor healing process in chronic ulcers.

Fig- 3: Venous ulcer covered by amniotic membrane



Fig- 4: Chronic venous ulcer shown in table/fig-2 healed in 3 weeks



Fig- 5: chronic venous ulcer



Fig- 6: Chronic Venous ulcer healed by amniotic membrane after 2 weeks showing complete epithelialization



Table 7: Tabulation of parameters observed in test group treated with amniotic membrane and control group treated with normal saline

Parameter	Normal saline (N=100)	Amniotic membrane (N=100)
Epithelialization		
At 1st week	0	18
At 2nd week	23	45
At 3rd week	31	25
No epithelialization (x)	46	12
Infection		
Absent	41	87
Present	59	13
Exudation		
Absent	29	69
Present	71	31
Biodegradation of membrane		
Not applicable (x)	100	12
Missing at 1 week	0	58
Missing at 2 week	0	28
Missing at 3 week	0	2

3.2 Infection

Infection was observed in 13 cases in amniotic membrane dressing group, of which 12 cases ulcers showed infection which were seen in cases where epithelialization failed as mentioned above. In one case, there was mild infection, which later subsided with appropriate antibiotic coverage as per culture sensitivity report. Rest of cases showed amniotic membrane dressing prevented infection and thus promoting wound healing. In control group, 59 cases showed infection in normal saline dressing and thus delaying healing process.

3.3 Biodegradation of amniotic membrane

There was loss of amniotic membrane structure by the end of 2 week with no residue or necrosis in almost 86 cases. In the non healing ulcer sites membrane was seen adherent to the wound as a transparent glistening layer. This layer was seen present until epithelialization began and was lost as scaling on its own. In due time the surface epithelialized and the membrane was totally missing (Fig- 4 & 5). There was no evidence of the membrane undergoing necrosis or lysis but it peeled off and lost adherence with the wound surface. This property could not be assessed in control group.

3.4 Exudation

There was no exudation in 69 cases treated with amniotic membrane. In contrast exudation was present in 70% of control cases treated with normal saline dressing leading to prolongation of wound healing. All the values are tabulated in table 3.

An inquisitive application of the amniotic membrane during our study was its application to an inguinal lymph node dissection site for carcinoma of penis, where non healing ulcer formed. A full thickness wound with fluid loss of about 100 ml a day was layered with amniotic membrane. By the 5th day soakage of the dressing was minimal and by 8th day the dressings were dry. The surface epithelialized in 21 days. (Fig. 6 & 7)

Fig- 8: Amniotic membrane dressing showing prevention of exudation and healing



Fig- 9: Non healing inguinal ulcer with exudation before application of amniotic membrane



Fig- 10: Amniotic membrane dressing to the ulcer shown in Fig-8, showing prevention of exudation and healing



4. Discussion

Amniotic membrane is unique in its anti-adhesive effect, bacteriostatic property, wound protection, pain reduction and epithelialization. Membrane becomes cohesive, on the wound and peels off on epithelialization on its own without any external intervention. This has been demonstrated by many studies using amnion for dressing in diverse clinical conditions.[1–3,7]

Histologically this membrane has amnion and chorion which are loosely held with each other. The inner amnion has cuboidal or flattened epithelial cells and outer chorion has extraembyonic mesenchymal connective tissue. Since it is formed from fetal ectoderm, Pigeon has stated that, this membrane is continuation of fetal skin.[7]Ultra structural details of amniotic membrane and fetal skin show similarities.[8] Hence amniotic membrane can be considered as fetal skin allograft.[9] This may account for beneficial effects of this membrane in wound dressing.

In the present study, we have used both the layers of amniotic membrane, in accordance with many previous studies.[9–12] Studies have shown that neovascularization of chorionic component occurs faster and that there will be lesser bacterial growth in the ulcer base under cover of amnion.[13–16] So we applied the amniotic side of the membrane to cover the ulcer. This improved the coverage, prevented desiccation and allowed neovascularization of the graft. The cohesiveness of the membrane was made use during vestibuloplasty in previous studies.[7] The granulation tissue seen was healthy and highly vascular as was proved by studies conducted by Faulk *et al* way back in the 1980s.[17] In the present study we too appreciated the cohesiveness and angiogenic property of the amniotic membrane in most of the successful healing cases. One of the main advantages of the biological dressing is cohesiveness and prevention of secondary infection. This was achieved in the present comparative study. The patients treated with convectional saline dressings showed 59 % of infection after 2 and 3 weeks into the study, as against 12 % in amniotic membrane dressing. Burleson *et al* found that the antibacterial effect of biological dressings was related to the adherence to the underlying wound bed.[18] Morris *et al* believed it was due to intimate closure of the open wound provided by the biologic dressing, preventing the further contamination and allowed the defense mechanisms to act effectively.[19]Studies have found that there was concomitant increase of leucocyte below the graft possibly the reason for lesser bacterial count.[20]We believe the intimate contact of the membrane with the ulcer base with obliteration of dead space and greater access of the defense mechanisms to the ulcer site by repeated mechanical debridement has role in decreased infection in the patients treated with amniotic membrane dressing.

5. Conclusion

Human amniotic membrane was efficiently used to treat chronic leg ulcers of various etiologies and found superior to conventional saline dressing in terms of greater epithelialization, graft uptake and prevention of infection at ulcer sites.

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