Section: General Surgery

Original Research Article

COMPARISON OF WOUND HEALING PROPERTIES BETWEEN HUMAN AMNIOTIC MEMBRANE DRESSING AND NORMAL SALINE DRESSING IN DIABETIC FOOT ULCERS: A PROSPECTIVE COMPARATIVE STUDY

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ABSTRACT

Background: Diabetic foot ulcers (DFUs) are a major cause of morbidity among patients with diabetes mellitus owing to impaired wound healing, recurrent infections, and an increased risk of lower extremity amputation. Human amniotic membrane (hAM) is a biologic dressing with anti-inflammatory, antimicrobial, and regenerative properties that may enhance wound healing. This study compared the wound-healing efficacy of hAM dressing with that of a conventional normal saline dressing in patients with diabetic foot ulcers. **Materials and Methods:** A prospective observational study was conducted in the Department of General Surgery at the Government Medical College, Kannur, India, between May 2024 and May 2025. Sixty patients with Wagner grade I–III diabetic foot ulcers were enrolled and allocated into two groups: hAM dressing (n=30) and normal saline dressing (n=30). Following wound debridement, patients received their respective dressings, and outcomes were assessed after two weeks. The primary outcome was a reduction in ulcer size, while secondary outcomes included the formation of healthy granulation tissue and complete epithelialization. **Result:** Baseline demographic characteristics, comorbidities, and ulcer severity were comparable between the two groups. The mean reduction in ulcer size was significantly greater in the hAM group than in the normal saline group (1.7 ± 0.8 cm vs 1.1 ± 0.6 cm; $p<0.01$). Excellent granulation tissue (+++) developed in 24% of patients treated with hAM compared with none in the saline group ($p=0.011$). Complete epithelialization occurred more frequently with hAM dressing (16.7% vs. 3.3%), although the difference was not statistically significant ($p=0.097$). No adverse events related to amniotic membrane application were observed. **Conclusion:** Human amniotic membrane dressing significantly improved wound healing by achieving greater ulcer size reduction and superior granulation tissue formation compared with conventional normal saline dressing. Its safety, low cost, and easy availability make it a promising biological dressing for diabetic foot ulcers, particularly in resource-limited settings.

INTRODUCTION

Diabetic foot ulcer (DFU) is a major complication of diabetes mellitus and remains a significant cause of morbidity, prolonged hospitalization, lower extremity amputation, and mortality. Impaired wound healing resulting from chronic hyperglycemia, neuropathy, peripheral vascular disease, and infection contributes to the persistence of these ulcers.[1] Approximately 1% of the adult population is affected by diabetic foot ulcers, and nearly 12% of chronic non-healing ulcers ultimately

require amputation.[1-3] Despite limb salvage, recurrent debridement, prolonged antibiotic therapy, and repeated hospital visits impose considerable physical, psychological, and economic burdens on patients.

Conventional normal saline dressing provides a moist wound environment but has limited biological activity to promote tissue regeneration [4]. Human amniotic membrane (hAM), a trilaminar biological dressing comprising an epithelial layer, basement membrane, and avascular mesenchyme, possesses several properties that facilitate wound healing. It is non-immunogenic and exhibits anti-inflammatory, antimicrobial, anti-fibrotic, and pro-epithelialization effects. These properties are mediated by suppression of pro-inflammatory cytokines, production of anti-inflammatory mediators, and antimicrobial proteins such as lactoferrin and β-defensins, thereby promoting granulation tissue formation and reducing scar formation.[5-7] Furthermore, hAM is readily available, cost-effective, and can be procured without sophisticated tissue banking facilities.[8]

The present study was done to compare the wound-healing efficacy of a human amniotic membrane dressing with that of a conventional normal saline dressing in patients with diabetic foot ulcers, with an emphasis on ulcer size reduction and the formation of healthy granulation tissue.

MATERIALS AND METHODS

This prospective observational study was conducted in the Department of General Surgery at the Government Medical College, Kannur, India, over a 1-year period from May 2024 to May 2025. The study aimed to compare the wound-healing efficacy of a human amniotic membrane (hAM) dressing with that of a conventional normal saline dressing in patients with diabetic foot ulcers (DFUs). A total of 60 patients were studied, 30 in each group. Adult patients aged 18–80 years with Type 1 or Type 2 diabetes mellitus presenting with Wagner grade I–III diabetic foot ulcers and requiring inpatient management were consecutively enrolled after obtaining written informed consent. Patients with fistulous communication to organs or cavities, discharging sinus from bone, exposed blood vessels, malignancy, tuberculous ulcers, gangrenous changes, uncontrolled diabetes mellitus, or terminal illness were excluded from the study.

At admission, all patients underwent detailed clinical evaluation, baseline laboratory investigations, glycemic assessment, and thorough wound examination. Following surgical debridement, wound swabs were obtained for culture and antibiotic sensitivity testing. Baseline ulcer dimensions were measured using a sterile measuring tape, and appropriate systemic antibiotics were administered according to culture sensitivity reports or institutional antibiotic policy. Glycemic control was optimised throughout the study period in consultation with the endocrinology team.

Patients in the amniotic membrane group underwent application of processed human amniotic membrane following wound debridement and irrigation with normal saline. The membrane was covered with a sterile three-layer gauze dressing, and dressing changes were performed every three days. In the control group, wounds were managed with conventional sterile normal saline dressings, which were changed daily after routine wound care. Human amniotic membrane grafts were prepared from placentas obtained during elective caesarean sections after informed maternal consent. Donor mothers were screened and confirmed negative for human immunodeficiency virus, hepatitis B, hepatitis C, and syphilis, and placentas from pregnancies complicated by premature rupture of membranes, endometritis, or placental abruption were excluded. The amniotic membrane was aseptically separated from the chorion, thoroughly washed with heparinized normal saline, treated with gentamycin and crystalline penicillin, preserved in glycerol, and refrigerated at 4°C for up to 5 days. Before application, the membrane was rehydrated in sterile normal saline for 10 minutes and placed directly over the ulcer bed. The primary outcome was the percentage reduction in ulcer size at two weeks compared with baseline, while the secondary outcome was the development of healthy granulation tissue. Patients demonstrating clinical deterioration or progression of the ulcer were managed with alternative treatment modalities as deemed appropriate.

Statistical analysis was performed using IBM SPSS Statistics version 20.0. Continuous variables were expressed as mean ± standard deviation or median (interquartile range), whereas categorical variables were presented as frequencies and percentages. Comparisons between the two groups were performed using the independent Student's t-test for continuous variables and the Chi-square test or Fisher's exact test for categorical variables, as appropriate. A two-tailed p-value <0.05 was considered statistically significant. The study protocol was approved by the Institutional Ethics Committee of Government Medical College, Kannur, and all procedures were conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent was obtained from all participants, and patient confidentiality was strictly maintained throughout the study.

RESULTS

Baseline characteristics: A total of 60 patients were included in the study, with 30 patients in each group. The mean age was comparable between the amniotic membrane and normal saline groups (65.3 ± 11.9 vs 65.4 ± 11.6 years; p=0.983). Both groups

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had identical sex distribution and comparable prevalence of hypertension, coronary artery disease, chronic kidney disease, and peripheral arterial occlusive disease [Table 1].

Ulcer characteristics: Baseline ulcer severity was similar in both groups, with 40% of patients presenting with Wagner grade III ulcers. The mean baseline wound size was comparable between the amniotic membrane and normal saline groups (4.7 ± 1.5 cm vs 4.8 ± 1.6 cm), indicating similar disease severity at enrolment [Table 2].

Primary outcome: After two weeks, patients treated with amniotic membrane dressing demonstrated a significantly greater reduction in wound size than those treated with normal saline dressing (1.7 ± 0.8 cm vs 1.1 ± 0.6 cm; t=3.52, p<0.01). Although the mean wound size at two weeks was lower in the amniotic membrane group (3.5 ± 1.6 cm vs 3.8 ± 1.7 cm), the principal improvement was reflected by the greater reduction in ulcer size [Table 3 and 4].

Secondary outcomes: Healthy granulation tissue developed more rapidly in the amniotic membrane group, with 24% of patients achieving excellent (+++) granulation compared with none in the normal saline group (p=0.011). Complete epithelialization at two weeks was more frequent after amniotic membrane dressing (16.7% vs. 3.3%), although this difference did not reach statistical significance (p=0.097) [Tables 5 and 6].

Table 1: Baseline demographic and clinical characteristics of the study population

Variable	Amniotic membrane (n=30)	Normal saline (n=30)	p value
Age (years), Mean ± SD	65.3 ± 11.9	65.4 ± 11.6	0.983
Male sex, n (%)	17 (56.7)	17 (56.7)	1.000
Female sex, n (%)	13 (43.3)	13 (43.3)	
Hypertension	10 (33.3)	13 (43.3)	0.426
Coronary artery disease	10 (33.3)	8 (26.7)	0.573
Chronic kidney disease	8 (26.7)	8 (26.7)	1.000
Peripheral arterial occlusive disease	4 (13.3)	5 (16.7)	0.718

Table 2: Baseline ulcer characteristics

Variable	Amniotic membrane (n=30)	Normal saline (n=30)	p value
Wagner Grade			
I	7 (23.3)	7 (23.3)	
II	11 (36.7)	11 (36.7)	
III	12 (40.0)	12 (40.0)	1.000
Initial wound size (Mean ± SD)	4.7 ± 1.5	4.8 ± 1.6	NS*

Table 3: Comparison of wound size after two weeks

Variable	Amniotic membrane (n=30)	Normal saline (n=30)	p value
Wound size after 2 weeks (Mean ± SD)	3.5 ± 1.6	3.8 ± 1.7	NS*
Median (IQR)	4 (2–4.75)	4 (2.5–5)	

Table 4: Comparison of wound healing outcomes

Outcome	Amniotic membrane (n=30)	Normal saline (n=30)	Test statistic	p value
Reduction in wound size (Mean ± SD)	1.7 ± 0.8	1.1 ± 0.6	t = 3.52	<0.01

Table 5: Development of healthy granulation tissue at 2 weeks

Granulation grade	Amniotic membrane n (%)	Normal saline n (%)	p value
Present (+)	10 (40.0)	20 (69.0)	
Present (++)	9 (36.0)	9 (31.0)	
Present (+++)	6 (24.0)	0 (0.0)	0.011

Table 6: Complete epithelialization at two weeks

Complete epithelialization	Amniotic membrane n (%)	Normal saline n (%)	p value
Yes	5 (16.7)	1 (3.3)	
No	25 (83.3)	29 (96.7)	0.097

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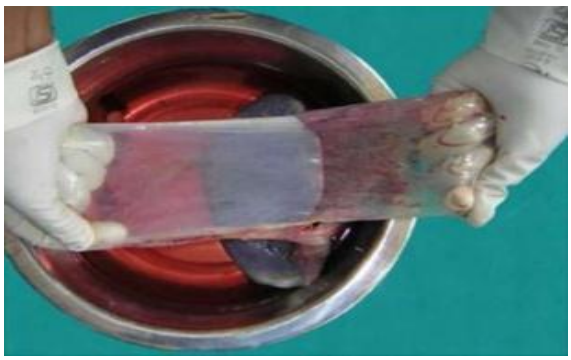

Figure 1: Amniotic membrane

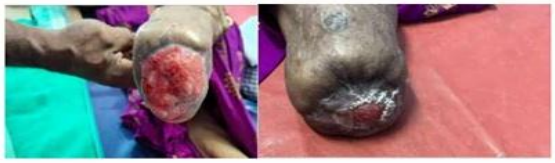

Figure 2: Diabetic foot before and after amniotic membrane dressing after 2 weeks

DISCUSSION

Diabetic foot ulcer (DFU) remains one of the most challenging complications of diabetes mellitus because of impaired wound healing, recurrent infection, and the high risk of lower extremity amputation.[5,9] The present prospective observational study compared the wound-healing efficacy of a human amniotic membrane (hAM) dressing with that of a conventional normal saline dressing in patients with Wagner grade I–III diabetic foot ulcers. Both treatment groups were comparable with respect to age, sex, baseline ulcer characteristics, and associated comorbidities, thereby minimizing potential confounding factors and allowing a reliable comparison of treatment outcomes. The study population predominantly comprised males (56.7%), with the majority of patients aged 60–79 years. Hypertension was the most common associated comorbidity, followed by coronary artery disease, chronic kidney disease, and peripheral arterial occlusive disease. Most ulcers were classified as Wagner grade III, reflecting the advanced stage of disease commonly encountered in tertiary care settings.

The principal finding of this study was a significantly greater reduction in ulcer size with amniotic membrane dressing compared with conventional normal saline dressing (1.7 ± 0.8 vs 1.1 ± 0.6; $p < 0.01$). Furthermore, complete epithelialization was achieved in five patients receiving amniotic membrane dressing compared with only one patient in the normal saline group.[10] Although the difference in complete epithelialization did not reach statistical significance within the short two-week follow-up period, the trend favoured amniotic membrane therapy. Similarly, the development of healthy granulation tissue was significantly superior in the amniotic membrane group, with 24% of patients achieving excellent (+++) granulation, whereas none of the patients in the normal saline group demonstrated comparable healing.[10,11] These findings indicate that hAM accelerates the proliferative phase of wound healing by promoting granulation tissue formation and epithelial regeneration, ultimately facilitating faster wound closure.[12]

The beneficial effects of the amniotic membrane can be attributed to its unique biological properties. Human amniotic membrane acts as a natural extracellular matrix scaffold and contains numerous growth factors and bioactive molecules that promote angiogenesis, epithelialization, fibroblast proliferation, and collagen deposition while simultaneously suppressing excessive inflammation. Its anti-inflammatory, antimicrobial, and anti-fibrotic characteristics create an optimal environment for wound healing.[10-12] In addition, the presence of progenitor cells further enhances tissue regeneration and repair.[13] Experimental and clinical studies have also demonstrated its ability to reduce bacterial colonization, decrease pain, promote neovascularization, and accelerate healing of chronic wounds, burns, and leg ulcers.[11,12] In the present study, no adverse reactions, graft-related complications, or treatment discontinuations were encountered, further supporting the safety of hAM dressing. Proper donor screening and standardised processing techniques ensured safe clinical application while minimizing the risk of disease transmission.

The findings of the present study are consistent with previous literature evaluating the role of amniotic membrane in chronic wound management. Hanumanthappa MB et al. reported that amniotic membrane dressing is a safe, effective, and economical alternative to conventional saline dressings for chronic non-healing lower-limb ulcers, particularly in resource-constrained settings.[14] Likewise, ElHeneidy et al. concluded that the amniotic membrane has superior wound-healing potential compared with tissue-engineered skin substitutes due to its biological activity and regenerative capacity.[15]Jaianand et al. also demonstrated that the amniotic membrane exerts anti-inflammatory, analgesic, and antimicrobial effects while reducing the risk of infection and enhancing wound healing in diabetic foot ulcers.[16] From a practical perspective, amniotic membrane offers several advantages, including ready availability from elective caesarean sections, minimal processing costs, long shelf life after preservation, ease of handling, and the potential to reduce treatment duration, hospital stay, and overall healthcare expenditure.[11,12,14-16] These advantages are particularly relevant in developing countries, where the cost of advanced wound care products remains a major barrier to optimal patient care.

Despite these encouraging findings, the present study has certain limitations. It was a single-centre observational study with a relatively small sample

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size and a short follow-up period of two weeks. Long-term outcomes such as complete wound healing, recurrence, need for amputation, quality of life, and cost-effectiveness were not evaluated. Larger multicenter randomized controlled trials with longer follow-up periods are required to validate these findings and establish standardised protocols for the routine use of amniotic membrane dressings in the management of diabetic foot ulcers.

CONCLUSION

Human amniotic membrane dressing demonstrated superior wound healing compared with a conventional normal saline dressing in patients with diabetic foot ulcers, with a significantly greater reduction in ulcer size and enhanced formation of healthy granulation tissue. Although complete epithelialization was more frequent in the amniotic membrane group, the difference was not statistically significant within the two-week follow-up period. The dressing was safe, well-tolerated, cost-effective, and readily available, making it a practical biological dressing for managing diabetic foot ulcers.

REFERENCES

1. DiDomenico LA, Orgill DP, Galiano RD, Serena TE, Carter MJ, Kaufman JP, Young NJ, Jacobs AM, Zelen CM. Use of an aseptically processed, dehydrated human amnion and chorion membrane improves likelihood and rate of healing in chronic diabetic foot ulcers: a prospective, randomised, multi- centre clinical trial in 80 patients. International Wound Journal. 2018 Dec;15(6):950-7.
2. Su YN, Zhao DY, Li YH, Yu TQ, Sun H, Wu XY, Zhou XQ, Li J. Human amniotic membrane allograft, a novel treatment for chronic diabetic foot ulcers: A systematic review and meta- analysis of randomised controlled trials. International wound journal. 2020 Jun;17(3):753-64.
3. Zelen CM, Gould L, Serena TE, Carter MJ, Keller J, Li WW. A prospective, randomised, controlled, multi- centre comparative effectiveness study of healing using dehydrated human amnion/chorion membrane allograft, bioengineered skin substitute or standard of care for treatment of chronic lower extremity diabetic ulcers. International wound journal. 2015 Dec;12(6):724-32.
4. Calhoun JH, Cantrell J, Cobos J, Lacy J, Valdez RR, Hokanson J, Mader JT. Treatment of diabetic foot infections: Wagner classification, therapy, and outcome. Foot & ankle. 1988 Dec;9(3):101-6.
5. Paggiaro AO, Menezes AG, Ferrassi AD, De Carvalho VF, Gemperli R. Biological effects of amniotic membrane on diabetic foot wounds: a systematic review. Journal of wound care. 2018 Feb 2;27(2):S19-25.
6. Friel NA, de Girolamo L, Gomoll AH, Mowry KC, Vines JB, Farr J. Amniotic fluid, cells, and membrane application. Operative Techniques in Sports Medicine. 2017 Mar 1;25(1):20-4.
7. Stern M. The grafting of preserved amniotic membrane to burned and ulcerated surfaces, substituing skin grafts: a preliminary report. Journal of the American Medical Association. 1913 Mar 29;60(13):973-4.
8. Mirza MA, Mazhar A, Bhutto W, Khan AS. Effectiveness of Amnion on Chronic Wounds Vs Saline Dressing. InProceedings 2021 Apr 21 (Vol. 35, No. 2, pp. 35-40).
9. Margolis DJ, Allen-Taylor L, Hoffstad O, Berlin JA. Diabetic neuropathic foot ulcers: the association of wound size, wound duration, and wound grade on healing. Diabetes care. 2002 Oct 1;25(10):1835-9.
10. Chintada GR, Kshirsagar A. A Prospective Comparative Study Between Human Amniotic Membranes Dressing Versus Routine Dressing In Ulcer. Surgical Chronicles. 2022 Oct 1;27(4).
11. Lakmal K, Basnayake O, Hettiarachchi D. Systematic review on the rational use of amniotic membrane allografts in diabetic foot ulcer treatment. BMC surgery. 2021 Feb 15;21(1):87.
12. Zheng C, Tang W, Ran X. Efficacy and Safety of Human Amniotic Membrane for Chronic Wounds: A Systematic Review and Meta-Analysis of Clinical Trials. Advances in Wound Care. 2025 May 10:21621918251411808.
13. Zelen CM, Gould L, Serena TE, Carter MJ, Keller J, Li WW. A prospective, randomised, controlled, multi- centre comparative effectiveness study of healing using dehydrated human amnion/chorion membrane allograft, bioengineered skin substitute or standard of care for treatment of chronic lower extremity diabetic ulcers. International wound journal. 2015 Dec;12(6):724-32.
14. Hanumanthappa MB, Gopinathan S, Suvarna R, Guruprasad RD, Shetty G, Shetty K, Shetty S, Nazar Z. Amniotic membrane dressing versus normal saline dressing in non-healing lower limb ulcers: A prospective comparative study at a Teaching Hospital. Journal of Clinical and Diagnostic Research. 2012 May 1;6(3):423-7.
15. ElHeneidy H, Omran E, Halwagy A, Al-Inany H, Al-Ansary M, Gad A. Amniotic membrane can be a valid source for wound healing. International journal of Women's Health. 2016 Jun 27:225-31.
16. Kannaiyan J, SuriyaNarayanan S, Palaniyandi M, Rajangam B, Chhabra H, Pandey A. Amniotic membrane as a scaffold in wound healing and diabetic foot ulcer: an experimental technique and recommendations. International Journal of Research in Medical Sciences. 2016 Aug;4(8):8.

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