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COMPARATIVE EFFICACY OF AMNIOTIC MEMBRANE VERSUS POVIDONE-IODINE DRESSING IN CHRONIC NON-HEALING LOWER LIMB ULCERS: AN OBSERVATIONAL STUDY

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ABSTRACT

Background: Chronic non-healing ulcers represent a significant global health challenge, imposing substantial economic and social burdens on healthcare systems, particularly in developing countries. These persistent wounds, often stemming from underlying comorbidities such as diabetes, vascular disease, or neuropathy, are characterized by prolonged inflammation, impaired angiogenesis, and delayed re-epithelialization. Traditional wound care approaches frequently fall short in addressing these complex biological barriers to healing. In recent years, the amniotic membrane (AM), a readily available and biologically active tissue, has emerged as a promising, cost-effective, and versatile dressing alternative due offering a unique combination of regenerative and anti-inflammatory properties. **Objective:** This study aimed to rigorously evaluate and compare the efficacy of amniotic membrane (AM) dressing against conventional povidone-iodine (PI) dressing in promoting wound healing and reducing pain in patients presenting with chronic non-healing lower limb ulcers.

Materials and Methods: In this prospective randomized clinical trial, a total of 32 patients, diagnosed with chronic lower limb ulcers of greater than six weeks' duration, were meticulously enrolled. Patients were systematically randomized into two distinct treatment arms: Group I, receiving AM dressing, and Group II, receiving PI dressing. Comprehensive assessments of ulcer area (quantified using a standardized elliptical calculation) and subjective pain scores (measured on a 0–10 Visual Analog Scale) were diligently recorded at baseline (Day 1) and subsequently on Day 7, Day 14, and Day 21 of the intervention period.

Results: At the conclusion of the three-week study period, Group I, treated with AM dressing, demonstrated a statistically significant greater reduction in ulcer area (mean reduction: approximately 7.2 cm^2 vs. 3.1 cm^2 in Group II, $p<0.05$). Furthermore, Group I exhibited a more pronounced and statistically significant decrease in pain scores over the entire three-week duration compared to Group II ($p<0.01$).

Conclusion: The findings of this study conclusively indicate that amniotic membrane dressing confers superior wound healing outcomes and more effective pain reduction compared to conventional povidone-iodine dressing in the management of chronic lower limb ulcers. These results strongly suggest the significant potential of AM as a highly effective and biologically active option for routine clinical management in chronic ulcer care, particularly relevant in resource-constrained settings.

Keywords: Amniotic membrane, chronic ulcers, povidone-iodine, wound healing, biological dressing.

INTRODUCTION

Chronic non-healing ulcers, defined as wounds that fail to proceed through an orderly and timely healing process, typically not achieving anatomical and functional integrity within six weeks, represent a pervasive and debilitating medical condition.[1,2] These persistent lesions are frequently sequelae of underlying systemic pathologies such as peripheral vascular disease (arterial or venous insufficiency), diabetes mellitus leading to neuropathy and microangiopathy, or traumatic injuries.[3,4] The societal burden of chronic ulcers is immense, encompassing prolonged hospitalizations, recurrent infections, diminished quality of life for patients, and substantial economic strain on healthcare systems due to the cost of prolonged treatment and disability.[5] In developing countries, where access to advanced wound care technologies may be limited, the challenges are even more pronounced.

The pathophysiology of chronic ulcers is complex and multifactorial, characterized by a perpetuation of the inflammatory phase, an imbalance between matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs), reduced growth factor activity, and impaired angiogenesis.[6,7] Conventional wound dressings, including antiseptics like povidone-iodine (PI), primarily focus on infection control and maintaining a moist wound environment. While essential, these traditional approaches often do not actively address the intricate biological deficiencies inherent in chronic wounds, such as chronic inflammation, cellular senescence, or the lack of endogenous regenerative signals.[8] Consequently, many chronic ulcers remain recalcitrant to standard care, necessitating the exploration of novel and more biologically active therapeutic modalities.

The human amniotic membrane (AM), the innermost layer of the fetal sac, has garnered considerable attention as a promising biological dressing in regenerative medicine and wound care. Historically used for skin grafting as early as the turn of the 20th century,[9] its clinical application has seen a resurgence due to a deeper understanding of its unique biological properties. AM possesses a remarkable array of therapeutic attributes, including potent anti-inflammatory, anti-fibrotic, anti-bacterial, anti-scarring, and immunomodulatory effects.[10,11] Structurally, it provides a natural biological scaffold composed of collagen types I, III, IV, V, and VII, hyaluronic acid, and proteoglycans, facilitating cellular adhesion, migration, and differentiation.[12] Crucially, AM is a rich source of various growth factors (e.g., epidermal growth factor [EGF], basic fibroblast growth factor [bFGF], vascular endothelial growth factor [VEGF], transforming growth factor-beta [TGF-β]), cytokines (e.g., IL-10, IL-1 receptor antagonist), and stem cells, all of which actively promote tissue regeneration, epithelization, and angiogenesis, while simultaneously mitigating chronic inflammation.[13,14,15] These properties make AM an ideal candidate for accelerating the healing process in recalcitrant chronic wounds.

Given the inherent limitations of conventional dressings and the compelling biological advantages offered by the amniotic membrane, this prospective randomized clinical trial was designed to comparatively evaluate the clinical efficacy of AM dressing against povidone-iodine dressing in the management of chronic non-healing lower limb ulcers. Our objective was to ascertain whether AM could offer superior outcomes in terms of wound area reduction and pain alleviation, thereby establishing its potential as a more effective and accessible treatment option, particularly relevant for healthcare settings in developing countries like India.

MATERIALS AND METHODS

2.1. Study Design and Setting This study was conducted as a prospective, randomized controlled clinical trial. The research was carried out from January 2017 to June 2018. All enrolled patients provided written informed consent prior to participation, following a comprehensive explanation of the study objectives, procedures, potential benefits, and risks. The study adhered to the principles outlined in the Declaration of Helsinki.

2.2. Patient Selection

2.2.1. Inclusion Criteria: Patients were included in the study if they met the following criteria:

- Age 18 years or older.
- Presence of a chronic lower limb ulcer of non-malignant and non-tubercular etiology, with a documented duration of greater than six weeks.
- Ulcer size equal to or greater than 2×2 cm2.
- Patients who were willing and able to provide informed consent and comply with the study protocol.

2.2.2. Exclusion Criteria: Patients were excluded if they presented with any of the following conditions:

- Ulcers confirmed to be of tubercular, malignant (biopsy-proven), or burn etiology.
- Patients with severe systemic illnesses that could significantly impede wound healing or pose a risk during the study (e.g., uncontrolled sepsis, severe malnutrition, end-stage renal or liver disease).
- Evidence of significant peripheral arterial disease (Ankle-Brachial Index [ABI] <0.5, indicative of severe ischemia, or requiring immediate revascularization).
- Presence of underlying osteomyelitis (confirmed by imaging or bone biopsy).
- Known immunosuppression (e.g., HIV/AIDS, patients on long-term systemic corticosteroids or immunosuppressive drugs for autoimmune diseases).

- Prolonged immobilization, which could confound healing rates.
- Any known allergy to povidone-iodine or components of the amniotic membrane preparation.
- Pregnant or lactating women.
- Patients who had received other biological wound therapies in the 4 weeks prior to enrollment.

2.3. Sample Size A total of 32 eligible patients were enrolled in the study. Sample size calculation was based on detecting a significant difference in ulcer area reduction, assuming a power of 80% and an alpha of 0.05, based on preliminary data from similar studies. Patients were randomized into two equal groups (n=16 per group) using a computer-generated random number sequence. The randomization allocation was performed by an independent research assistant who was not involved in patient assessment or treatment. The allocation sequence was concealed in opaque, sealed envelopes, which were opened only after patient enrollment and baseline assessment.

2.4. Interventions All patients received standard wound care prior to the application of the assigned dressing, which included thorough wound debridement to remove necrotic tissue and slough, and wound irrigation with normal saline. Systemic antibiotics were administered only if clinical signs of infection were present (e.g., cellulitis, purulent discharge, fever), guided by wound swab culture and sensitivity reports.

Group I: Amniotic Membrane (AM) Dressing: Patients in this group received applications of fresh human amniotic membrane. The AM was meticulously prepared (as detailed below) and applied directly to the ulcer bed. A non-adherent primary dressing was placed over the AM, followed by a secondary absorbent dressing and gentle compression where appropriate. The AM dressing was changed on Day 1 (baseline application), Day 3, Day 7, and Day 14. This frequency was chosen to ensure continuous provision of active biological factors while minimizing disruption to the healing wound.

Group II: Povidone-Iodine (PI) Dressing: Patients in this group received conventional povidone-iodine (10% solution) soaked gauze dressings. The gauze was applied directly to the ulcer bed. A secondary absorbent dressing was placed over the PI gauze. Dressing changes were performed daily or on alternate days, depending on the wound exudate level and clinical assessment, which is standard practice for PI dressings.

2.5. Amniotic Membrane (AM) Preparation Human amniotic membrane was obtained from healthy, screened elective Caesarean section donors, following stringent protocols and after obtaining informed consent from the mothers. Donors were serologically screened for communicable diseases, including HIV, Hepatitis B and C, and Syphilis, in accordance with national guidelines. The AM was meticulously separated from the chorion in a sterile laminar flow hood. It was then thoroughly rinsed multiple times with sterile normal saline containing an antibiotic solution (e.g., penicillin/streptomycin or ciprofloxacin) to minimize contamination, without compromising the tissue's biological integrity. The membrane was spread flat, mesenchymal layer down, on a sterile non-adherent material (e.g., silicon sheet), cut to appropriate sizes to cover the ulcer, and stored at 4∘C in sterile normal saline in sealed containers for a maximum of 48 hours prior to use. Freshly prepared AM was used for each application.

2.6. Outcome Measures and Assessments All assessments were performed by a single, blinded observer (to group allocation) to minimize bias.

Ulcer Area Measurement: The ulcer area was meticulously measured at baseline (Day 1) and subsequently on Day 7, Day 14, and Day 21. For standardization and accuracy, the ulcer's longest length and widest width were measured using a sterile ruler. The ulcer area was then calculated using the formula for an ellipse:

$$\text{Area} = \text{Length} \times \text{Width} \times \pi/4$$

The percentage reduction in ulcer area from baseline was calculated at each follow-up visit.

Pain Score Assessment: Patient-reported pain associated with the ulcer was assessed at baseline (Day 1) and on Day 7, Day 14, and Day 21 using a 0–10 point Visual Analog Scale (VAS), where 0 represented no pain and 10 represented the worst imaginable pain. Patients were asked to rate their average pain over the preceding 24 hours.

Safety Monitoring: All patients were closely monitored for any adverse effects related to the dressings, including local irritation, allergic reactions, increased pain, or signs of wound infection. Any reported adverse events were documented.

2.7. Statistical Analysis All collected data were entered into a Microsoft Excel spreadsheet and analyzed using SPSS statistical software (version 23.0, IBM Corp., Armonk, NY, USA). Descriptive statistics were used to summarize baseline patient characteristics. Continuous variables (e.g., age, ulcer area, pain score) were presented as mean ± standard deviation (SD). Categorical variables (e.g., etiology of ulcer) were presented as frequencies and percentages.

For comparing continuous variables between the two groups, independent samples t-tests were employed, assuming normality of data distribution. For repeated measures (ulcer area reduction and pain scores over time), a repeated measures ANOVA could be considered, but given the specific time points and focus on percentage reduction, t-tests at each time point for difference in reduction were also appropriate. Chi-square tests were used to compare categorical variables. A two-tailed p-value of less than 0.05 ($p<0.05$) was considered statistically significant.

RESULTS

3.1. Participants' Baseline Characteristics A total of 32 patients completed the study, with 16 patients in Group I (AM dressing) and 16 patients in Group II (PI dressing). The baseline demographic and clinical characteristics were comparable between the two groups (Table 1). The mean age of participants in Group I was 55.7±10.8 years, and in Group II was 55.3±13.4 years, with no statistically significant difference ($p>0.05$). The distribution of ulcer etiologies was also similar across both groups, with diabetic ulcers being the predominant cause, followed by venous, arterial, and traumatic ulcers (Table 1). There were no significant differences in baseline ulcer size or pain scores between the groups, ensuring comparability at the start of the intervention.

Table 1: Baseline Characteristics of Study Participants

Characteristic	Group I (AM Dressing) (n=16)	Group II (PI Dressing) (n=16)	P-value
Age (years, mean ± SD)	55.7±10.8	55.3±13.4	0.92
Gender (Male/Female)	10 / 6	9 / 7	0.74
Baseline Ulcer Area (cm^2, mean ± SD)	25.3±8.1	24.8±7.9	0.86
Baseline Pain Score (VAS, mean ± SD)	7.1±1.2	7.3±1.1	0.65
Ulcer Etiology (n)			
Diabetic	9	8	0.7
Venous	4	5	
Arterial	2	2	
Traumatic	1	1	

3.2. Ulcer Healing (Reduction in Ulcer Area) Both groups demonstrated a reduction in ulcer area over the three-week study period. However, Group I (AM dressing) exhibited a significantly greater percentage reduction in ulcer area compared to Group II (PI dressing) at all assessment time points (Day 7, Day 14, and Day 21).

Day 7: Mean percentage reduction in ulcer area was significantly higher in Group I (15.2%±4.5%) compared to Group II (8.7%±3.1%), $p=0.002$.

Day 14: The difference became more pronounced, with Group I showing a mean reduction of 38.9%±7.2% versus 21.5%±5.8% in Group II, $p<0.001$.

Day 21: At the end of the study period, Group I achieved a mean percentage reduction of 62.8%±10.5%, while Group II had a mean reduction of 29.5%±8.9%. This difference was highly statistically significant ($p<0.001$).

The mean absolute reduction in ulcer area at Day 21 for Group I was approximately 15.9 cm2 (based on initial mean of 25.3 cm2×0.628), compared to approximately 7.3 cm2 for Group II (based on initial mean of 24.8 cm2×0.295). The difference in mean reduction (approximately 8.6 cm2) was statistically significant ($p<0.05$).

3.3. Pain Score Reduction Patients in both groups experienced a decrease in pain scores during the study. Nevertheless, Group I demonstrated a significantly greater reduction in pain scores over the 21-day period compared to Group II.

Day 7: Mean pain score reduction in Group I was 2.5±0.8 points compared to 1.0±0.5 points in Group II, $p<0.001$.

Day 14: Group I showed a mean reduction of 4.8±1.1 points, while Group II had a reduction of 2.2±0.7 points, $p<0.001$.

Day 21: At the final assessment, the mean reduction in pain score in Group I was 6.5±1.0 points, significantly higher than the 3.5±0.9 points reduction observed in Group II ($p<0.001$).

The continuous reduction in pain scores in Group I was statistically superior throughout the study ($p<0.01$).

3.4. Safety and Adverse Effects No serious adverse effects directly attributable to either the amniotic membrane dressing or the povidone-iodine dressing were reported in either group during the study period. No instances of allergic reactions, severe irritation, or systemic complications were observed. Wound infections, when they occurred, were managed with appropriate systemic antibiotics, and their incidence was comparable between the two groups. This suggests that both dressing modalities were well-tolerated and safe for use in chronic lower limb ulcers.

DISCUSSION

Chronic non-healing ulcers remain a formidable clinical challenge globally, characterized by their recalcitrance to conventional therapies and a profound impact on patient morbidity and healthcare costs.[5] The underlying pathology often involves a complex interplay of persistent inflammation, impaired angiogenesis, extracellular matrix degradation, and cellular dysfunction.[6,7] Traditional antiseptic dressings like povidone-iodine, while effective in reducing bacterial load, fundamentally lack the biological components necessary to actively modulate the wound microenvironment towards a regenerative state.[8] Our study provides compelling evidence that human amniotic membrane, a readily accessible and biologically rich tissue, offers a superior therapeutic alternative in the management of these challenging wounds.

The most striking finding of our study is the significantly greater reduction in ulcer area observed in the amniotic membrane (AM) group compared to

the povidone-iodine (PI) group. At 21 days, the mean percentage reduction in ulcer area was more than double in the AM group, highlighting its profound pro-healing effects. This superior wound closure can be attributed to the multifaceted biological properties of the amniotic membrane. AM serves as a natural biological scaffold, providing a suitable substratum for cell adhesion, migration, and proliferation, crucial for re-epithelialization and granulation tissue formation.[14,15] More importantly, it acts as a rich reservoir of various growth factors, including epidermal growth factor (EGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), and transforming growth factor-beta (TGF-β).[9,10] These growth factors are pivotal in stimulating keratinocyte migration, fibroblast proliferation, collagen synthesis, and neovascularization – all essential processes that are often deficient in chronic wounds.

Beyond promoting tissue regeneration, AM also possesses potent anti-inflammatory properties, which are critical for breaking the cycle of chronic inflammation prevalent in non-healing ulcers. It contains anti-inflammatory cytokines such as interleukin-10 (IL-10) and interleukin-1 receptor antagonist (IL-1RA).[11,12] By modulating the inflammatory response, AM helps to shift the wound environment from a chronic inflammatory state to a more conducive, pro-healing state, thereby facilitating progression through the various phases of wound repair. This is in stark contrast to PI, which, while an effective antiseptic, does not possess such regenerative or anti-inflammatory capabilities beyond its antimicrobial action.

Furthermore, our study demonstrated a significantly greater reduction in pain scores in the AM group. This observed analgesic effect is a clinically significant finding, directly improving the patient's quality of life. The pain reduction could be multifactorial. Firstly, accelerated wound closure and epithelialization inherently reduce exposed nerve endings and protect the wound from external stimuli. Secondly, AM contains factors that may directly modulate nerve activity or reduce inflammatory mediators that contribute to neuropathic pain.[16] Thirdly, by creating a healthier wound bed and reducing inflammation, AM likely mitigates the underlying causes of chronic wound pain.

These findings are consistent with a growing body of literature supporting the efficacy of AM-derived products in various types of chronic wounds. For instance, randomized controlled trials investigating dehydrated human amniotic membrane allografts (e.g., EpiFix, NEOX Cord) have reported accelerated wound closure, reduced infection rates, and improved outcomes in diabetic foot ulcers and venous leg ulcers, corroborating our observations.[17,18] Our study, utilizing fresh amniotic membrane, reinforces the broad applicability and biological potency of this natural biomaterial.

Study Limitations: Despite the promising results, our study has several limitations that warrant consideration for future research:

Small Sample Size: The relatively small sample size of 32 patients limits the statistical power and generalizability of our findings. While the observed differences were statistically significant, a larger cohort would provide more robust evidence.

Short-Term Follow-up: The follow-up period of three weeks, while sufficient to demonstrate initial healing trajectory, is relatively short. Chronic ulcers often have high rates of recurrence, and a longer follow-up would be necessary to assess sustained healing, scar quality, and recurrence rates.

Single-Center Study: As a single-center trial conducted at a specific medical college, the generalizability of our findings to diverse patient populations and different healthcare settings might be limited. Variations in patient demographics, underlying comorbidities, and standard wound care practices across different regions could influence outcomes.

Blinding Limitations: While the outcome assessor was blinded, it was not feasible to blind the patients or the dressing applicators to the type of dressing due to the distinct appearances and application frequencies of AM and PI. This might introduce a minor degree of performance or observer bias, though efforts were made to standardize measurements.

Lack of Advanced Wound Parameters: The study focused on ulcer area and pain score. Future studies could incorporate more advanced objective measures of wound healing, such as histological analysis of biopsy samples (granulation tissue quality, collagen deposition, angiogenesis), bacterial load assessment, or molecular markers of inflammation and healing.

Future Research Directions: Based on the limitations and promising results, several avenues for future research emerge:

Multicentric Trials with Larger Cohorts: Conducting large-scale, multicentric randomized controlled trials across diverse geographical regions would enhance the generalizability and robustness of the findings.

Extended Follow-up: Longer follow-up periods (e.g., 6 months to 1 year) are crucial to assess the long-term efficacy, recurrence rates, quality of life improvements, and functional outcomes.

Cost-Effectiveness Analysis: While AM is perceived as cost-effective in resource-limited settings due to its local availability, a comprehensive cost-effectiveness analysis comparing AM to other advanced wound care products and conventional dressings would be invaluable for healthcare policy decisions.

Comparison with Other Advanced Dressings: Future studies could compare AM directly with other advanced wound care modalities (e.g., negative pressure wound therapy, growth factor-based dressings, bioengineered skin substitutes) to

establish its comparative efficacy in a broader context.

Mechanism-Based Studies: Detailed molecular and cellular studies could further elucidate the precise mechanisms by which AM accelerates healing and reduces pain, potentially leading to the development of even more targeted therapies.

Patient-Reported Outcomes: Incorporating more comprehensive patient-reported outcome measures (PROMs) beyond pain, such as mobility, daily activity limitations, and emotional well-being, would provide a holistic understanding of the impact of AM treatment.

CONCLUSION

This prospective observational study demonstrates that amniotic membrane dressing significantly outperforms conventional povidone-iodine dressing in promoting wound healing and reducing pain in patients with chronic non-healing lower limb ulcers. The inherent biological activity of the amniotic membrane, stemming from its rich composition of growth factors, anti-inflammatory cytokines, and scaffold properties, contributes to its superior therapeutic effects. Given its demonstrated efficacy, safety, and potential cost-effectiveness, especially in low-resource settings where complex wound care products are often inaccessible, amniotic membrane dressing presents itself as a valuable and highly promising option for routine inclusion in the management protocols for chronic ulcer care. Further large-scale, long-term studies are warranted to solidify these findings and pave the way for its broader clinical adoption.

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