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General Surgery

EFFECTIVENESS OF AMNIOTIC MEMBRANE IN HEALING OF CHRONIC LEG ULCERS

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ABSTRACT The amniotic membrane (AM) creates an optimal environment for the regeneration of chronic ulcers by promoting epithelialization and reducing pathologic fibrosis. **Objective:** This study aimed to investigate whether AM scaffolds could improve wound healing by promoting tissue remodeling instead of scar formation. **Methods:** AM was harvested and processed for patients with chronic leg ulcers, which were further randomly allocated into two groups to the intervention. Sixteen patients completed 24 weeks of treatment: group I (control): n = 12, treated with conventional dressings daily for a total duration of eight weeks. Group II (experimental) consists of 12 patients managed by AM fixed with a secondary dressing daily fixing. Healing rates, ulcer size and pain were followed up on day 0, 7,14,21,30,45 and 60 along with ulcer imaging measurements. **Results:** Group I exhibited no reduction in ulcer size with the bottom of it remaining unchanged. Granulation was healthy in both ulcers (17.2%) and absent in nine ulcers (82.8%). The pain levels either worsened or had no improvement in any of the 12 ulcers. All 12 ulcers in group II healed completely after an average time of 34.3 ±14.8 days and the healing rate empirically calculated to be (0.065 ±.656 cm^2/day). Among 12 ulcers, healthy granulation was noticed in the base of ulcer in 11(92.9%) and absent only one (7.1%). Of these, three ulcers were grade 1 (21.4%) and the remaining eleven ulcers were grade 2 (78.6%). These ranged from rates of 1.7 ±0.438 cm^2/day in the mild ulcers to those of only 0.673 ±0.498 cm^2/day in the moderate group subsets healing by secondary intention. Improvement in their pain level on a scale from 0 to 10 was observed in eleven cases, accounting for 78.6%. **Conclusions:** AM grafts have a major impact in wound healing.

KEYWORDS :

INTRODUCTION

Amniotic membrane (AM) is an appealing form of wound grafting due to its unique qualities, which include anti-inflammatory properties, bacteriostatic wound protection, reduced scarring and discomfort, and epithelialization initiating capacity. Furthermore, AM is more readily available and less expensive compared to other bioengineered skin analogues. Human AM was utilized in 2,308 ophthalmologic reconstruction in Germany in 2008. [1] Its unique success in ophthalmology could be attributable to the immune-privileged features of the AM. [2,3]AM's anti-inflammatory properties appear to be due to the creation of antiinflammatory protein and the suppression of the release of transforming growth factors B and cytokines that are pro-inflammatory, such as interleukin 10.[4,5]AM also generates B-defensins, elastase inhibitors, which are elastin, and lactoferrin, which assist to it's anti-inflammatory and antibacterial actions. [6,7]The decrease in scarring after applying AM to wounds might be related to the anti-inflammatory actions, acceleration of epithelialization, [4,5,8] and inhibition of fibrosis. [9]Accelerated reepithelialization was also demonstrated by Maral et al [10] after covering split thickness skin graft with AM in rats. Loeffelbein et al [11]AM-treated wounds showed faster development of basement membranes, which might be attributed to the releasing of growth factors. One of the most essential qualities of AM as a skin replacement is pain relief, which can be attributable to decreased inflammation, greater hydration of the bed of a wound and the shielding of exposed nerve endings. [11]AM expresses few antigens, which accounts for its good tolerability and the absence of rejection reactions.[12]AM signifies several neurotrophic and angiogenic components: endothelin-2 and -3, vascular endothelial growth factors, vascular endothelial growth factor-B, Tie-2 angiopoietin receptor, ephrin-A2, ephrin receptors A2, B1, B3, B4, B5, neuropilin-2, nerve growth factor and its receptor, and semaphorin-F19, as well as erythropoietin and its receptor that aid in wound healing.[13,14] Some studies demonstrated the effectiveness of AM graft for healing of wounds. Mermet et al [15] put an AM graft for 15 chronic leg ulcers and healing occurred in all patients. Pesteil et al [16] used cryopreserved AM in eight patients with resistant vascular ulcers. Tolerance to the graft was excellent with healing of six out of eight patients with significantly improved pain. Alsina-Gibert and Pedregosa-Fauste [17] used AM for four refractory ulcers with a mean 81.93% reduction of ulcer size after 16 weeks. Litwiniuk et al [18] suggested the potential role of matrix metalloproteinase inhibitors present in radiation-sterilized amnion dressing in healing of 23 out of 25 patients with chronic venous ulcers. Sheikh et al [19] used dehydrated amnion to provoke healing of chronic wounds in four patients and healed wounds did not recur on long term follow-up. A similar study was done by Zelen et al [20] who used dehydrated AM in diabetic foot ulcers with complete healing of 37 out of 40 ulcers. With respect to the low cost, wide availability, and easy preparation, AM can be an ideal graft for chronic refractory ulcers.

Method

Research Design: This research was an experimental comparative RCT undertaken with the aim to examine the efficacy of AM in managing chronic non-healing wounds. Patient Description and Data negligible rejection. Also, AM releases several neurotrophic/angiogenic factors crucial for wound healing, such as endothelin-2/-3, vascular endothelial growth factor (VEGF), angiopoietin receptor Tie-2, and ephrins, to name a few. It has been identified that most prior investigations have shown significant improvement in wound healing when AM grafts are used. For instance, Mermet and others used AM grafts on 15 patients with chronic leg ulcers; all these patients 'ulcers healed. Pesteil et al. applied cryopreserved AM in eight patients with refractory vascular ulcers, which resulted in the patients 'reduced pain and improved healing process. Alsina-Gibert and Pedregosa-F'austel have reported their trial on refractory ulcers and that AM has reduced average ulcer size by 81% on the four ulcers treated. 93% after 16 weeks. The authors, Litvinyuk et al. proposed matrix metalloproteinase inhibitors to be used in AM bands for treating chronic venous ulcers. Four patients with chronic wounds caused by dehydrated AM healing were reported by Sheikh and his team, and no reappearance was observed from the long-term follow-up. In the same manner, Zelen et al. , attained the closure of 37 out of 40 diabetic foot ulcers by treating them with dehydrated AM. Due to low cost, increased availability and readiness for preparation, AM is recommended for chronic refractory ulcers, where grafts are required.Collection: The detail work was done in the Department of General Surgery, Navodaya Medical College and Research Center, Raichur, Karnataka, over a period of one year from August 2022 to August 2023. Sources of sample included out-patient clinic or in-patient department of the hospital and each patient volunteered for the study by providing informed consent.

The study was designed to be ethical, and patients were then randomly assigned to groups. Control Group I consisted of 12 patients diagnosed

with chronic leg ulcers dressed conventionally and with dressing changes done at least once per day for a duration of 8 weeks. Group II (experimental) comprised 12 patients suffering from chronic leg ulcers with AM applied on the wound base and covered by a secondary dressing, which was changed every day. The criteria for sample selection were: open leg ulcers with a duration of over 3 months and failure to heal with standard treatment protocol and age ranged between 26 and 43 years. Patients with ulcers having active infection including diabetic ulcers were excluded from the study. Medical history including prior treatment and surgery, known diagnosed diabetes, hypertension, ulcer etiology, type, and duration all formed part of the clinical assessment.

Follow-up also entailed assessment of the ulcer healing and pain, evidenced by the thickness of the healed area and overall healing rate. Wound sizes on the animals were captured using Image J software to determine the percentage of healed area relative to the initial and final wound sizes after the 2nd week and at the 2nd month. The intensity of the pain was determined by the patient using a pain intensity scale ranging from 0 (no pain) to10 (worst possible pain).

RESULTS

Data from all patients were pooled for statistical analysis.

Am Isolation, Preservation, Grafting, And Follow-up Of Patients:

Human AMs were derived from placentas collected from full term, normal deliveries by cesarean section. Maternal and neonatal infection, preterm labor before 34 weeks of gestation, and rupture of membranes for more than 12 hours was considered as an exclusion criteria. Written informed consent was obtained from the donors. AM tissue obtained from 1 placenta is 4 to 5 pieces with a diameter of 5 cm. Enumeration was done in a class D area using class A microbiological safety cabinet. After cesarean section the placenta was rinsed with saline and remained in contact with the antibiotic solution for 2 hours. The membrane structure was placed in a sterile packaging container and then positioned in a size appropriate to the carious lesion in the tooth; it was then cut into small pieces and preserved in cryoprotectants at -80°C. AM swabs were collected for culture and sensitivity while blood samples of the mother was taken to screen for the presence of serological markers. The final validation of AM was carried out after confirmation by repeat serological test of the donor after 120 days. AM is stable within the dry ice for the duration of 24 hours and in the saline solutions, room temperature for the 2 hours before the use. Wounds and ulcers were washed, then mechanically debrided; the membrane preservation solution was rinsed with saline. Direct application on the ulcer base was made with a petroleum jelly dressing and the patients lay in bed for 2 hours before mobilization with moderate levels of activity measured in 5 days.

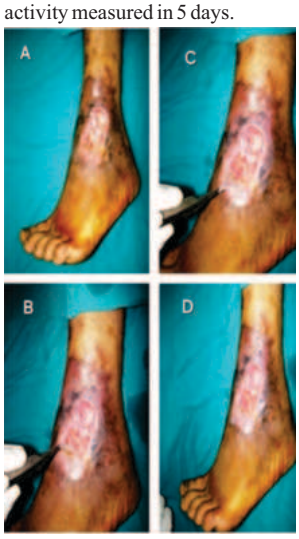

Fig:1

Fig:2

Further evaluation was done to check the healing rate and size of the ulcer, pain rating, and to capture ulcer images on days 0, 7, 14, 21, 30, 45, and 60. The statistical analysis of all data was conducted by using the SPSS software version 15. 0 for Windows (SPSS Inc, Chicago, IL, USA). In a two-sided test, the value of P is denoted as P<0. 05 was considered statistically significant for all analyses. We used mean ±SD to describe continuous variables.

Fig 4:- Healing Of Two Ulcers After Amniotic Membrane Application

(A) Two leg ulcers (upper small ulcer and lower big one). (B) AM applied covering both leg ulcers. AM graft was taken in the upper ulcer while it was not in the lower one. (C) Reduction in ulcer size on follow up with complete healing of the upper ulcer, and 70% reduction in size and healing of the lower one,.

RESULTS

The Sample - Demographic Data

Patients were all male, aged 26 to 43 years. Group I, composed of 12 leg ulcers with ages ranging from 26 to 43 years (mean age:34.45 ±7.03 years). Among these, 10 (81.8%) were venous ulcers and 2 (18.2%) had traumatic etiology; Group II: 12 leg ulcers, ages ranging from 26 to 43 years (mean age:32.86 ±6.94 yrs) Twelve ulcers (85.7%) were venous, and two ulcers (14.3%) traumatic in this group of patients None of the patients in both groups suffered from diabetes, were smokers or had a history of hypertension and other medical diseases.

Control Group

Conventional treatment was given to the control group only. In total, the duration of leg ulcers ranged from 24 up to as long as 62 months. Original ulcer size averaged 4.9 ±0.66 cm^2 (mean±SD). Results The averaged provided percentage of improvement was zero with no decrease in the ulcer size (Table 1,Table 2) and the floor still maintained. Granulation was healthy in 2 ulcers (17.2%) while it was unhealthy with ischaemia, necrosis or fibrotic reaction seen, the remaining 10 of them-81.8%. None of the 12 ulcers experienced a reduction in pain score (Table 3).

Table 1:-

PARAMETERS OF HEALING	GROUP 1	GROUP 2
Chronicity of leg ulcer (range)	24 - 62 MONTHS	24 - 80 MONTHS
Chronicity of leg ulcer (mean + SD)	46.82+14.11 months	50.37 + 16.40 months
Reduction of ulcer size with treatment	0%	100% Reduction in size
Reduction of ulcer size with treatment (mean + SD)	0.0 + 0.0	100.0+0.0
Healing rate cm2/ day (range)	0.0 - 0.0	0.068 - 2.42
Healing rate cm2/ day (mean + SD)	0.0 + 0.0	0.898 ± 0.648

Abbreviation: SD , standard deviation

Table 2:- Percentage Of Healed Ulcers

ULCER HEALING	GROUP 1		GROUP 2	
No Healing	12	(100%)	0	(0%)
Complete healing	0	(0%)	12	(100%)
Incomplete healing	0	(0%)	0	(0%)

Note: Group 1 included patients without amniotic membrane application, while Group 2 included patients with amniotic membrane application.

Study Group

The study group, consisting of 12 leg ulcers, was treated with AM directly applied to the ulcers. The chronicity of these ulcers ranged from 24 to 80 months. The initial ulcer area was 5.1 ±0.48 cm^2 (mean ± SD). All 12 ulcers achieved complete healing within 14–60 days, with a mean healing time of 33.4 ±14.8 days. The healing rate varied from 0.068 to 2.42 cm^2/day, with an average of 0.898 ±0.648 cm^2/day, leading to a 100% reduction in ulcer size. The ulcer floor improved in all cases. Healthy granulation tissue was found in 12 ulcers (92.9%) and was absent in 1 ulcer (7.1%). Of the ulcers, 3 (21.4%) were mild (grade 1), and 12 (78.6%) were moderate (grade 2). Mild ulcers had a faster healing rate (1.8 ±0.438 cm^2/day) compared to moderate ulcers (0.674 ±0.499 cm^2/day). Pain levels improved in 12 cases (78.8%), with 3 patients reporting no pain (Tables 1–3; Figure 4). The AM graft was successfully integrated in 4 cases (28.6%), and in the remaining 10 cases (71.4%), ulcers still showed complete healing during follow-up.

The reduction in ulcer size was significantly greater in the study group compared to the control group (P=0.001), where only AM was used.

Table 3:- Pain Level Improved Or Remained The Same From Day 0 Till The End Of Study

PAIN LEVEL	GROUP 1		GROUP 2	
No pain	0	(0.0%)	3	(22.5%)
Improved	0	(0.0%)	9	(77.5%)
The same	12	(100%)	0	(0.0%)

DISCUSSION

Persistent leg ulcers are described as a skin defect, beyond the level of the knees and over the foot, that persists for 6 weeks or longer.[22]A prior study indicated that ~60%-80% of chronic leg ulcers had a venous component, 10%-30% were connected with arterial insufficiency, and other variables included diabetes and rheumatoid arthritis. Arterial and venous insufficiency mix in 10%-20% of patients.[22]

Chronic leg ulcers frequently healed inadequately if there is no revascularization. Various treatment options are centered on optimal local wound care, including washing, debridement and dressings, compressive treatment, and skin grafting. AM grafts can be employed since placental tissues have a high concentration of growth factors. AM decreases the risk of the fibrosis by downregulating fibroblasts' production of TGF-β and its receptors. As a result, an AM scaffolding can influence wound healing by stimulating tissue repair instead of scar tissue development.[4]

AM is a natural substrate that serves as a supporting matrix for cells and tissues, making it a crucial component of tissue healing with a variety of therapeutic uses. Furthermore, the AM possesses various biological features necessary for tissue regeneration, such as anti-inflammatory, antibacterial, anti-fibrosis, anti-scarring, and low immunogenicity, as previously mentioned. AM may thus be seen as a bio-therapeutic material made up of one single layer of epithelial cells residing on a basement membrane and a non-vascular collagenous stroma. AM's therapeutic benefits are attributed to these three components, which include antiadhesive, bacteriostatic, wound protection, pain relief, and epithelialization actions.[23–25]

The AM epithelial cells reside on the inner layer of the AM, while amniotic mesenchymal stromal cells form the outer layer.[26]

Results obtained from our study group showed complete healing of 14 ulcers in 14–60 days with a mean of 33.3±14.7; healing rate range was 0.064–2.22 and mean 0.896±0.646 cm2/day with a 100% reduction in ulcer size. Ulcer floor improved in all ulcers. Healthy granulations were present in 13 ulcers (92.9%) and absent in one ulcer (7.1%). Three ulcers (21.4%) were of mild severity (grade 1 ulcers) while eleven ulcers (78.6%) were of moderate severity (grade 2 ulcers). Eleven cases (78.6%) showed improvement in their pain level on a scale from 1 to 10. AM graft was taken in four cases (28.6%), while AM was not taken in ten cases (71.4%).

The current study results were supported by the results of Mermet et al [15] in a prospective pilot study, in which they evaluated the safety, feasibility, and the effects on healing of AM graft in 15 patients with chronic venous leg ulcers. The percentage of granulation tissue increased significantly (from 17% on day 0 to 69% on day 14, P,0.0001), along with a significant decrease of fibrinous slough (from 36% at day 0 to 16% at day 14, P,0.001). There was significant reduction in ulcer size and pain level as well. Also, Alsina Gilbert and Pedregosa-Fauste [17] performed AM transplantation for four refractory vascular ulcers. Complete wound reepithelialization was achieved for one ulcer by week 8; in the other three cases, there was a 50% reduction in size compared to baseline. At week 16, the mean reduction in wound size for the four ulcers was 81.93%. The corresponding reduction in pain intensity was 86.6%. No adverse effects were observed.

CONCLUSION

AM graft can be an ideal choice instead of tissue-engineered skin equivalents to be used in wound healing. In addition to being an excellent scaffold, it has unique biological properties that are important for tissue repair, including antiinflammatory, antimicrobial, anti fibrosis, anti scarring, as well as a reasonable cost and low immunogenicity. Furthermore, the presence of its own progenitor cells help in tissue repair.

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