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A Study of Amniotic Membrane Graft in Treatment of Chronic Venous Leg Ulcers

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Abstract

Aim: To evaluate the efficacy of Amniotic Membrane graft in the treatment of chronic venous leg ulcers.

Research Design and Methods: Prospective, randomized, non-blinded study. Eligible patients were randomized to one of 2 groups: Group I: (Control group) included 11 chronic leg ulcers, in which ulcers were treated with conventional moist wound dressings and multilayer compressive bandages. Group II: (Amniotic Membrane group) included 14 leg ulcers. Amniotic membrane was placed in contact with ulcer and held in place with secondary dressing and multilayer compressive bandages. all patients were evaluated for healing rate and change in ulcer size.

Results: Healing rate showed significant difference between group I and group II (p = 0.001). Group II (AM) patients demonstrated near complete healing of 14 ulcers in 14-60 days with mean of 33.3 ± 14.7, healing rate range was 0.064- 2.22 and mean of 0.896 ± 0.646 cm^2/day with 94% reduction in ulcer size. Taken AM grafts were seen in 28.6% (n=4) of group II. Reduction in ulcer size in taken AM graft was 94.7%, while 93% in non-taken AM graft.

Conclusions: Our results suggest that using AM in treat leg ulcers not responding to conventional multilayer compression in patients with non- option chronic venous insufficiency.

Keywords: Amniotic membrane; Venous; Leg ulcers

Introduction

Chronic wounds represent a significant health problem with an incidence of 5-7 million cases per year in the United States alone [1]. Point prevalence of active ulceration ranges from 1.48 to 3.05/1000 internationally. Ulcers are considered chronic if they persist for more than 6 weeks. Causes of chronic leg ulcers are venous (60-80%), arterial (10-30%) and other factors such as diabetes mellitus and rheumatoid disease. Arterial and venous components coexist in 10-20% of cases [2,3].

Chronic venous disease (CVD) results from a disturbance of blood flow within the veins of the lower extremity because of obstruction or reflux. The resultant venous hypertension initiates a cascade of events that cause a visible deformation of the veins and a chronic inflammatory state that leads to a multitude of dermatologic changes with Its most severe manifestation is cutaneous venous ulceration. The incidence of venous ulceration varies from 0.05% to 1.52% in the total population. Isolated superficial reflux is capable of producing severe CVD but responds well to appropriate therapy. The clinical appearance of ulceration resulting from postthrombotic CVD is often indistinguishable and can have a completely different management plan and prognosis [4].

Wound healing is a complex multi-phase process involving a variety of cell types, growth factors and extracellular matrix. It passes through three phases namely inflammatory, proliferative and remodeling phases [5]. The use of amniotic membrane (AM) in chronic wounds stems from its proven efficacy in similar ophthalmic indications such as corneal ulcers, where it serves as a promoter of epithelization and inhibitor of fibrosis. This is due to down-regulation of TGF-β and its receptor expression by fibroblasts. Furthermore, AM can act as a biologic dressing and a natural scaffold for cellular therapy of chronic wounds. Hence, an AM scaffold can modulate the healing of a wound by promoting tissue reconstruction rather than promoting scar tissue formation [6]. The present work aims to evaluate the efficacy of AM graft in the treatment of chronic venous leg ulcers.

Patients and Methods

The current study is a prospective, randomized, non-blinded study evaluating the regenerative effect of amniotic membrane graft in treatment of chronic venous leg ulcers. The study design conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was reviewed and approved by our Institutional Ethical Committee. Patients were recruited from vascular surgery department, Assiut University Hospital. Each patient signed an informed written consent before any study involvement. Inclusion criteria were the presence of Venous leg ulcers for more than 6 weeks, with no improvement despite treatment of underlying cause of ambulatory high venous pressure in the form of superficial / perforator system ablation or trial deep system reconstruction considering our cohort as "non option venous leg ulcer patients ". Exclusion criteria were Ulcers with ongoing active infection, Malignant ulcers (ulcers with increasingly recent changes in the size and everted edges), and ulcers showing good response to conventional treatment.

Randomization

Eligible patients were randomized using closed envelope method to one of 2 groups; Group I: (Control group) included 11 chronic leg ulcers, in which ulcers were treated with conventional moist wound dressings that were changed every other day for eight weeks and multilayer compressive bandages. Group II: (Amniotic Membrane group) included 14 leg ulcers. Amniotic membrane was placed in contact with ulcer and held in place with secondary dressing changed every other day and multilayer compressive bandages.

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Wound evaluation, ulcers were assessed for

1- Signs of wound healing and epithelial regeneration. 2- Percentage of the healed wound area and healing rate: = [(original wound area - final wound area) / original wound area] %. Healing rate /day = (original wound area - final wound area)/ time consumed to reach final wound area.

3- Wound size: The greatest length (head to toe) and the greatest width (side to side) were measured using a centimeter ruler, and surface area was calculated in square centimeters (cm^2).

Obtaining Amniotic Membrane and its preparation: Human AM was prepared from placenta obtained after scheduled delivery by cesarean section following a normal pregnancy. On the day of the cesarean section, test tubes containing blood from the mother were collected for the following serology tests: HIV, HCV, HTLV, syphilis: VDRL-TPHA, HBV: HBs antigen- HBc antibody.

The AM was then cut into different sizes and AM tissue fragments were obtained and immersed immediately in sterile phosphate buffered saline with 10% antibiotics and antifungal (Sigma). Three AM samples were collected for bacteriological examination. Final validation of the AM was performed after negative serology tests.

Covering the ulcer

The defect to be covered was cleansed by saline. All exudate and devitalized tissues were removed with minor debridement. AM was then placed in contact with ulcer bed. Small longitudinal incisions were made in the membrane to allow any discharge to get out. A secondary dressing was applied over to prevent AM dislodgment.

Study outcomes

Primary endpoints were reduction in ulcer size and percentage of completely healed ulcers within 60 days follow up period. Complete healing was defined as complete epithelialization and coverage of ulcer area.

Follow up

All patients were evaluated by the researchers (A. O, A. E) for healing rate and change in ulcer size. AM Graft was evaluated on Day 2 whether taken or not. Taken graft was recognized when the AM is adherent to the ulcer bed without any underlying discharge or debris. Ulcer image acquisition was performed on Days 0, 7,14, 21, 30, 45, and Day 60.

Statistical Analysis

Statistical analyses of all data set were performed with SPSS software version 15.0 for Windows (SPSS Inc., USA). The differences in the primary endpoints between groups were compared by Mann-Whitney U Test in comparison between the two groups. Chi-square test or Fisher's Exact Test were used to compare categorical data. Statistical significance was set at $P < 0.05$.

Results

The eligible 25 patients (25 chronic leg ulcers) were randomly assigned to one of the two study groups; Group I (Control, n=11, mean age= 34.4), Group II (AM, n=14, mean age= 32.8). All patients in the current series were male. 18 patients (72%) were smokers, 9 cases (36%) were positive for hypercoagulability. 13 ulcers (52%) were of post-thrombotic venous origin. Patient characteristics among study groups are demonstrated in Table 1.

	Smoking	Hypercoagulability	Post thrombotic
Group I	8	3	5
Group II	10	6	8

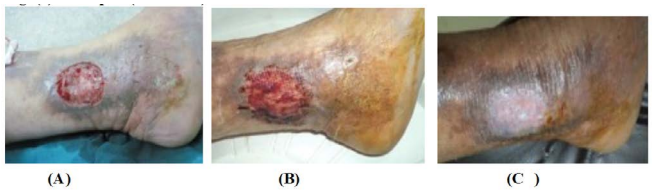
Table 1: Patient characteristics.

Group	No. of ulcers	Ulcer size reduction %	
I	11	29.6	P<0.001
II	14	94.0	

Table 2: Reduction in ulcer size among different study groups.

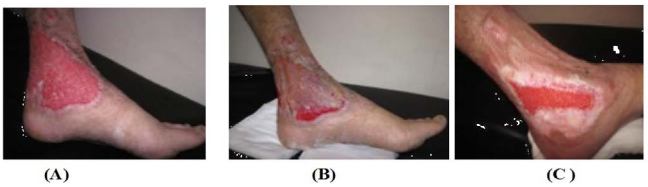
Group	No. of ulcers	Cm^2/day	
I	11	0.107	P<0.001
II	14	0.896	

Table 3: Ulcer Healing rate among different groups.



(A): leg ulcer before application of AM. (B): Ulcer 2 days after AM application and AM graft was taken. (C): follow up showed complete healing after 14 days.

Figure 1: Group II (taken AM graft).



(A) Two leg ulcers (upper small ulcer and lower big one). (B) AM applied covering both leg ulcers. AM graft was taken in upper ulcer while 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.

Figure 2: Partially taken AM graft.

Chronicity of leg ulcers ranged from 24 to 60 months with mean of 45.82± 14.01 in group I, from 24 to 84 months with mean of 50.57±16.43 in group II with no statistically significant difference in chronicity between the studied groups.

Comparing the reduction in ulcer size among study groups, there was a significant difference between group I and groups II (p=0.001). Healing rate showed significant difference between group I and group II (p = 0.001).

Group II (AM) patients demonstrated near complete healing of 14 ulcers in 14-60 days with mean of 33.3 ± 14.7, healing rate range was 0.064- 2.22 and mean of 0.896 ± 0.646 cm^2/day with 94% reduction in ulcer size. Reduction in ulcer size and healing rate among study groups are presented in Tables 2 and 3.

Taken AM grafts were seen in 28.6% (n=4) of group II (Figure 1). Reduction in ulcer size in taken AM graft was 94.7%, while 93% in non-taken AM graft (Figure 2). Healing rate in taken AM graft was 0.992 cm^2/day, and 0.764 cm^2/day in non-taken AM graft, while 0.1 cm^2/day when AM was not used in treatment. Table 4 shows the impact of taken versus non-taken AM grafts as regards ulcer size reduction and healing rate.

	Reduction in ulcer size		
	Number of leg ulcers	Ulcer size reduction%	
Graft not taken	10	93%	0.910
Graft taken	4	94.7%	
	Rate of healing in cm^2/day		
	Number of leg ulcers	cm^2/day	
Graft not taken	10	0.764	0.238
Graft taken	4	0.992	

Table 4: The impact of taken versus non-taken AM grafts as regards ulcer size reduction and healing rate.

Discussion

Patients with non-healing wounds of long duration tend to respond slowly and require repeat treatments. Given the chronic nature of these wounds and often associated co-morbidities seen in those patients, it is not surprising that rebuilding of affected tissues will take considerable time. The current study is a clinical study assessing the healing and regenerative effects of AM in treatment of chronic venous leg ulcers.

Amniotic membrane graft can serve a number of functions in the treatment of chronic wounds. It acts as a promoter of epithelization, inhibitor of fibrosis through down-regulation of TGF-β and its receptor expression by fibroblasts, and more importantly, acts as a biological scaffold for healing tissues. That was supported by studies found that AM cell transplants have been attributed not only to the direct replacement of lost cells, but also to factors secreted by the cells which may serve a protective or reparative function [7-9].

In the current study, group II (AM) showed near complete healing of 14 ulcers in 14-60 days with mean of 33.3 ± 14.7, healing rate range was 0.064- 2.22 and mean of 0.896 ± 0.646 cm^2/ day with 94% reduction in ulcer size. Amniotic membrane graft was taken in 4 cases 28.6% while AM was not taken in 10 cases (71.4%).

These results were supported by those obtained by Isabelle et al in a prospective pilot study, where they evaluated the safety, feasibility, and the effects on healing of AM graft in 15 patients with chronic venous leg ulcers [10]. Faulk et al. [11] have reported the benefits of AM in wound management [11]. Histological analysis of leg ulcers before and 5 days after treatment with AM revealed an increase in granulation tissue and in connective tissue, formation of basement membrane, and vascular development. Increased expression of factor VIII synthesized by endothelial cells was also observed [11]. Somerville has reported the formation of capillary neovessels following use of AM after storage at 4°C [12]. AM expresses many neurotrophic and angiogenic factors: endothelins 2 and 3, vascular endothelial growth factor (VEGF), VEGF-B, Tie-2 angiopoietin receptor, ephrin-A2, ephrin receptors A2, B1, B3, B4, B5, neuropilin-2, nerve growth factor (NGF) receptor, and semaphorin-F19 as well as erythropoietin and its receptor [13].

In a study conducted by Hanumanthappa et al., on 200 varicose ulcer patients, they divided their patients into 100 control treated with conventional dressing and compression stockings and 100 test cases treated with AM and compression stockings. They found that 81% of cases showed epithelialisation by the end of 3rd week ($P<0.005$) and in 80% of cases, there was absence of wound infection ($P<0.048$). In 63% of cases, significant drop in exudation was observed by the end of 1st week ($P<0.034$) [14]. Robson, Krizek, Koss and Samburg in 1973 [15] observed rapid ingrowth of epithelium from the wound edges in full thickness defects and increased rate of reepithelialisation of partial thickness burns by the use of AM [16,17]. This stimulatory effect on epithelialisation has been considered to be mediated by growth factors and progenitor cells released by AM [10,18,19]. Small sample size is considered the main limitation in the current study; as we aimed patients suffering from chronic non healing venous leg ulcers despite trials of conventional compressive treatment and failed correction of high ambulatory venous pressure through targeting superficial, perforator or deep venous system errors. Chronic non healing leg ulcers represent a great challenge for concerning physicians and huge economic burden for age working group in both developed and developing communities. Regenerative medicine presents a significant hope for this patient group when treatment of underlying causes is not possible describing it as "non- option leg ulcer patients". With continuous improvements in techniques of isolation and delivery of amniotic membrane and promising results are seen in the horizon, continuous reporting of novel techniques and results in non option leg ulcer patients are mandatory [20-22].

Conclusions

Using amniotic membrane represent effective therapeutic approach for healing of chronic venous leg ulcer. Our results suggest that using AM in treatment may be the best choice in treating chronic venous leg ulcers not responding to conventional multilayer compression in patients with non- option chronic venous insufficiency.

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