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### Original Article

# Amniotic membrane dressing versus conventional dressing in lower limb Varicose ulcer: A prospective comparative study

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#### ABSTRACT

**Aim:** To evaluate the safety & effectiveness of the amniotic membrane dressing in the management of varicose ulcers. **Materials & Methods:** A prospective comparative study was conducted in the department of surgery, A.J. hospital & research centre, India, from Jan 2010 to Jan 2012. 200 cases with leg varicose ulcers were studied, which were equally & randomly divided into control and test group. Test group received amniotic membrane dressing with compression stocking, while control group had saline dressing with compression stocking. They were evaluated on 7th, 14th & 21st day for epithelialisation, granulation tissue, control of wound infection, control of wound exudation & local pain control. Amniotic membrane grafts were prepared from placentas harvested during caesarean section after obtaining written informed consent. **Results:** Out of 100 cases in test group, 81 (81%) cases showed epithelialisation by the end of 3rd week ( $P < 0.005$ ) & in 80 % of cases, there was absence of wound infection ( $P < 0.048$ ). In 63 (63%) cases, significant drop in exudation was observed by the end of 1st week ( $P < 0.034$ ). Local pain score in test group dropped from 70 (mean) to 10 (mean). The percentage of granulation tissue increased significantly from 20% to 80% in 3 weeks time. Granulation tissue was healthy & highly vascular in the majority of the cases. No adverse effects were observed. **Conclusion:** Widespread availability, negligible cost, ease of use, absence of adverse effects and facilitated wound healing make amniotic membrane dressing superior to conventional dressing in the management of varicose ulcers.

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### 1. Introduction

Varicose ulcers (VU) are the most prevalent form of chronic wounds in the Western world [29]. Their successful treatment represents a special clinical challenge because of their high prevalence, refractory nature, impact on patients' quality of life, morbidity & economic consequences on the health care system [30]. The optimal treatment of VU is not clear, but compression therapy is the mainstay of medical management [31]. Autologous skin graft accelerates tissue repair, but harvesting of autograft creates a donor wound & repeated harvesting results in scarring at the donor site. Bioengineered skin substitutes have been

successfully tested & are commercially available [2], but their cost remain too high for routine clinical use.

Amniotic membranes (AM) are tissues of particular interest for several reasons. It has been considered as an ideal biological dressing because it promotes epithelialization [28], controls fluid loss from the wound, relieves pain & provides moist environment for healing [18,21,26]. It has got antibacterial property as well [6, 9, 11]. It is also readily available at negligible cost to the patient, which is relevant in developing countries [26, 27]. AM contains angiogenic factors which contribute to faster granulation [19].

Although it is biological, it is never rejected by the receiving tissue because AM does not express antigens of histocompatibility [23, 25].

The amniotic membrane has been in use for corneal and conjunctival reconstruction [24]. Based on the success observed in ophthalmology, we wished to evaluate AM as a wound dressing in varicose ulcer. In the literature, a few studies have been documented about AM dressing in the management of VU & in

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most of the studies the sample sizes were not big enough. We thus undertook a prospective comparative study (AM dressing Vs conventional dressing, 100 cases each) on patients with leg VU ulcers to evaluate the effects and the safety of AM dressing.

## 2. Materials and Methods

This prospective comparative study was carried out in the department of surgery, A.J. hospital & research centre, Karnataka, India from Jan 2010 to Jan 2012, after obtaining approval from local ethical committee.

### 2.1. Patients

Total no of cases studied were 200, which were equally & randomly divided into control and test group. Patients presenting with varicose ulcers in the lower limb were the subjects for the study. Written informed consents were obtained.

Inclusion criteria were: age 18 years or older; the presence of at least one varicose ulcer with a minimum size 4x4cm; no tendency for healing in the past 3 months despite conventional medical treatment; presence of varicosity on clinical examination & confirmed by venous duplex scan.

Patients were excluded if they had deep vein thrombosis, deep venous insufficiency on venous duplex scan, significant arterial insufficiency, severe neuropathy in the reference leg, tendon or bone exposure in the reference ulcer, severe systemic disease.

Selected patients were treated as outpatients. They underwent detailed clinical examination. Routine haematological investigations & culture sensitivity of wound swab were performed for all the cases, while the special investigations like x-ray of the part, & edge biopsy were carried out as & when required.

They underwent treatment for a period of one to two weeks before the actual study. During this period, appropriate medical and surgical line of treatment like diabetic control, control of infection by appropriate antibiotic based on culture sensitivity report, surgical debridement, and correction of medical illness were performed.

Prior to the study, a repeat culture swab was taken from ulcer. They were treated with appropriate antibiotics if culture revealed organisms and then subjected for the study. Then the eligible patients were divided randomly & equally into test and control group.

### 2.2. Amniotic membrane (harvesting, preservation & its application)

AM grafts were prepared from placentas harvested during caesarean section. Eligible donor mothers were accepted for AM donation after a medical interview and after obtaining written informed consent. Their blood samples were tested for HIV, Hepatitis B&C, syphilis, toxoplasmosis, and cytomegalovirus. Those who tested negative for the aforementioned diseases with no premature rupture of membranes were chosen for the donation.

The AM was separated from the chorion of placenta under sterile aseptic conditions (fig 1). The AM was cleared of all gross tissue attachments and blood clots by washing in copious amounts of normal saline. The membrane was then placed in large bottles containing 85% glycerol and stored at room temperatures for 24 hours and then stored at 4° C in the refrigerator until use. The membranes were tested for bacterial count and culture sensitivity prior to the use. At the time of application, the AM was thawed by soaking it in normal saline for 10 minutes.

### 2.3. Method of application of dressing

**2.3.1. Test group:** The ulcers were cleaned and irrigated with normal saline, AM was then applied with rough (chorionic) surface facing the surface of the ulcer & 3 layered gauze dressing was done. Compression stocking (level 2 or 3) was applied over the dressing. The dressing was left in place for 4 days and was observed for any exudation. Redressing thereafter was done once in 3 days and evaluated on 7th, 14th & 21st day.

**2.3.2. Control group:** cases were subjected for normal saline dressing once or twice daily depending on exudates. Over the dressing, compression stocking (level 2 or 3) was applied.

### 2.4. Method of evaluation of the wound

At the end of 1st, 2nd & 3rd week, test group & control group were evaluated & compared. The parameters noted at each evaluation were epithelialisation of the ulcer, percentages of granulation tissue, local pain score, exudation & presence of wound infection.

Local pain score was assessed using a 101-point (0-100) visual analogue scale, with 0 indicating no pain and 100 the worst pain imaginable.

Foul smelling purulent discharge and the surrounding erythema with local signs of inflammation were taken as infection. Soakage of 3 layered gauze dressing was taken as the presence of exudation.

### 2.5. Statistical analysis

Chi-squared & P-value were calculated using SPSS 17 version software.

## 3. Results and Discussion

Selected 200 cases with varicose ulcers were randomly divided into test group & control group. The clinical details are given in tab 1.

Altogether, 100 AM grafts were applied on 100 varicose ulcers. At the end of 1st, 2nd & 3rd week, test group (AM dressings) & control group (conventional dressing) were evaluated & compared. The parameters compared were epithelialisation of the ulcer, percentages of granulation tissue, pain control, exudation & prevention of wound infection.

**Tab 1: Clinical details of test & control group**

Feature	Test group (N=100)	Control group (N=100)
Male	78	80
Female	22	20
Mean age in years (range)	46.5 (18-75)	45.5 (18-73)
Mean duration of ulcer in months (range)	4.5 (3-6)	5 (3-7)
Co-morbidity:		
DM	32	27
Cardiac diseases	18	15
Others	5	4

### 3.1. Epithelialisation

Robson, Krizek, Koss and Samburg [17] in 1973 observed rapid ingrowth of epithelium from the wound edges in full thickness defects and increased rate of re- epithelialisation of partial thickness burns by the use of AM [14, 18-21]. This stimulatory effect on epithelialisation has been considered to be mediated by growth factors and progenitor cells released by AM [1, 3, 4].

In our study, this property was very well noticed, as 81 (81%) cases in test group showed epithelialisation by the end of 3rd week compared to 40 (40%) cases in control group (tab 2). This difference was statistically highly significant ( $P < 0.005$ ).

**Tab 2: AM dressing: Effect on epithelialisation**

Epithelialisation	Control group (N=100)	Test group (N=100)
On 7th day	2	19
On 14th day	20	38
On 21st day	18	24
No epithelialisation	60	19

Chi-squared=7.371 P-value<0.005 (Statistically highly significant)

### 3.2. Antibacterial property

This is another important feature of AM & is thought to be due to the presence of antibodies and lysozyme (a bacteriolytic protein) in AM [8].

AM has got a high thrombin activity which allows a rapid and efficient attachment of AM to granulating tissue [5, 10]. This close adherence allows restoration of lymphatic integrity which protects circulating phagocytes from exposure and allows removal of surface debris and bacteria [11]. Furthermore, adherence to the wound surface eliminates its exposed status which in turn lowers bacterial count [6, 7, 9].

Our study revealed absence of wound infection in 80% of the cases in test group against 39% in control group ( $P < 0.048$ ) (tab 3).

**Tab 3: AM dressing: Effect on infection control**

Infection	Control group (N=100)	Test group (N=100)
Absent	39	80
Present	61	20

Chi-squared=3.582

P-value<0.048 (Statistically significant)

### 3.3. Exudation

By providing secure coverage, AM reduces exudation from the wound. This property is particularly important in burn wound management where there will be a lot of tissue fluid loss.

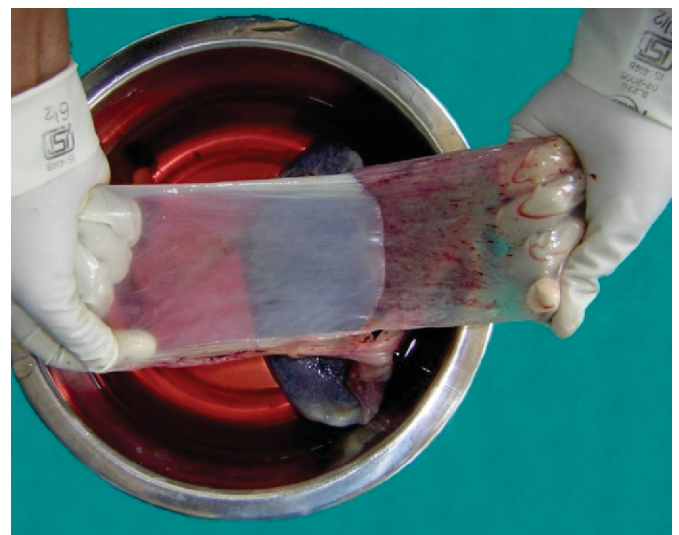
This feature was obvious in our study also, as in 63 (63%) cases in test group, dressings were dry by the end of first week compared to 25% in control group & this difference was statistically significant ( $P < 0.034$ ) (tab 4).

**Table 4: Exudation control by AM**

Exudation	Control group (N=100)	Test group (N=100)
Absent	25	63
Present	75	37

Chi-squared=3.870

P-value<0.034 (Statistically significant)

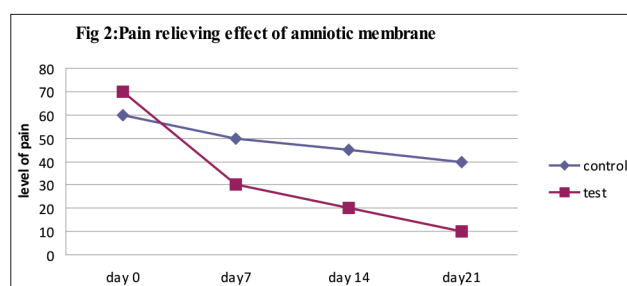
**Figure 1: peeling of amniotic membrane from placenta**

### 3.4. Pain relief

Pain relieving is one of the well recognized properties of AM when used as a skin substitute [12-14]. It is possibly due to decreased inflammation, better state of hydration of wound bed [15] & protection of the exposed nerve endings from external irritant [16].

Local pain score in test group dropped from 70 (mean) to 10 (mean) in 3 weeks time. Maximal effect was observed in first week (fig 2).

**Fig 2: Pain relieving effect of amniotic membrane**

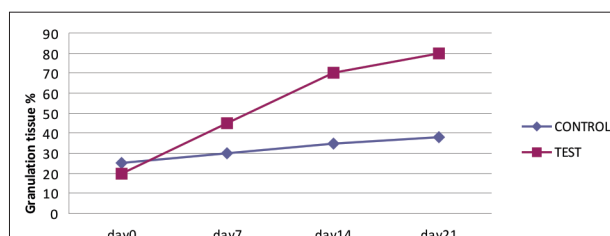


### 3.5. Angiogenesis/Granulation

The most striking effect noted by Faulk et al [19] using AM on chronic leg ulcer was the development of new vessels which they thought was due to the presence of some angiogenic factors [5]. Burgos [22] confirmed the presence of angiogenic and mitogenic factors in amniotic membrane [5]. In our study, this property was observed in most of the cases.

The percentage of granulation tissue increased significantly in test group from 20% (mean) to 80% (mean) in 3 weeks compared to control group (fig 3). The maximal effect was observed in first 2 weeks in test group. Granulation tissue was healthy & highly vascular in the majority of the cases.

**Fig 3: Granulation stimulating effect of AM**



### 4. Conclusion

We conclude that amniotic membrane dressing promotes epithelialisation & granulation tissue development, while controlling infection & exudation from the wound. Also, the membrane is easily obtained at negligible cost & it can be used as on ambulatory treatment without immobilization. AM dressing may thus be considered as safe, cheap & effective alternative method for treating varicose leg ulcers, particularly in developing countries where the cost of dressing material is the major concern.

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