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"A Comparative Study of Conventional Dressing over Dehydrated Human Amniochorionic Allograft in Chronic Leg Ulcers"

AUTHOR

Abstract
INTRODUCTION
Human amniotic membrane is a uniquely suited material for use as an allograft in wound management. It is used in its natural form , later in preserved preparations the material assists in the healing process through a number of properties such as physical, biochemical and molecular biological pathways to promote regenerative healing while simultaneously reducing scar formation.
AIM:
1. *To compare the outcome of conventional dressing over dehydrated human amniochorionic allograft in chronic leg ulcers.*
OBJECTIVES:
1. To study ulcer healing rate.
2. To study duration of hospital stay.
3. To study graft survival in conventional dressing and human amniochorionic allograft dressing.
MATERIALS AND METHODS
Of a total of 100 patients, half of them are subjected to conventional normal saline dressings, and another half of them will be given amniochorionic allograft dressings.
Offloading of pressure from the affected area will be done for both groups. Culture and sensitivity of ulcers will be obtained before and after the dressings. The patients will be followed on a daily basis for three weeks in both groups. The size of the ulcer will be recorded at the end of 3 weeks.
OBSERVATION AND RESULTS:
The graft takes up in the study group is 96%, whereas, in the control group, it is 18%. The results were analyzed by the chi-square test, which showed a very high significant value.($p<0.0001$)
CONCLUSION
The ulcers in subjects treated with amniochorionic allograft graft take up was more than the wounds in the control group (96% vs 18%; $P= < 0.0001$, very significant) which indicates that amniochorionic allograft dressing is an effective modality for chronic leg ulcers.
Keywords
Chronic leg ulcers, amniochorionic allograft, graft take up.

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I. Introduction

Leg ulcers have plagued mankind since ancient times and still pose a considerable burden for both patients and carers in most countries of the world. Ulcers can be defined as wounds with a "full-thickness depth" and a "slow healing tendency." Ulcers of skin can result in complete loss of the epidermis and often portions of the dermis and even subcutaneous fat. Chronic leg ulcer disease also known as chronic lower-limb ulcer is a chronic wound of the leg that shows no tendency to heal after 3 months of appropriate treatment or is still not fully healed at 12 months. According to most of the Western and European studies, the most common type of leg ulcer is venous ulcer, the others being neuropathic ulcer and arterial ulcers .These three kinds of ulcers account for almost 90 % of cases of lower-leg ulceration. Approximately 70 % of the limb ulcers are caused by venous diseases. Rest 30 % are due to vascular diseases, diabetic, malignant ulcers, traumatic ulcers, chronic lymphedema, and a few medical conditions . Chronic ulceration of the lower legs is a relatively common condition among adults. The spectra of symptoms in chronic leg ulcer disease include increasing pain, friable granulation tissue, foul odour, and poor wound healing. This not only results in social distress and considerable increase in healthcare and personal costs but also loss of productivity and poor quality of life .

Amniotic Membrane Structure and Function

The amniotic membrane develops from extra-embryonic tissue and consists of a fetal component (the chorionic plate) and a maternal branch (the deciduas).

The amniotic membrane is composed of 2 distinct layers that surround the embryo: the amnion that lines the inner surface of the amniotic sac interfacing with the fetus, and the chorion that is the outer layer in contact with maternal tissue.[1,2] TheThese two parts are held together by the chorionic villi and connect the cytotrophoblastic shell of the chorionic sac to the deciduas basalis. The amniochorionic membrane forms the outer limit of the sac that encloses the fetus, while the innermost layer of the sac is the amniotic membrane. It is about 10-15mm thick, consisting of two fetal membranes; the inner amniotic membrane and the outer chorion. The innermost layer, nearest to the fetus, is called the amniotic epithelium and consists of a single layer of cells uniformly arranged on the basement membrane. The ultrastructure of the amniotic membrane is a thin, tough, transparent, avascular composite membrane. It constitutes three major layers: a single epithelial layer, a thick basement membrane, and avascular mesenchyme consisting mainly of collagen. The amniotic membrane contains no blood vessels or nerves; instead, the nutrients it requires are supplied by direct diffusion out of the amniotic fluid and the underlying decidua. The amniotic epithelial cell layer is a single layer of flat, cuboidal, and columnar cells in direct contact with amniotic fluid. From this layer, amniotic MSC(AMSC) is isolated and stored for regenerating tissues. The amniotic mesoderm layer consists of macrophages and fibroblast-like mesenchymal cells. The amnion's basement membrane is very similar to the basement membrane found in the other parts of the body, like the conjunctiva or gingiva. Some of the many factors present in this bioactive ECM include platelet-derived growth factors (PDGFs), transforming growth factor-b1 (TGF-b1), basic fibroblast growth factor, and granulocyte colony-stimulating factor, as well as interleukin-4 (IL-4), IL-6, IL-8, and IL-10.[7] The wounds treated with AM responded to a protocol that allowed coverage of tissues as diverse as exposed bowel, pleura, pericardium, blood vessels, tendon, nerve, and bone. Wounds unresponsive to standard therapeutic measures have also responded to application of AM, and human AM dressings have become a useful adjunct in the care of complicated wounds [11.]

Features of Amniotic Membrane

Immunogenicity of amniotic membrane : Amniotic membrane has low or no antigenicity. The native ACEs express the non-polymorphic, non –classical human leukocyte antigen (HLA-G).. Immune barrier is due to lack of expression of co-stimulatory cell surface molecules such as CD80 and CD8, mostly in humans.

Anti-Inflammatory and Antimicrobial Property

The amniotic membrane reduces inflammation. The AM stromal matrix markedly suppresses the expression of the potent pro-inflammatory cytokines, IL-1α and IL-β. Matrix metalloproteases(MMPs) are expressed by infiltrating polymorphonuclear cells and macrophages. Hyaluronic acid is a high molecular weight glycosaminoglycan in large quantities in the AM and acts as a ligand for CD44, which is expressed on inflammatory cells and plays an essential role in adhesion of the inflammatory cells, including lymphocytes, to the AM stroma. ACEs also can produce β- defensins. The β3 defensin is the predominant defensin in the amniotic epithelial cells. The AM anti-inflammatory action may be mediated in part by interleukin-10 **(IL-10)**, of which we detect significant amounts in AM extracts. IL-10 is known to suppress or counteract the actions of pro-inflammatory cytokines such as IL-611 and tumor necrosis factor-alpha (TNF-a) (12). IL-10 also suppresses amniotic cell production of IL-8,13 which is a pro-inflammatory chemokine attracting the migration of neutrophils. TCF-13 superfamily provides the protein substrates for production of inhibin and activin. Activin promotes the production of prostaglandin **PGE2**. [12,14]

The two low molecular mass elastase inhibitors, secretory leukocyte proteinase inhibitor(SLPI) and elafin, are expressed in the AM. In addition to their anti-inflammatory properties, elafin and SLPI have antimicrobial actions and act as components of the innate immune system to protect related surfaces from infection. Adherence of amnion to the burn wound by eliminating its exposed status may manifest itself in lower bacterial count in the wound. The close adherence of the membrane to the wound is said to be via fibrin and elastin. The amniotic membrane has a high thrombin activity, which allows a very rapid efficient attachment to the living dermis or granulating tissue..

Reduction of pain

AM has a unique ability to reduce pain during the surgical procedure. It diminishes inflammation and provides a better hydration state that soothes the wound bed to promote faster healing. The soft mucoid lining of the amniotic membrane also protects the exposed nerve endings from an external irritant that reduces pain sensation by preventing nerve stimuli.

Increase Vascularisation or Revascularization

The rapidity of growth epithelium from the wound's borders in full-thickness defects, and the rate of epithelization of partial-thickness burns appears to be increased by using the amniotic membrane. There is an enhanced induction of vascular endothelial growth factor (VEGF) both for VEGF receptors 1 and 2 by the AM cells. The release of angiogenic factor-like insulin derived growth factor (IGF) helps develop tissue-engineered vascular grafts useful in revascularization of ischemic tissues, chronic ulcers, repair of bone, and cartilage.

Preparation of Amniotic Membrane

The fresh membrane is obtained from the placenta at the delivery time, either vaginal or caesarian section. Robson and Krizek rinsed the membrane in a 0.025% solution of sodium hypochlorite and stored at 4C in a sterile solution containing penicillin. They showed that membranes remained sterile for up to 6 weeks. Preservation with 1:40 dilution of sodium hypochlorite revealed no positive cultures until 30 days. Typically, amniotic membrane is harvested upon scheduled cesarean section from donors who undergo rigorous screening for viral and infectious diseases, including human immunodeficiency virus 1 and 2, hepatitis B and C, human T-cell lymphotrophic virus, and syphilis[8,9]After washing and cleaning harvested tissues in buffered solutions, amniotic membrane grafts can be prepared as fresh or preserved allografts. When using fresh amniotic membrane grafts, immediate transplantation is often necessary[10]; therefore, preservation techniques such as cryopreservation or dehydration have been utilized to increase storage time.

AIM:

1. To compare the outcome of conventional dressing over dehydrated human amniochorionic allograft in chronic leg ulcers.

OBJECTIVES:

1. To study ulcer healing rate.
2. To study duration of hospital stay.
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Study design: a randomized controlled study
Study duration: one year
Source of data: cases which are admitted in general surgery wards in S. V . R. R. G. G. Hospital, Tirupati.
Sample size: 100 cases.

INCLUSION CRITERIA :

1. Patients of age 20-60 years of age.
2. Size of ulcer >5cm to <15cm.
3. Duration of ulcer more than 2 weeks.

EXCLUSION CRITERIA :

1. Chronic peripheral vascular disease of limb.
2. Immunocompromised patients.
3. Associated bone involvement(osteomyelitis)
4. Malignant ulcer of skin.
5. Ulcer leg with metabolic disease (uremia and diabetic ketoacidosis)
6. Ulcer having bone or tendon injury on the floor.

II. Method

After a detailed clinical examination and relevant investigations, the initial size of the wound will be recorded after sharp debridement. Both groups will be subjected to daily dressings. Of a total of 100 patients, half of them are subjected to conventional normal saline dressings, and another half of them will be given amniochorionic allograft dressings.

Offloading of pressure from the affected area will be done for both groups. Culture and sensitivity of ulcers will be obtained before and after the dressings.

The patients will be followed on a daily basis for three weeks in both groups. The size of the ulcer will be recorded at the end of 3 weeks.

DRESSING TECHNIQUE

The patients who are given normal saline dressing are allotted odd numbers, and amniochorionic allograft is given even numbers.

For the conventional dressing:
The ulcer was cleaned with normal saline and saline-soaked with gauze piece kept over the ulcer, covered with pad and roller bandage.
For amniochorionic allograft dressing:
The ulcer was cleaned with normal saline. A single sheet of dehydrated amniochorionic allograft, which is commercially available, is spread over the ulcer. The dressing was opened after four days, rate of healing, contracture of the wound were assessed.
At the end of 21 days, the groups' wounds were assessed. The final area was evaluated in both groups by planimetry using a transparent graph sheet and subjected to statistical analysis.

Statistical methods
Chi square test and student t test are used for statistical analysis of results wherever applicable. The statistical software named SPSS software version 21.0 is used for the analysis of the data and Microsoft word and excel sheet have been used to generate graphs, tables.

III. Observation And Results

SSG Survival * Group

			Group		Total
			Group-A	Group-B	
SSG Survival	NO	Count	41	2	43
		% within SSG Survival	95.3%	4.7%	100.0%
		% within Group	82.0%	4.0%	43.0%
	YES	Count	9	48	57
		% within SSG Survival	15.8%	84.2%	100.0%
		% within Group	18.0%	96.0%	57.0%
Total		Count	50	50	100
		% within SSG Survival	50.0%	50.0%	100.0%
		% within Group	100.0%	100.0%	100.0%

Chi-square value = 62.056, p<0.0001 (Very High Sig.)

In group-A, 41 (82.0%) patients hadn't SSG survival, and 9 (18.0%) patients had SSG survival. In group-B, 2 (4.0%) patients hadn't SSG survival, and 48 (96.0%) patients had SSG survival. However, there was a statistically significant association between the SSG survival and group (P<00001, Very High Sig.)

T-Test

	Group	**N**	**Mean**	**Std. Deviation**	**t-value**	**p-value**
Area of Ulcer	Group-A	50	29.33	17.58	0.418	0.677 (Not Sig.)
	Group-B	50	27.93	15.92		
Final Area of Ulcer	Group-A	50	26.39	17.33	1.033	0.304 (Not Sig.)
	Group-B	50	23.03	15.19		
Area of Reduction	Group-A	50	2.94	1.23	-7.894	<0.0001 (VHS)
	Group-B	50	4.90	1.26		

The mean ± S.D. age (Years) was higher in group-B (43.04 ± 8.82 years) than the group-A (41.54 ± 10.10 years). However, there was no statistically significant difference between the groups for the mean of age (P=0.431, Not Sig.).

The mean ± S.D. number of the day in the hospital (days) was higher in group-A (19.02 ± 2.69) than the group-B (13.90 ± 2.55). However, there was a statistically significant difference between the groups for the mean number of days in the hospital (P<0.0001, Very high Sig.).

The mean ± S.D. ulcer initial length was higher in group-B (6.22 ± 2.24) than the group-A (6.11 ± 2.07). However, there is no statistically significant difference between the groups for the mean of ulcer initial length (P=0.810, Not Sig.).

The mean ± S.D. ulcer initial width was higher in group-A (4.77 ± 1.73) than the group-B (4.41 ± 1.34). However, there was no statistically significant difference between the groups for the mean of initial ulcer width (P=0.248, Not Sig.).

The mean ± S.D. area of the ulcer was higher in group-A (29.33 ± 17.58) than the group-B (27.93 ± 15.92). However, there was no statistically significant difference between the groups for the mean area of ulcer (P=0.677, Not Sig.).

The mean ± S.D. final area of the ulcer was higher in group-A (26.39 ± 17.33) than the group-B (23.03 ± 15.19). However, there was no statistically significant difference between the groups for the mean of the ulcer's final area (P=0.304, Not Sig.).

The mean ± S.D. area of reduction was higher in group-B (4.90 ± 1.26) than the group-A (2.94 ± 1.23). However, there was a statistically significant difference between the groups for the mean of age (P<0.0001, Very high Sig.).

The mean ± S.D. area of reduction was higher in group-B (4.90 ± 1.26) than the group-A (2.94 ± 1.23). However, there was a statistically significant difference between the groups for the mean of age (P<0.0001, Very high Sig.).

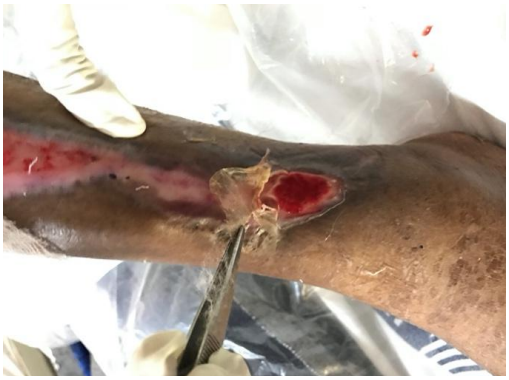
ANALYSIS OF DATA

The mean rate of the reduction area was higher in the study group(4.90) than the control group(2.94). The chi-square test results wereanalyzed, which showed a highly significant difference in the rate of the area of reduction of ulcer size.(p<0.0001)

The graft takes up in the study group is 96%, whereas, in the control group, it is 18%. The results were analyzed by the chi-square test, which showed a very high significant value.(p<0.0001)

The total number of days in the hospital for the patient to stay is also compared. The mean number of days of hospital stay in the control group was 19 days, and that in the study group was 13 days. The results were analyzed by the chi-square test, which showed a very high significant difference in the number of days of hospital stay (p<0.0001

An ulcer of size 5*3 cm was applied amniochorionic allograft.



The ulcer dressing was removed after four days, the rate of reduction of ulcer size and granulation tissue formation was noted. Later the ulcer was put graft after four days. The length of hospital stay was ten days. Graft survival also was good.

This is an ulcer of size 8*3 cm noted over the sole. The ulcer is applied amniochorionic allograft.
the dressing of the ulcer was opened after four days; the granulation tissue appeared within

IV. Discussion

According to Mirvat El Ansary. et .al this is a comparative study between conventional wound dressing and amniochorionic allograft. The study was conducted on a total of 25 patients. Out of eleven patients were given a conventional wound dressing, and fourteen patients were given amniochorionic allograft. The parameters compared were healing rate, detection of ulcer size, assessment of pain. In group I, given conventional treatment, leg ulcer chronicity varied from 24 to 60 months. The ulcer area at the beginning of the study was4.8+0.65cm^2(mean+SD). The mean percentage of healing rate was 0%, and all ulcers in this group showed no reduction in their size, and the ulcer floor remained the same. Healthy granulations were present in two ulcers and absent in nine ulcers. There is no improvement in pain level in the eleven ulcers.

parameters of healing	group I	group II
chronicity of leg ulcer(range)	24-60 months	24-84months
chronicity of leg ulcer(mean+SD)	45.82+14.01mon	50.57+16.43mon
reduction of ulcer size with treatment	0%	100%
healing rate cm^2/ day (range)	0.0-0.0	0.064-2.22
Ulcer healing.		
No healing	11 100%	0 0%
Complete healing.	0 0%	14 100%
Incomplete healing	0 0%	0 0%

Group, I included patients without amniochorionic allograft, and group II included patients with amniotic membrane application.

In the study group, the amniochorionic allograft was directly applied to leg ulcers; this group included 14 leg ulcers. The chronicity of leg ulcers varied from 24-84 months. The ulcer area at the start of the study was 5.1+0.48cm^2(mean+SD). The study group results 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 cm^2/ day with a 100 % reduction of ulcer size. The 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 I ulcers), while eleven ulcers(78.6%) were of moderate severity. Eleven cases (78.6%) showed improvement in their pain level on a scale from 1to 10. Three patients had no pain. AM graft was taken in four cases, while AM was not taken in ten cases in the days following the graft application. In these ten cases, the ulcers also showed complete healing on follow up. Reduction in ulcer size shows a significant difference between group I (control group) in comparison to group II in which we use AM alone.

The critical difference between the present study and Mirvat et al.'s study in the latter study included assessing pain after amniochorionic allograft.

According to Peer et al , utilised commercially available placental tissues namely EpiFix and Grafix . Three case reports are currently available for EpiFix , which is a dehydrated human amniochorion , also known as dHACM. In total , 12 patients were treated in these studies who had chronic wounds with a wound age> 4 weeks and of various etiology . of 12 patients , 4 had surgical dehiscence, 3 had neuropathic DFUs, and 5 other patients had venous leg ulcers , scleroderma, snake bite, or traumatic or arterial insufficiency wounds.

Three patients with neuropathic chronic DFUs were described in Shah study. The initial wound sizes were 0.42, 3.42, and 1.32cm^2 with a duration of 4, 7-8, and 3 months, respectively.

No advanced wound therapy use was reported before application of EpiFix . overall, after one application of EpiFix , two of three patients (66.7%) reached complete closure at 4 and 5.5 weeks postapplication .the patient with the 3.42cm^2 wound reached 50% of reduction of wound size 4 weeks post treatment.

The use of Grafix is reported in a retrospective single-center study.[14] The analysis included 66 patients with 67 wounds, among them 27 patients with chronic DFUs, 34 patients with VLUs, and 6 patients with other types of chronic wounds (*e.g.*, surgical, traumatic).

Twenty-three of 27 DFU patients (85.2%) previously failed different types of advanced therapies, including collagen matrices , skin grafting , cellular skin substitutes , topical growth factors, hyperbaric oxygen therapy, and negative pressure wound therapy. The mean DFUs wound size was 3.97cm^2, and the mean wound age was 24.5weeks. by week 12, 85.2% of DFU patients (23/27) reached complete wound closure.

	EpiFix	Grafix
No of patients	3	27
Wound Size	case 1 : 0.7×0.6 = 0.42cm^2 case 2 : 1.9×1.8 = 3.42cm^2 case 3: 1.2×1.1= 1.32cm^2	3.97±3.08cm^2(mean±SD)
wound age	case 1:16 case2: 28-32 case 3 : 12	24.5±49.2(mean±SD)
Exclusion criteria	end stage renal failure, previous graft failure, infection , autoimmune diseases	Infection; ischemia and malnutrition were addressed before application
Previous advanced therapy	0/3(0) failure, failed /total(%)	23/27(85.2)
Complete wound closure, closed/total(%)	2/3(66.7)	23/27(85.2)
Time to wound closure	case 1: 4 weeks Case 2: not reported Case 3: 5.5 weeks	6.2±2.6 weeks(mean±SD)
No. of application	1	3.8(mean)

V. Conclusion

Amniochorionic allograft can be ideal instead of tissue engineering 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 anti-inflammatory, antimicrobial, anti-fibrosis, anti-scarring, and a reasonable cost low immunogenicity. The present study concluded that amniochorionic allograft dressing helped in the formation of granulation tissue, decrease in ulcer area, and decreased hospital stay length. It helps to take up of graft well than the conventional dressing. Thus amniochorionic allograft dressing can be considered as a superior treatment option for chronic leg ulcers.

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