Use of Amnion Transfer in Resistant Nonhealing Venous Leg Ulcers

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Abstract In the treatment of venous leg ulcers, after adopting many treatment modalities, at times we are still left with a local indolent problem, “the resistant nonhealing venous ulcer.” With repeated surgical procedures failing to obtain a healed ulcer, constant pain, persistent discharging wound, infections, etc., many a patient turn hostile. Also, there comes a dimension of despair in the health feeling of the patient. This prospective study was conducted in 40 patients, who were within the inclusion criteria (January 2006–January 2010, 12 males and 28 females, mean age 45 years). A single amniotic membrane (AM) transfer was done after preparation of the wound, and the parameters were assessed periodically. Follow-up for a minimum period of 1 year in all cases and a maximum period of 3 years in many cases (38) was done. Significant clinical response was defined as an ulcer healing greater than 75 % of the original area. Treatment failure was taken as a persistence or recurrence of more than 25 % of the ulcer area in a 1-year follow-up period. The preliminary results of good ulcer healing and no recurrence are encouraging, even up to 3-year follow-up. This proof-of-concept study demonstrates that AM transfer is useful, safe, inexpensive, readily available, and well accepted by patients, with tremendous potential in promoting epithelial healing in “the resistant nonhealing chronic venous leg ulcer.”

Keywords Resistant nonhealing venous ulcers · AM transfer · Epithelization · Wound infection · Healing · Recurrence

Introduction

In a significant number of patients with venous leg ulcers, even after correction of factors such as saphenofemoral incompetency, saphenopopliteal incompetence, clinically identified above knee and below knee perforator incompetency, edema, cellulitis, stiff joints, anemia, etc., we are still left with a local indolent problem, “the resistant nonhealing venous ulcer” [1, 2]. Conventional autograft obtained by harvesting from healthy skin areas is considered the optimum wound cover [3]. However, repeated harvests and split skin graft (SSG) failures are not so uncommon and, ironically, result in added burden of nonhealing donor wounds.

At this point, the patient is not agreeable for any type of further surgical intervention, either on the leg or for the ulcer. Attempts for pain control and phase-adapted wound healing, using different surgical dressings [4] (membranes, polymer sponges, hydrogels, hydrocolloids, collagen products, foils), become the principle of management entailing high costs. Replacing missing tissue with biological skin substitutes is ideal (allograft, cultured allograft, amniotic membrane (AM), xenograft, de-epidermized cadaver skin). Clinical trials and animal experiments have documented that, of the biological dressings, the AM is superior to both allograft and xenograft skin [5–8].

Management of chronic wounds by AM was abandoned because of the risk of bacterial and viral infection. However today, with scheduled prenatal bacteriological and serological testing in all cases, safe AMs can easily be obtained for use [9, 10]. We provide an update on AM therapy, its mechanisms of action, and potential advantages in healing of

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the resistant nonhealing venous ulcer, by means of a literature review and the authors' own preliminary study.

Materials and Methods

This study was conducted through a 4-year time frame (January 2006–January 2010) in 40 patients (12 males and 28 females) (Table 1) with “the resistant nonhealing venous leg ulcer,” falling within the inclusion criteria. In this study, the parameter points evaluated for AM included application, tolerance, adherence on the ulcer surface, and effectiveness of AM as a biological dressing. The parameter points evaluated for the ulcer included pain scale, bacterial colonization, epithelization in the ulcer, reduction in the baseline ulcer area, healing percentage, and recurrence. Also, the study incorporated the change in the health feeling dimension and the patient's quality of life.

Inclusion Criteria

- Patients aged between 40 and 50 years, with presence of resistant nonhealing chronic venous leg ulcer of size 3 cm or more, for more than 3-month duration, despite surgery for varicose veins and/or repeated SSG
- Absence of significant arterial insufficiency, neuropathy, or malignancy
- Absence of specific wound infection by *Streptococcus pyogenes*
- Well-controlled systemic diseases, if any, such as diabetes, hyperlipidemia, or hypertension
- No healing despite Bisgard's management or conventional medical treatment
- Patient unwilling for further surgical intervention

Table 1 Demographic table

Character	Category	Number of patients
Age	40–45 years	18
	45–50 years	22
Sex	Male	9
	Female	31
Site of ulcer	Leg	6
	Ankle and foot	34
Duration of ulcer	3–6 months	8
	>6 months	32
Size of ulcer	3–5 cm	14
	>5 cm	26
Ulcer number	Single	29
	Multiple	11

Exclusion Criteria

- Patients not satisfying the inclusion criteria

Preparation of the Amniotic Membrane and Amnion

The AM is made up of two membranes: the inner amnion and the outer chorion. The chorionic side of the membrane is rough and mucinous. The amnion is a thin, smooth, shining, elastic but tough, transparent membrane that can easily be separated from the chorion.

AM was obtained with sterile precaution from placentae, harvested during cesarean section, on patients tested negative for the following tests: human immunodeficiency viruses 1 and 2, hepatitis C virus, syphilis, venereal disease research laboratory–rapid plasma reagin, *Treponema pallidum* hemagglutination test, hepatitis B surface antigen, and hepatitis B core antibody antenatally. Amnion was separated from the chorion by blunt dissection and then cleaned by rinsing in sterile saline, to remove traces of blood. The AM was put for 1 h in a collection container with 500 mL sterile saline and heparin, after adding 80 mg of gentamicin, before application onto the ulcer.

Preparation of the Patient Before AM Transfer

With the approval of an ethics committee, the patient, who was within the inclusion criteria of the study, was explained that an option of inducing a healing with AM transfer, in the otherwise resistant nonhealing venous ulcer, could be attempted. A written informed consent was obtained from the patients.

Preparation of the Wound Before AM Transfer

A swab was taken from the ulcer surface. An oral antibiotic was started. During the 3 days of waiting for culture sensitivity results, removal of actual eschars, frankly nonviable tissue, and exudative component was done. Superoxidized solution was sprayed on the wound surface.

Fig. 1 Application of amniotic membrane (AM)

Table 2 Parameters assessed while patient in hospital and the results obtained

SI no.	Parameter	Observation	Number	Percentage
1	Procedure acceptability	Very keen	40	100
2	Availability of AM and price	Sufficient quantity and free	40	100
3	Harvesting character of amnion	Very easy	40	100
4	Application character of AM	Very easy	40	100
5	Pain scale: pain disappearance	(a) Total	12	30
		(b) 75–100 %	17	42.5
		(c) 50–75 %	6	15
		(d) 25–50 %	4	10
		(e) <25 %	1	2.5
6	AM adherence to the wound bed: assessment on first opening after 48 h	(a) Total	14	35
		(b) 75–100 %	11	27.5
		(c) 50–75 %	9	22.5
		(d) 25–50 %	2	5
		(e) <25 %	4	10
7	Bacterial colonization based on C&S: assessment on day 5	Decreased	38	95
		No change	2	5

Application of the AM

No local or general anesthesia was required. Observing aseptic measures, AM was cut to dimensions and applied on the wound, with the glistening side of amnion in contact with the ulcer. A frill of amnion was used to overlap the margins of the ulcer. Care was taken to ensure that no air bubbles were trapped between membrane and wound, by smoothening out air or fluid blebs (Fig. 1). After total contact of AM to the ulcer

bed was ascertained, the wound was covered by bandage and strict bed rest was followed for 6 h with limb elevation.

Follow-up After AM Grafting

The oral antibiotic that commenced pretransfer or modified based on culture report was continued. The first wound inspection was carried out after 48 h, and adherence of the membrane to the floor of the wound was ascertained (Table 2).

Table 3 Parameters assessed while patient on follow-up in OPD and results obtained

SI no.	Parameter	Observation	Number	Percentage
1	Epithelization tendency in the wound: assessment on day 10	(a) >75 %	17	42.5
		(b) >50 %	19	47.5
		(c) >25 %	3	7.5
		(d) <25 %	1	2.5
2	Reduction in the baseline ulcer area and healing during a 3-month follow-up period	(a) >75 %	19	47.5
		(b) >50 %	13	32.5
		(c) >25 %	6	15
		(d) <25 %	2	5
3	Hypertrophic scar or keloid during the healing in a 1-year follow-up period	Nil	40	100
4	Ulcer recurrence in 1–3 years of follow-up period (Fig. 4)	No recurrence	13	32.5
		<10 % previous size recurred	8	20
		<25 % previous size recurred	11	27.5
		25–50 % previous size recurred	5	12.5
5	Patient satisfaction, improvement in health feeling, and quality of life in 1–3 years of follow-up period	>50 % previous size recurred	3	7.5
		Very good/very happy	32	80
		Good/happy	5	12.5
6	Adverse effects	Unhappy	3	7.5
		None	40	100

Any membrane bleb that was identified was punctured. AM surface cleaned by sterile saline and nonadherent portion of the membrane, if any, including the frill was trimmed. Second bacterial examination swab was taken and wound was left open, after spraying the wound surface with superoxidized solution. On days 3 to 5, similar dressings were carried out. Crusting became obvious in almost all ulcers where the AM became adherent. Patients were discharged on the fifth day, with caution to avoid any pressure or trauma on the wound and to continue with superoxide solution spray, after saline soaks two times a day. Continuation of daily routine was encouraged and limb elevation, for at least 30 min once in every 4 h, during the next 1 week.

Patients were called in for follow-up assessments to the surgery OPD after 10, 30, and 90 days (Table 3) (Fig. 2). Long-term follow-up to 1 year was done in all cases (Fig. 3). Three-year follow-up was recommended to all and was possible in 38 patients.

Results

1. One hundred percent patient acceptability and availability of amnion in sufficient quantity.
2. One hundred percent easy harvesting, application, and handling character of amnion.
3. Greater than 50 % analgesic effect noticed in 87.5 % of patients within the first 48 h.
4. Greater than 50 % AM adherence to ulcer bed in about 85 % of patients.
5. Greater than 50 % new granulation tissue precipitated in AM nonadherent areas in 85 % of patients.
6. Greater than 90 % wound infection and bacterial colonization decreased by day 5.
7. Greater than 50 % epithelization tendency in the wound occurred in 90 % of patients by day 10.
8. Ninety-five percent of patients had a sloping wound edge by day 10.
9. Greater than 50 % reduction in baseline ulcer area and re-epithelization in 80 % of patients by day 90.

Fig. 2 After 90 days

Fig. 3 After 1 year

10. No ulcer recurrences in greater than 30 % of patients in 1–3 years of follow-up (Fig. 4).
11. Only <25 % ulcer size recurrence in an additional 45 % of patients in 1–3 years of follow-up.
12. Greater than 90 % patient satisfaction and improvement in quality of life in 1–3 years of follow-up.
13. Hypertrophic scar/keloid complication was nil during 1–3 years of follow-up.
14. No patient underwent any further surgery for ulcer in 1–3 years of follow-up.

Additional Observations Made when AM is Used as a Biological Dressing

1. Low-cost biological dressing with histology similar to skin.
2. Can be applied as an ambulatory treatment without immobilization.
3. Low immunogenicity and negligible allergic/rejection reactions.
4. A one-time dressing only, since wound kept open after 48 h.
5. Bacteriostatic function in the wound, which enhanced healing.
6. Patient acceptability since neat, dry, and odorless.
7. Well-recognized quality reduction in pain.
8. Reduced treatment costs, shorter hospital stays, and medications.

Fig. 4 Ulcer recurrence in 1–3 years of follow-up period

9. Many of AM properties complement each other to promote healing.

Discussion

Chronic ulcers have pathogenetically what is termed *necrotic burden*, consisting of nonviable tissue and exudates. Typical surgical dressings cannot replace missing tissue. Conventional autograft obtained from healthy skin areas is considered the optimum wound cover, in that its viability yields immediate take (incorporation into the wound bed) and resistance to wound infection. However, repeated SSG failures, in setting of resistant nonhealing venous ulcers, are not uncommon and will warrant repeated harvests. With repeated surgical procedures failing to obtain a healed ulcer and also with infections, persistent discharging wounds, constant pain, contour defects, scarring, etc., many a patient turn hostile. Sometimes later, ironically, the main wound and the donor wound can end up as nonhealing ulcers, leading to a desperate health feeling in the patient.

Developed wound dressings are only temporary dressings due to their synthetic or chemical components, limited persistence on the wound surface, and foreign-body character. Current avenues of research are based on the development of angiogenesis and the use of growth factors to promote healing. Ideally, biological skin should be the dressing material. In 1980, Bennett, Matthews, and Faulk reported the use of fresh AM in the management of leg ulcers as a preparation for skin grafting. Clinical trials and animal experiments have documented that of the biological dressings, the AM is superior to both allograft and xenograft skin [7, 11].

Mermet et al., in a prospective pilot study, evaluated the safety, feasibility, and the effects on healing of AM graft in 15 patients with chronic venous leg ulcers. AM transplantation seems to function as a safe substrate, promoting proper epithelization while suppressing excessive fibrosis. Further advantages of biotherapy with AM are its easy and low-cost production and that it can be applied as an ambulatory treatment without immobilization [12]. Embryologically, the membrane is derived from fetal ectoderm and hence can be considered analogous to fetal skin allograft. It has no nerves, blood vessels, or lymphatics. Quinby et al. emphasized that the immunogenicity of AM is low and rejection is rare [6]. Histological analysis and histopathology biopsy studies on ulcers after treatment with AM have revealed an increase in granulation tissue, connective tissue, formation of basement membrane, and vascular development [7, 8]. AM also possesses antifibrotic, anti-inflammatory, angiogenic, bacteriostatic, and thrombolytic properties. It is also documented that the thick-walled vessels had a reversion toward normal vasculature characterized by vessel wall thinning and widening of lumen induced by AM transfers [13]. Bailo et al.

summarized that their findings suggest that amnion and chorion cells may represent an advantageous source of progenitor cells with potential applications in a variety of cell therapy and transplantation procedures [14].

The contents of the AM include factors such as neuropilin-2, nerve growth factor receptor, semaphoring-F19, allantoin, endothelins 2 and 3, erythropoietin, factor VIII, Tie-2 angiotensin receptor, ephrin receptors, glycosaminoglycan, vascular endothelial growth factor VEGF-B, hyaluronic acid, chondroitin sulfate, dermatan sulfate, heparin sulfates, and a host of others. The ability of AM to express unique properties, combat infection, and sterilize a wound has been documented in many clinical and laboratory experiments [15–17].

Toda et al. analyses showed that amnion-derived cells have the potential to differentiate into all three germ layers both in vitro and in vivo. Their ability to integrate into the tissue and respond to the degenerated environment suggests that amnion-derived cells retain the plasticity to respond to these potential signals from the nearest environment. These data suggest that amnion-derived cells are a useful cell source for developing therapeutic strategies such as cell transplantation, gene therapy, and artificial organs to treat various disorders [18].

In the light of this discussion, it is beyond doubt that AM is a superior biological dressing. The results of our study confirm the superior capability of AM in the treatment of resistant nonhealing venous ulcers.

Conclusion

The preliminary results of analgesic effect, new granulation tissue precipitation, increased epithelization tendency, significant reduction in wound infection, definitive ulcer healing, no need for any further surgery for the ulcer, minimized chances of ulcer recurrence, significant patient satisfaction, and improvement in the patient's quality of life are encouraging.

In our study, the “use of amnion transfer in resistant nonhealing venous leg ulcers” is designed as a proof-of-concept study. The results of the healing obtained are encouraging. AM is inexpensive and readily available in developing countries, such as India. It should be used in the treatment of the resistant nonhealing venous ulcers.

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