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Maximizing the safety of glycerol preserved human amniotic membrane as a biological dressing

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ABSTRACT

Introduction: Preservation of human amniotic membrane (HAM) in glycerol 85% has been used clinically but the use of glycerol 98% can give the maximum virucidal activity and increases the safety of HAM.

Objective: To determine the degree of clinical efficacy of HAM preserved in glycerol 98% as a biological dressing in management of donor site of split thickness skin graft (STSG).

Patients and methods: 40 subjects were enrolled in this randomized, controlled study conducted in Al-Azhar University Hospitals from August 2013 to June 2014. We compared HAM preserved in glycerol 98% to vaseline gauze. Patients were randomly allocated to STSG donor site dressing with one of these materials. Outcome measures included pain scores at postoperative days 2, 6 and 10, time to re-epithelialization, and incidence of infection.

Results: Both groups were homogenous regarding age, gender, cause of burn and size. The HAM group showed significantly less pain on postoperative days 2 and 6 (4 and 2.7 vs. 5.6 and 4.2 respectively with p value <0.05). Shorter time to re-epithelialization was also found in the HAM group (11.7 vs. 15.4 with p value <0.05). No significant difference was found between both groups in the incidence of infection.

Conclusion: HAM preserved in glycerol 98% is clinically effective as a biological dressing. The higher glycerol concentration increases the safety of HAM with retaining the clinical effect at the same time.

1. Introduction

The use of human amniotic membrane (HAM) in burn and wound management was reported in the literature approximately hundred years ago [1–3]. The object of these early attempts was to achieve durable wound coverage and, although the body ultimately rejected the membrane, lack of infections and alleviation of pain were noted [4].

In 1952, Duglas reported the use of amniotic membranes to temporarily cover burn wounds [5]. In the following decades, the advantages of amnion as a temporary dressing became more evident [6–10].

Since the early 1990s, there has been an increasing body of literature addressing the use of amnion in chronic wounds

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and burns. Moreover, a review by Kesting et al. found 31 articles dealing with the use of amnion in burns published in the main international burn journals in the period 1987–2007 [11].

The proven properties of HAM as a biological temporary dressing include promotion of epithelialization and wound healing due its content of growth factors [12,13] along with analgesic effects [14–16]. Dressing of burns with HAM limits fluid and protein loss which reflects on general patient's outcome [17,18].

When compared with many other dressing materials, HAM had significantly stronger effect in suppression of bacterial proliferation [18–20]. Furthermore, the antigenicity of HAM is very low and was found of no sequence [21]. HAM can also help to reduce scar formation. In experimental study on merino lambs, Fraser et al. found that HAM resulted in reduced scar tissue as assessed histopathologically [22].

There are multiple methods of HAM preservation including; cryopreservation [23], glycerol preservation [24] and freeze–drying (lyophilization) and gamma-irradiation [18,25]. Of these methods, glycerol preservation has the advantage of simplicity and low cost. Glycerol also has antibacterial and antiviral activity giving it additional advantage [26]. The efficacy of antibacterial and antiviral properties of glycerol is directly proportionate to the concentration of glycerol and the initial storage temperature [27,28].

We conducted this study to determine the degree of clinical efficacy of HAM preserved in glycerol 98% as a biological dressing. We compared its effect as to that of the chlorhexidine impregnated vaseline gauze in management of donor site of split thickness skin graft (STSG). The parameters used were rapidity of healing, degree of pain and incidence of infection.

2. Patients and methods

2.1. *Preparation of HAM*

Amniotic membranes were harvested only from placentas delivered by caesarian sections to ensure sterile harvesting conditions. An informed consent is provided by all donors and a blood sample is simultaneously taken so that the donor is screened for human immuno-deficiency virus, hepatitis B and hepatitis C viruses.

The membrane was separated from the placenta, washed thoroughly from blood and then kept in povidone iodine 10% for half an hour. The membrane was then washed again with saline and transferred to a sterile sealed container filled with glycerol 98%. The containers were labeled with donation data including the donor's name, medical recording number, date of harvest and the name of harvesting resident. The HAM containers were kept in room temperature for 1 month to gain the maximum antibacterial and antiviral effects of glycerol. During this month the membranes from donors with positive serology for viral diseases were excluded.

After this period, the HAM containers were stored in refrigerator and were ready for use. At the time of dressing, the glycerolized HAM is taken from its container, washed thoroughly with saline. It is then kept in saline for 20 minutes before use to get rid from any glycerol remnants (Fig. 1).

2.2. *Study design*

Forty subjects were enrolled in this randomized, controlled clinical trial conducted in Al-Azhar University Hospitals in the period from August 2013 to June 2014. We included patients indicated for split thickness skin grafting due to thermal burns or trauma. We excluded cases with raw areas due to other causes as postinflammatory raw area.

We limited the study to patients between ages of 10 and 50 years. We excluded those who had other comorbidities affecting wound healing as well as patients with burns of more than 40% of total body surface area (TBSA) or associated inhalation injuries.

Subjects were randomly allocated to one of the two groups according to the method of donor site dressing. Each group is formed of 20 patients.

The first group was called the HAM group. Dressing of patients in this group was achieved by covering the donor site with the HAM as a primary dressing. The membrane was then covered with paraffin gauze wrapped with cotton gauze dressing and held by bandage.

The second group of patients is called the control group. Dressing of patients in this group was achieved by covering the donor site with chlorhexidine-impregnated paraffin gauze as a primary dressing then covered with cotton gauze dressing and held by bandage.

For both groups dressing change was done after 48 hours. We opened the external dressing and then the primary dressing was checked. If adherent, the primary dressing was left undisturbed and if not, it was changed. Afterwards, wound care was done day by day in the same way.

Outcome measures included pain scores at postoperative days 2, 6 and 10, time to re-epithelialization, and incidence of infection.

Pain score was measured by asking the patient to grade the pain on a scale from 1 to 10. This pain scoring system is valid for children above 9 years old as well as for adults [29].

Donor site was considered infected when there are local signs of infections as sever intolerable pain, surrounding erythema, induration, purulent discharge or bad odor.

Results of the study were assessed for significance using the independent *t*-test and the Chi-square test. The independent *t*-test was used to compare continuous variables among groups of patients. The Chi-square test was used to compare nominal variables. All p values were considered significant if less than 0.05.

3. Results

Both groups were homogenous regarding age (27 ± 13 in HAM group and 23 ± 10 in control group) with p value >0.05 using independent *t*-test. Gender distribution and cause of burn had no statistically significant difference between the groups with p value >0.05 using Chi-square test. The difference in the size of the open area, presented as percent of total body surface area, was not statistically significant too ($7.4 \pm 2.6\%$ TBSA in HAM group and $6.1 \pm 2.6\%$ TBSA in control group) with p value >0.05 using independent *t*-test.