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HUMAN AMNIOTIC MEMBRANE: A BIOLOGICAL COST EFFECTIVE DRESSING IN CHRONIC WOUNDS.

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ABSTRACT… Objectives: The use of human amniotic membrane is essential new concept in wound healing which functions as a biodegradable scaffold on wound surface, as it is a rich hub of stem cells which play an important role in wound healing. **Study Design:** Randomized Control Trial. **Setting:** Department of Surgery THQ Hospital Gojra. **Period:** 1st January 2019 to 30 September 2019. **Material & Methods:** Experimental study using clinical trial. A case series of 50 patient cases were picked from surgical OPD. Who fall in criteria of chronic non-healing wound with at least three months duration comprising of diabetic, venous ulcers and traumatic non healing wound and neuropathic ulcers. All located on lower limbs. **Results:** All 50 patient were treated with standard protocol by applying freshly prepared amniotic membrane out of which 4 chronic wounds more than 4 year duration were not healed and 2 cases escaped from the study. HAM dressing was changed after every 7 days and its effect were studied by seeing measuring the reduction in wound size and improvement in pain, swelling and mental stress. Success rate was found about 90% with complete healing. **Conclusion:** There is a dire need in developing countries to promote the use of HAM, in chronic non healing wounds which is a biological membrane, readily available (free if fresh) with simple sterilization techniques, easy storage and easy application with ultimate goal in achieving speedy cost effective wound healing.

Key words: HAM (Human Amniotic Membrane), AFSC, AF

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INTRODUCTION

The search for an ideal wound cover as a substitute for the patient's skin began more than a century ago and continues today.[1] There is evidence to graft pieces of amniotic sac on to granulating wounds[2] and treatment of burned patient with pieces of amniotic membrane. The main objective of study is to promote use of human amniotic membrane to achieve cheaper dressing which has added advantages of providing bacterial barrier[3] and decreased infection rate[4] along with increased angiogenesis and reduction in pain.[5] In essence objective is to restore tissue integrity, and function, while avoiding infection and morbidity, and minimizing scarring. The objective in wound healing is to convert chronic wound into acute wound achieved by creating balance between cell degradation and synthesis, eradicating fibrosis promoting granulation and completed at epithelialization.[6] Regenerative medicine is based on the concept to regenerate the lost tissue by giving stem cell or by stimulating endogenous stem cells.

Amniotic membrane attracts and recruits stem cells to the area of chronic wound. Chronic wounds are a constant source of mental stress and burdensome in terms of effects on the patient quality of life and financial stress. There is need for effective affordable and durable technique for rapid wound closure as a priority. The concept of HAM is as of a scaffold, "which is supporting matrix upon which cells and tissue grow" this biological micro scaffold is made of granulation tissue matrix includes the hylauronidase and growth factors naturally present in amniotic fluid and amniotic tissue which provide in vivo wound covering. Several experimental studies have been done by using amniotic membrane as scaffold. HAM is a biodegradable scaffold

and has characteristic for nerve regeneration.[7] Ulcers on the leg are serious and have significant complication in diabetic patients leading to increase in overall cost of a health care.[8]

Actual surgery is wound care. No one can ignore the importance of conventional wound care. Non-healing is labelled as less than 20% decrease in wound area after standard wound care within 2 weeks. Chronic wounds is labelled if a wound does not heal after proper wound care in 3 months.[9] The main objective in wound management of an open wound is to obtain a clean and closed wound in shortest time. HAM is a non-vascular tissue. It is made of epithelial cells, collagen, which are essential in wound healing. It has additional property of being anti-fibrosis, anti-scaring and mechanical property to play as scaffold. The mechanism of healing by HAM can be understood by subject of foetal healing. Wounds happening on the foetus during development in epidermis/dermal tissues gain full tensile strength without visible scars.[10] The regenerating capacity in humans is ultimately replaced by fibrosis and in adults wound is repaired and replaced by fibrous collagen tissues, the healed tissues gains 70% of its original tissue strength.[11] The regenerative capacity in foetal development and adults cannot be explained. It is predicated upon the degree to which body's inflammatory responses are activated. Foetal wounds allows very low inflammatory response to wounds in late gestation period and almost no response in early gestation period.[10] The application of HAM in adults slows the inflammatory response as in foetal tissues. This anti-inflammatory properties is enhanced by anti-bacterial, non-immunogenic characteristics, also it is saturated with a lot of main growth factors[12] chronic wounds are constant source of mental stress and burdensome, in terms of effects the patient's financial stress and quality of life.

MATERIAL & METHODS

Study will be done on 30 cases with open diabetic wounds and chronic non-healing wounds.

Fresh amniotic membranes will be obtained from obstetric operation theatre with following criteria;

- Donor must be seronegative for HBs antigens and syphilis.
- Only membranes of cesarean sections will be used.
- Meconium stained amniotic membranes will be rejected.

The method for measuring the wound closure.
% of wound closure = wound area on day 0 – Wound area on day n (100)/wound area on day 0
Where n = no of days 4^{rth}, 8^{th}, 12^{th}, 16^{th}, 20^{th},

Method for preparation of amniotic membrane.
Fresh membrane obtained from placenta at the time of delivery after caesarian section.

Sterile jar containing 1 liter of normal saline will be taken. With aseptic technique membrane will be removed from placenta and spread on the back of kidney tray and will be rinsed with normal saline 4*4 moist gauze square and membrane will be washed four times with normal saline. All blood, mucous and debris will be removed and after that membrane will be rinsed with a solution of 200000 units of crystalline Penicillin/100ml of normal saline and placed in petri dish containing the same solution. Petri dish will be sealed and stored in refrigerator at 4 degree centigrade. Robson and krizek showed that membrane remain s sterile after six weeks. In our study, we used the prepared amniotic membrane for two weeks then again prepared fresh membrane.[13] In 1965 Dino et al proved that amniotic membrane can be sterilized and stored at 4 degree centigrade and applied on skin donor sites.[14]

RESULTS

In our study 50 cases were picked from surgical OPD which fall into definition of chronic wound. Freshly prepared HAM was applied in operation theatre in the form of overlay or patch "by preparing the wound" means the chronic wounds were brought in acute phase by serial wound wash and debridement along with free of infection by giving broad spectrum antibiotics. The HAM was applied on well granulated prepared wounds and application was done with chorionic site towards wound surface. The HAM sticked to wound surface once applied by removing any bubble beneath the HAM. Cotton gauze was applied

by soaking in normal saline and crepe bandage applied.

The dressing changed after one week. 90% of the wounds healed after 2nd dressing. 8% healed after 4th dressing. 2% of the wounds showed slow healing response with exudation and rejection of the HAM. Usually green exudation was found probably due to pseudomonas or rejection of the HAM.

Number of HAM Dressing	Percentage of Healed Wounds
2	90%
3	8%
12	2%

Table-I. Percentage of healed wounds.

We selected 50 well granulating lower limb chronic wounds and applied HAM and got following results.

Out of 50, 40 chronic wounds healed completely. Out of 50, 2 cases escaped from study after single dressing. Out of 50, 4 cases chronic wounds more than 4 year duration were not healed.

Wounds Healed or not	No of Wounds Healed or not
Completely healed	44
Escaped cases	2
Not healed at all	4
total	50

Table-II. Total number of patients healed or not.

A try was made by us by applying the HAM after removing the chorionic layer after which no rejection was found in most cases but two cases rejected this HAM also.

Preparation of HAM with glutaraldehyde increases the resistance of membrane to breakdown we proved in our study.

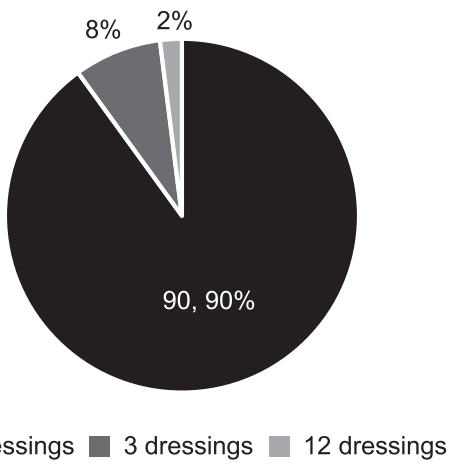

Figure-1. Percentage of healed wounds due to HAM dressing.

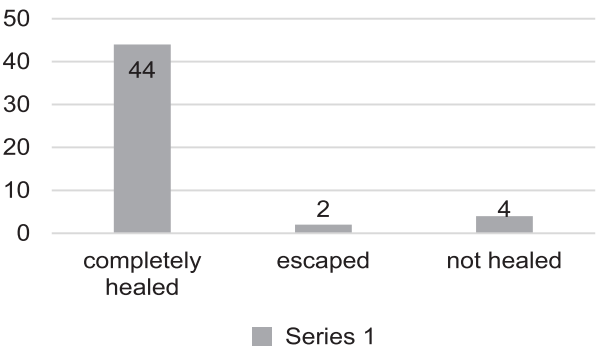

Figure-2. No of chronic wounds healed or not.

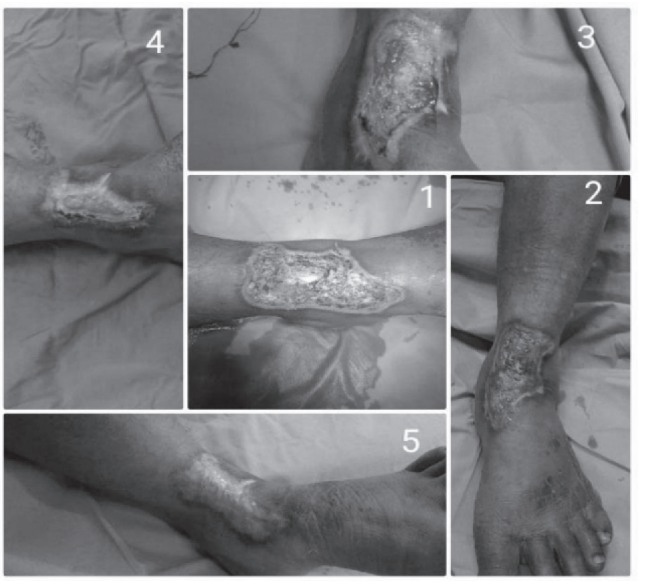

Figure-3. Chronic wound left lower leg for 02 years showing HAM response (pictures 1 to 5 showing progress in healing process from open wound to complete healing).

Figure-4: Showing HAM application response in diabetic foot ulcer (pictures 1 to 4 showing diabetic ulcer till complete healing of ulcer).

Figure-5. Showing failure of HAM in a chronic wound of 4 year duration (pictures showing 1 to 4 open wound showing HAM rejection after its application rejecting after pseudomonas infection).

DISCUSSION

Amniotic stem cells were 1st discovered in 2007.[15] Amniotic stem cells are different in cellular concentration and location, so they are new cells to be studied and extensive research is required for human medical purposes.[16] Stem cells are categorized into two type's adult stem cells and embryonic. Embryonic stem cells are derived from inner mass of blastocyst. They have potential of renewal and plasticity, they have been proposed as potential treatment for many human diseases.

Up till now embryonic stem cells has not been used for treatment because of controversies from means of obtaining them. Adult stem cells are located in almost all organs in human tissues other than gonads.[15] They have limited differentiation and potential and proliferation capacity. Adult stem cells represent possible resources for research and medicine. The adult stem cells are easily obtained and there is no controversy in obtaining them.[16] There are social and ethical constraints to use embryonic stem cells as treatment. The other challenge is to grow embryonic stem cells in vitro and maintain them. There is a need for alternative source of stem cells which provide same potential with easy availability.[17] Amniotic fluid cells are being cultured for prenatal genetic diagnoses stem cell population has been discovered in amniotic fluid and their role for cellular therapy has been proved. Stem cells in amniotic fluid represent the origin from all three germ layers. Amniotic fluid is safely collected during elective C-section without contamination or injury to the baby, in this way we get good volume and huge number of stem cells. These cells can be further grown and stored for future use. So they can be safely stored in stem cell bank.[18] The use of pregnancy specific biological substance as cell therapy is popular in regenerative medicine for treatment of non-healing ulcers whether traumatic or due to diabetes. They are easily collected by gynecologists or surgeon. The human amniotic membrane has been successfully used in burns and chronic non healing ulcers.[19]

These amniotic membrane is rich in stem cells cytokine growth factors and has anti-inflammatory properties which keeps in healing process through repair and regeneration of lost tissue. On May 2005 US FDA regulated the manufacture and use of HAM under 21 code of federal regulation (CFR). Part 1271 and section 861 of the public health service act. Although very little comparative studies about HAM are available, HAM is recognized as natural biological dressing with no risk of rejection.[20] The concept of using freshly prepared HAM was successfully generated by

Bhattacharya et al 1999 like embryonic stem cells amniocyte have high power of proliferation no report is cited for teratoma or tumor formation.[21]

USA is leading the world in use of HAM for cutaneous wound healing on searching state websites three studies available all in Europe with zero matches. One study available in India. European and Indian are not using commercial product but locally research material as in this study.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

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