Assessment of outcome of using amniotic membrane enriched with stem cells in scar formation and wound healing in patients with burn wounds

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ABSTRACT

Background: In recent decades, various techniques include flaps and grafts have been deployed to heal deep burn wounds. All efforts tend to use a method which will cause faster epithelization and fewer scars. Amniotic membrane and stem-cells are shown to have unique properties in epithelization and wound healing for depth wounds. Objective: There are many studies in which the prevalence of using fresh amniotic membrane has been discussed as a biological dressing. In this study, we used frozen amniotic membrane enriched with stem-cells to evaluate the outcomes of this biological combination on epithelization rate, wound healing and scar formation in third-degree burn wounds. Method: In this study, we evaluated outcomes of using amniotic membrane enriched with stem-cells in the repair process of third-degree burn for 180 days after the reconstructive surgery. We divided the burn area into two section of control (skin mesh graft only) and case (skin mesh graft with the dressing of amniotic membrane enriched with stem-cells). All patients were followed-up for 180-days to analyze the risk of infection, scar formation, and pain score during this period. Results: Our findings showed that there is a significant difference between pain score in case and control group only during the first 7-days of follow-up. The mean Vancouver scar scale score in case group is less than it in control group and has a statistically significant relationship with it. Prevalence of infection in case and control group showed no statistically significant difference and yet, there was a significant difference between satisfaction in both groups. Conclusion: Using frozen amnion, in addition, to maintain the properties of fresh amnion, may reduce the risk of infection transfer. Also because of the unique properties of stem cells in reepithelization and wound healing, using amniotic membrane enriched with stem-cells may be an ideal dressing to treat deep wounds.

Keywords: Graft, Burn, Wound healing, Amniotic membrane, Stem cell.

INTRODUCTION

In recent decades, various methods have been deployed in order to heal the deep wounds of burn. At the same time, it has always tried to use a technique that will cause faster epithelization and fewer scar. Burn wound healing is a complex process that has led to doing many studies in this field. In burn patients, pain, disorders, and risk of infection in the burn area, have undesirable physiological effects. There are different techniques to treat deep burn wounds includes using skin flaps and grafts. All efforts tend to treat burn patients by deploying innovative methods with minimal complications both on donor and burn area.

Since 1910, Amniotic Membrane (AM) has been used as an effective dressing for wound healing. Wound management by protecting proteins, electrolytes, and liquids, accelerating epithelization rate and also decreasing the risk of infection, are some benefits of using AM. In the other hands, failure in wound healing may inflict huge costs on patients; in a study on 974 patients with burn wound, the cost of dressing change is ten times more costly than while using amniotic membrane. Furthermore, the biological features of AM are caused them to be used for wound healing in the cornea, orthopedic, dental, ENT surgeries and burn wound management.

Amniotic membrane showed desirable results in the management of bedsores. It is also used as a safe and effective allograft for chronic non-healing wound. For split-thickness skin-graft donor sites, human amniotic membrane caused improved esthetic results and inhibitory effects on the rate of wound closure. AMs also have great potential for improving burn wound care.

Studies show the effectiveness of amniotic membrane enriched with stem cells for the treatment of the thermal burn, lung injury, neuronal regenerative therapy, wound healing and skin regeneration.

In this study, we evaluated the effects of using amniotic membrane enriched with stem cells on
patients with third-degree chemical or thermal burn. The results may help both groups of surgeons and patients to manage burn injuries.

MATERIAL AND METHODS

We studied 30 patients with third-degree chemical or thermal burn who were referred to Zare burn center of Sari and AJA hospital of Tehran during 2017-2018.

Third-degree chemical or thermal burn in an anatomical area or in two mirror organs except for hands and head and neck with the minimum size of 10*3cm or 5*3cm, respectively, were our inclusion criteria.

Patients with severe vascular trauma and amputation indication, burn in areas of hands and head and neck, the inability to follow-up and patient dissatisfaction with the use of amniotic membrane were our exclusion criteria. Steps of the procedure were as follows.

I. Preparation of Amniotic membrane enriched with stem cell

Amniotic membrane is the inner layer of the placenta with the thickness of 0.02 to 0.05mm made up of three sub layers. The epithelial cells layer include fibroblast growth factor (FGF), platelet derived growth factor (PDGF), epidermal growth factor (EGF) and vascular endothelial growth factor (VEGF). The thick basement membrane layer includes various types of collagen V, VI, VIII, II, III, IV and bioactive factors secreted from an extracellular matrix such as collagen, proteoglycan lamina, fibronectin, and glycosaminoglycan. The stroma layer (vascular mesenchymal tissue) includes transforming growth factor (TGF) beta, anti-inflammatory factors, and stem cells.

Stem cells are on the amniotic membrane of mesenchymal cells. These cells have the ability to be differentiated between the cells of the graft site. The mesenchymal cells of the amniotic membrane have the ability to be differentiated into keratinocyte cells (epidermis of the skin), adipogenesis, chondrogenesis, osteogenesis, angiogenesis, and myogenesis.

To prepare the amniotic membrane dressing, a placenta of healthy women who underwent C-section delivery is used. All steps of processing the placenta were done in the laminar hood in the clear room. After processing, the amnion was stored in special containers and freezing temperature of -80 degree C. This method is vital for preserving the integrity of the tissue and its biological effects.

The quality control system includes a comprehensive and completed structure of screening donors and conducting accurate tests for viral and serological transmission of infectious diseases that are in accordance with the global standards of EU-Directive 23/2004. This tests include HIV 1, 2 A / HCVAb / HBCAb / HBSAb / HBsAg / HTLV 1, 2 Ab / RPR / Endotoxin / MYCOPLASMA.

II. Steps of The Procedure

The approval of MAZUMS and AJAUMS ethics committee was received before starting the study with ethics codes of IR.AJAUMS.REC.1396.21 and IR.MZUMS.REC.96.D100, respectively. Thirty patients among whose total body surface area (TBSA) was less than 30%, with third-degree chemical or thermal burn in an anatomical area or in two mirror organs except for hands and head and neck with the minimum size of 10*3cm or 5*3cm, respectively, were candidates for this study.

Physical examination was performed for all patients and their medical history was recorded. The burn degree was diagnosed clinically. Initial treatments include cleaning the burn area with a sterile saline solution, debriding tissues that have necrosis and removing foreign particles from the burn area, were performed. Information, pain score and the Vancouver Scar Scale Score form were allocated to each patient.

Split thickness skin graft was harvested from the donor site. Depends on some parameters such as age, sex and physical conditions of each patient, the donor site is a difference. First of all, we divided the burn area into two sections. Skin mesh graft was placed on both sections to cover the burn area. Then the first section with a minimum size of 5*3cm was dressed by amniotic membrane dressing enriched with stem cells and the second one with the same size was dressed by sterile Vaseline gauze. Both of them were fixed by using sterile gauze in order for the graft to be taken. During the hospitalization period all patients were examined for infection and discharge. They were followed-up over time intervals of 7, 14, 30, 90 and 180 days to screen the wound healing and scar forming. Results of comparison were recorded and statistically analyzed by SPSS.

RESULTS

We divided the burn area into two groups of control (the area that was covered by skin mesh graft only) and case (the area that was covered by skin mesh graft and dressed by stem cells derived amniotic membrane). Results are as follows:

Gender: From 30 patients in our study, 23 and 7 of them were male (76.67%) and female (23.33%), respectively (Figure 1(a)).
Comorbidity: In our study, one patient had diabetes and blood hypertension (3.33%), two other patients had only blood hypertension (6.66%) and the remaining 27 patients (90.01%) had no comorbidity. (Figure 1b).

Age: The youngest and the oldest patient and the average age of all 30 patients were 16-, 60- and 33.20-yrs old, respectively. Figure 2(a) shows the histogram of age for all patients.

TBSA: As you can find in Figure 2(b), maximum, minimum and the average TBSA were 1%, 14%, and 9.03%, respectively.

Burn Anatomic Area: In 30 patients in this study, burn was occurred on arm (6.66%), forearm (16.66%), thigh (13.35%), leg (16.66%), back (16.66%), chest (10%), abdomen (3.35%) and foot (6.66%). Also, there were 3 patients (10%) whose burn was placed on minimum two anatomic areas.

Vancouver Scar Scale Score (VSSS): After 180 days after the reconstructive surgery, average VSSS was 5.73 and 3.96 in control and case group, respectively. Demographic data of all patients have been analyzed using SPSS Ver (23). To evaluate VSSS, we used the Kolmogorov-Smirnov test. Then we run the non-parametric Wilcoxon test in order to study the effects of using stem cell derived amniotic membrane (Table 1). Results showed that the mean VSSS in case group is less than it in control group and has a statistically significant relationship with it (P-Value < 0.05).

Pain score: We rated the pain score from 0 to 10. Zero indicates no pain and 10 shows a severe pain.

Table 1 The comparison of VSSS is case and control group

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean</th>
<th>N</th>
<th>Std. Deviation</th>
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<td>VSSS in Case Group</td>
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<tr>
<td>VSSS in Control Group</td>
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<td>2.049</td>
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</table>

Figure 1  Gender (a) and comorbidities (b)

Figure 2  Age (a) and TBSA (b)

Figure 3  Mean pain score during post-op

Figure 4  The process of scar formation on lower legs burn at the time of surgery, 90- and 180-days after the surgery
In Figure 3, we plotted mean pain score changes for all patients during 7, 14, 30, 90 and 180 days after the surgery.

We evaluated pain score in both groups of case and control using Wilcoxon test during follow-up periods (7-, 14-, 30-, 90- and 180-days) (Table 2). Results showed that there is a significant difference between pain score in case and control group only during the first 7-days of follow-up (P-Value < 0.05).

Infection: One patient from the case group (3.33%) and three patients from the control group (10%) were infected in the burn area. All of them were treated as the infection was diagnosed. Prevalence of infection in case and control group showed no statistically significant difference (P-Value > 0.05) (Table 3).

Satisfaction: After 180-days from the surgery, 21 patients in the case group were satisfied with using stem cells derived amniotic membrane on their burn area and 9 patients reported no difference between using or not using stem cells derived amniotic membrane on the burn area. In the control group, Seventeen patients were satisfied by using regular dressing, 4 patients were dissatisfied with using regular dressing and the rest reported no difference.

Table 4 presents the satisfaction of reconstructive surgery results between two groups of case and control. Non-parametric Kendall test showed a significant difference between satisfaction in both groups (P-Value < 0.05).

In Figure 4, you can find the process of burn wound healing in a patient's legs at the time of surgery, 90- and 180-days after the surgery. Case and control group are defined in the figure.

**DISCUSSION**

Burn wound treatment is a difficult medical problem in which various techniques are deploying to achieve the best results. Risk of infection and sepsis, scarring and joint problems is some important complications which must be considered during the management of third-degree burn wounds. This kind of wound required to be covered with a mesh graft in order to prevent skin deformity and improve the function of the injured area.

There are different of dressing which is used to cover the mesh graft placed in the burn area. However, affordability, availability, and effectiveness are some parameters which must be considered during the treatment.

Burn patients suffer from pain in the burn area. This will be more painful while dressing changes. Studies show that post-op pain is significantly associated with wound healing. Wound healing is also associated with infection occurrence. So an ideal approach in such these patients is to decrease the time of wound healing to minimize the risk of infection and post-op pain.

Since 2005, when the Food and Drug Administration approved the use of amnion membrane, there are many studies in where fresh amniotic membrane has been deployed as a dressing.
on the burn area. Nowadays, it has been proven that using frozen amnion in which all serology and virology tests are performed, may minimize the risk of infection transfer while having all properties of fresh amnion.31–36

Amniotic membrane increases keratinocyte migration speed from the wound edge. This property may expedite wound healing.37 Growth factors released by the amniotic membrane and the integrity of the stromal matrix also facilitates reepithelization.38,39

In the other hands, because of various properties of stem cells in an expedition the epithelization process and etc.,33–36 we used amniotic membrane enriched with stem cells in our study. Researchers reported that using amniotic membrane dressing is associated with reduced pain in burn patients.40,41

In 2016, Eskandar Lou and his colleagues released the result of their study on the effects of amniotic membrane on donor site in burn patients.42 In the analysis of pain score, 5 days after the surgery, mean pain score in the case group was 1.4 less than in the control group. In our study, the difference between the mean pain score in the control and case group after 7-days was 0.4 that shows a better effect of stem cells derived amniotic membrane.

In a study by Salehi and his colleagues in 2013, amniotic membrane has been deployed as dressing on donor site of 52 burn patients.43 Two patients experienced an infection under control and no infection was reported in the case group. In another study by Ludwik and colleagues, they found no relations between infection and using amniotic membrane as dressing in the treatment of pediatric partial-thickness facial burn.44 Considering that our study was in a burn on different anatomic areas, we experienced an infection in three patients of control and one patient in the case group.

Mohammadi and his colleagues evaluated the scar forming in 54 patients with TBSA between 4% and 15% during 6-months after the surgery.45 They divided all patients into two groups of the case (whose burn area was covered with the skin graft and amniotic membrane) and control (whose burn area was covered with the skin graft and regular dressing). They reported less hypertrophic scar formation in 64.81% of the cases in the case group. In our study, but the mean scar scale score in case group was significantly less than in the control group (P-Value <0.05).

CONCLUSION

Many studies have been run on using fresh amnion as a dressing of burn wounds. Recent studies show that fresh amnion may increase the risk of virus transferring because of the lack of enough time for performing microbiology and serology tests. Using frozen amnion, in addition, to maintain the properties of fresh amnion may reduce the risk of infection transfer. Also because of the unique properties of stem cells in epithelization and wound healing, using stem-cell-derived amniotic membrane may be ideal to treat depth wounds. Our study showed the faster reduction of pain in patients whose wound was dressed by amniotic membrane enriched with stem cells in the short time. Also, we found faster epithelization in this group and less scar formation during the post-op period.

CONFLICT OF INTEREST

None.

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