HISTOPATHOLOGICAL CHANGES OF AUGMENTATION CYSTOPLASTY WITH ACELLULAR HUMAN AMNIOTIC MEMBRANE IN RABBITS

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ABSTRACT

We aimed to evaluate the histopathological changes in Human Amniotic membrane in respect of transitional metaplasia or infiltration, and smooth muscle creation under the transitional layer, following Augmentation cystoplasty with human amniotic membrane in rabbits. Materials and Methods: This study was conducted on 12 healthy New Zealand white female rabbits aged between 12-15 months with the weight of approximately 3-3.5 Kg. Fresh amniotic membrane grafted at the dorsal wall of rabbits bladder. Then the histopathological changes of the graft assessed every two weeks over three months. Results: After Augmentation cystoplasty with amniotic membrane, reconstruction of transitional epithelium layer of bladder, lamina propria, muscle layer and neovascularization, observed through granulation tissue proliferation and rep epithelialization with mild to severe inflammatory response, wound contraction and necrosis. Conclusion: Amniotic membrane was necrosed without metaplasia and transitional layer was repaired with rep epithelialization, also the smooth muscle was created under transitional layer. Amniotic membrane as a biologic scaffold can form a base membrane for bladder reconstruction.
KEYWORDS: Augmentation cystoplasty, amniotic membrane, repithelialization, scaffold, transitional layer.

INTRODUCTION

Bladder is a cone organ which performs two major functions of urine storage with low pressure to prevent renal damage and the proper voiding of urine. These activities are supervised by neuromuscular and neurological system and in the case of congenital disease, trauma, infection, cancer and radiation problems, its normal functioning undergoes changes that can be manifested as a bladder lowcapacity and high pressure or combination of two. Bladder low capacity and high pressure treatments include conservative medical management, injection, Botox, and ultimately Augmentation cystoplasty. Augmentation cystoplasty is a surgical procedure in which a part or the entire bladder replaced by bowel, stomach or urinary system. Intestinal augmentation cystoplasty accompanies serious complications such as urinary intestinal diversion, electrolyte imbalance, impaired renal tests, intestine histological changes, abnormal absorption of drugs, osteomalacia, infection, stones, nutritional deficiencies and cancer when exposed to urine. Efforts have been made to find an alternative tissue for gastrointestinal augmentation cystoplasty, one of which is Amniotic membrane. Due to Amniotic membrane characteristics such as availability, maintenance for a few months (below 80° c), antimicrobial, inflammatory and low immunogenicity, it has been applied as a biological scaffold and tissue transfer in many surgeries.

MATERIALS AND METHODS

This study aimed to evaluate the histopathological changes in Human Amniotic membrane in respect of transitional metaplasia or infiltration, and smooth muscle creation under the transitional layer, following Augmentation cystoplasty with human amniotic membrane in rabbits, so the amniotic membrane was separated from placenta and washed in normal saline (0.9 %) four times. The normal saline solution with crystal penicillin 20000 units per 100 ml concentration was used for sterilizing the tissue. Then it was maintained in normal saline solution containing penicillin at 4° c in which it could be kept more than a week. This study was conducted on 12 healthy New Zealand white female rabbits aged between 12-15 months with the weights of approximately 3- 3.5 Kg. All twelve rabbits were fasted two hours prior to surgery. After premedication of rabbits by intramuscular injection of acepromazin (1mg/kg) for sedation, the animal abdominal area was shaved, scrubbed, and antiseptic
preparation was performed from sternum to pubic. Indwelling urinary catheter (8F) with aseptic conditions was entered the bladder (the inserted catheter remained in the bladder for 14 days). General anesthesia was carried out by using a mixture of ketamine (70mg/kg), acepromazin maleate (1mg/kg) and xylazine (10mg/kg). Afterward, the animals were positioned on supine and the draping performed. After midline incision of the skin, and entering the peritoneal cavity, four stay sutures with 8/0 silk on the four corners of supposed square (4×4) located to determine the posterior bladder and they were held by assistant surgeon. A longitudinal incision (2cm) at the middle of supposed square was created, then prepared piece of amniotic membrane in the form of square was sutured to the cutting edges by absorbable 8/0 silk with simple single pattern. The free edges of grafts with simple continuous pattern were sutured to free edges of bladder in watertight technique. Then, the leakage of bladder was checked out by methylene blue infusion through Foley catheter which had been fixed in bladder. Finally, the operation was ended with removing the four stay sutures. Foley catheter was fixed at vulve edges by marsupialization. The incision layers of abdomen were sutured by simple continuous pattern (poly glycolic 4/0) at inner and subcutaneous layers. Then the skin was sutured by nylon 3/0 simple single pattern. Intramuscular Antibiotics (gentamicin 2mg/kg over 3 days and cefazolin 20mg/kg over 5 days) before and every 12 hours after the surgery were used to prevent the postoperative infections. To reduce the post-operative pain, tramadol1mg/kg was used every 12 hours over 3 days. 14 days after the surgery, the catheter and skin sutures were removed. After the surgery all the animals were kept under standard management over three months. Two rabbits were underwent the second surgery every 2 weeks. Finally, the rabbits were underwent laparotomy at the site of prior surgery. After releasing the bladder, a piece of graft with the distance of 0.5 cm from the graft edges and bladder original tissue was resected. In order to histopathological examination the collected samples were placed in formalin buffer (10%) then they sent to pathological laboratory for lamella preparation. The samples were stained by standard method and studied histopathological.

**HISTOPATHOLOGICAL RESULTS**

**15 days after the operation:** The presence of inflammatory cells (lymphocytes, granulocytes and eosinophils) and necrotic tissue which was the indicative of repair process was observed at the sites of surgery and the migration of bladder transitional cells under amniotic membrane was begun. The necrosis process was seen in amniotic membrane.
30 days after the operation: Inflammatory process slightly decreased, two edges of wound reached closer and the migration of transitional cells under Amniotic membrane graft observed (repithelialization). Also, the presence of inflammatory cells around the suture was seen. (Fig. 4-5).

45 days after the operation: Inflammatory process decreased. Amniotic membrane necrosis and its remains were observed at the graft. The presence of increasing smooth muscle cells were seen separately in bladder. Granulation tissue (blood vessels, fibroblasts, soft tissue) with lymphocytic aggregation under smooth muscles and eosinophil cells (caused by suture) was observed focally. (Fig. 6-8).

60 days after the operation: The transitional layer regenerated and muscle cells were observed. Focal lymphocytes and previous granulation tissue were existed in the repair sites. Some cysts were seen in granulation tissue with one to more cell layers. (Fig.9).

75 days after the operation: The repair of transitional layer and smooth muscle completely covered the wound in the bladder and the reconstruction was achieved approximately. Some inflammatory cells were observed in submucosa. Epithelium had enough continuation. No suture was seen. (Fig.10).

95 days after the operation: The complete reconstruction of transitional layer and smooth muscles without inflammatory cells were observed. (Fig.11).

Fig.1. Amniotic membrane on the wound and interstitial layer.
Fig. 2. Transitional layer and the necrosis of amniotic membrane.

Fig. 3. The stitch and the inflammatory process.

Fig. 4. The migration of transitional cells under necrosis site.

Fig. 5. The migration of transitional cells under the wound and the inflammatory cells around the stitch.
Fig. 6. Necrosed amniotic membrane

Fig. 7. The Granuloma created by suture under bladder muscles.

Fig. 8. The granuloma, giant cells and eosinophil cells

Fig. 9. Regenerated bladder epithelium, remains of granuloma and bladder cysts.
10. Fig. The bladder epithelium with proper cohesion.

11. Fig. The layers of urothelium, submucosa and smooth muscles in bladder without inflammatory process.

DISCUSSION

Similar researches were conducted on Augmentation cystoplasty in which the repair of ureter with amniotic membrane and the regeneration of urethelium, lamina propria, and blood vessels were concluded.[2] Moreover, proliferation of granulation tissue, and mild to severe inflammatory response were observed.[14] In this study the histopathological changes in Human Amniotic membrane in respect of transitional metaplasia or infiltration, and smooth muscle creation under transitional layer were reviewed. In some Prior studies, lack of necrosis, contraction[7] and degeneration[14] were reported. Whereas, in this study, gradual increasing of inflammatory cells, amniotic membrane necrosis and wound contraction were observed at microscopic study between 15 and 95 days. In some researches, postoperative regeneration of urethelium was observed over 3 months[2,8,14] that corresponds with histopathologic process of this study within 95 days. In this research, the migration of transitional cells under amniotic membrane were increased gradually up to 45 days that the transitional tissue was completed, inflammatory process was reduced and the accession of granulation (blood vessels, fibroblast cells and soft tissues) was being beginning. In a prior
study the presence of smooth muscles under transitional layer was not observed\cite{2}, but it was seen in other studies\cite{7,8} which is similar to the results of this research that repair process was less inflammatory and smooth muscles of fibroblasts were increased gradually after 45 days. In 60 days of repair, the cohesion of transitional tissue with smooth muscle was observed. In 75 postoperative days transitional layer covered the bladder completely and smooth muscle was more proliferated. In 95 days after the graft, no signs of inflammatory cells and repair process were found and the repair of bladder including transitional urethelium, detrusor layer and blood vessels were completed. In a study, the adhesion of bladder and intestine was observed\cite{2} that corresponds with this study that the adhesion of the bladder and uterus was seen in all rabbits.

**CONCLUSION**

In this study, amniotic membrane was necrosed without metaplasia and transitional layer was repaired with repithelialization, also the smooth muscle was created under transitional layer. We concluded that amniotic membrane with characteristics such as availability, maintenance for a few months (below 80° c), antimicrobial, anti-inflammatory and low immunogenicity, it can be applied as a biological scaffold and tissue transfer in bladder reconstruction. In this research, due to the small bladder of rabbits, urodynamic study was not carried out and performing it on animals with larger bladder may reveal significant information about reconstructed bladder with amniotic membrane. Further Researches on the utility of amniotic membrane as a biological scaffold for urinary system defects is recommended. More studies in this area may reveal the complications of using amniotic membrane in the future.

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**CONFLICT OF INTEREST**

There is no conflict of interest for authors of this study.

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