Research Article

Impact of Freeze-Dried Amnion Membrane and Human Amnion Stem Cell Seeding on TGF-β and Collagen Type III in Vesicovaginal Fistula

Jojor Sihotang[1], Eighty Mardiyan[2], Widjiati[3], Amiruddin Hidayatullah[1]

[1]Departement of Obstetry and Gynecology Faculty of Medicine Universitas Riau
[2] Departement of Obstetry and Gynecology, Faculty of Medicine, Universitas Airlangga Dr. Soetomo General Hospital Surabaya
[3]Departement of Anatomy Faculty of Veterinary Universitas Airlangga Surabaya

Abstract

Objective: To analize how freeze-dried amniotic membrane and human amniotic stem cell seeding affect TGF β and type III collagen expression in suturing a New Zealand rabbit vesicovaginal fistula model.

Metode: This experimental study employed New Zealand rabbits and a vesicovaginal fistula model with a post-test only control group design. The rabbits were divided into 3 groups: vesicovaginal fistula suturing alone, suturing with freeze-dried amniotic membrane, and suturing with freeze-dried amnion-seeded stem cells. After 7 days of treatment, specimens near the repaired vesicovaginal fistula were collected for immunohistochemical analysis of TGF β and collagen type III expression.

Result: TGF β expression was significantly higher in the freeze-dried amniotic membrane with stem cell seeding group (p=0.001) compared to the freeze-dried amniotic membrane without stem cell seeding group (p=0.017) and the suturing-only group (p=0.049). Additionally, type III collagen expression was significantly elevated in the freeze-dried amnion membrane and stem cell seeding group (p=0.001) compared to the freeze-dried amnion group without stem cell seeding (p=0.09) and the suturing-only group (p=0.026).

Conclusion: The expression of TGF β and type III collagen was higher in rabbits with vesicovaginal fistulas treated using freeze-dried amnion and amniotic stem cell seeding compared to those without amniotic stem cell seeding and vesicovaginal fistula suturing alone.

Keywords: freeze-dried amnion, stem cell, vesicovaginal fistula.

Correspondence author : Jojor Sihotang. Departement of Obstetrics and Gynecology Faculty of Medicine Universitas Riau.
Email.jojorsihotang@lecturer.unri.ac.id

INTRODUCTION

Vesicovaginal fistula is a health concern within the field of urogynecology that can significantly impact patients' psychosocial and sexual well-being. Its symptoms often manifest as continuous urinary leakage from the vesicovaginal opening. A case report from the Urogynecology Reconstruction Division of dr. Soetomo Hospital for the years 2016 to 2018 revealed a notable recurrence rate of 31.2% for repeated fistula suturing. Gynecologic fistula cases resulting from iatrogenic causes remain a significant challenge, yet advancements in labor monitoring have led to a substantial decrease in obstetric fistula cases following childbirth.[1]

While surgical intervention through the transvaginal approach is favored over the transabdominal method in the treatment of vesicovaginal fistulas, the current scenario presents a challenge due to persistently high failure rates.[2-4] The dissatisfaction with this surgical procedure has led to the exploration of alternative approaches involving biomaterial application to replace the lost biological structure resulting from the defect. This is expected to enhance the repair process and sustain the normal functionality of the compromised tissue through tissue engineering. An emerging biomaterial in the field of urogynecology is the amnion membrane, chosen for its abundant presence in obstetrics and its non-invasive nature.[5,6] The amnion membrane

can be directly applied to the wound defect or in the form of stem cells. Human amnion stem cells have also been extensively researched and hold significant potential for transplantation and tissue engineering applications. They possess immunomodulatory properties, self-renewal capabilities, and the ability to differentiate through the production of growth factors such as TGF β, which is commonly found in the bladder's stromal layer.[7-9] The role of TGF β can stimulate the formation of type III collagen during the initial phases of wound healing. Thus, the application of amnion membrane stem cells is expected to reinstate the function and form of the layer to its original state or near-original state in vesicovaginal fistula cases.[10,11]

METHODS

An experimental study was conducted using New Zealand rabbits as the animal model for vesicovaginal fistula. The research design followed a post-test only control group approach. The study comprised three groups: a control group, which underwent vesicovaginal fistula suturing alone; treatment group 1, which underwent fistula suturing and received freeze-dried amnion membrane without amnion stem cell seeding; and treatment group 2, which underwent fistula suturing and received freeze-dried amnion membrane with amnion stem cell seeding. On the seventh day post-treatment, all rabbits were euthanized, and specimens were collected. These specimens were subjected to immunohistochemical staining to determine the expression of TGF β and type III collagen. The measurements of the rabbits' body weights were analyzed using the Shapiro-Wilk normality test, revealing normally distributed data ($p > 0.05$). The test for homogeneity of variance yielded a p-value greater than 0.05, indicating no significant variation in the body weights of rabbits among the different groups.

RESULTS

The analysis of the comparison of TGF β expression revealed significant differences among the groups, with the most significant difference observed between treatment group 2 and the control group, exhibiting the largest mean difference of 2.4.

Table 1. The Results of the Rabbit's Body Weight Analysis

Groups	Mean + SD	Normality	Homogenity test
Control	3.37 ± 0.16	0.103	
Intervention 1	3.37 ± 0.14	0.2	0.668
Intervention 2	3.47 ± 0.14	0.065	

Table 2. The Outcomes of the Statistical Analysis of Immunohistochemical TGF β Expression

Groups	Mean	Min	Max	*P-value*
Control	2.57	1.2	4.1	
Intervention 1	3.6	2.4	6.0	0.001
Intervention 2	5.0	2.9	7.4	

One Way Anova test

Table 3. The Results of the intergroup comparison analysis of TGF β expression

Groups	Mean	*P-value*
Intervention 1 VS Control	1.09	0.049
Intervention 2 VS Control	2.4	0.001
Intervention 2 VS Intervention 1	1.3	0.017

Kruskal-Wallis test

The analysis of the comparison of type III collagen expression revealed significant differences among the groups, with the most notable distinction observed between treatment group 2 and the control group, demonstrating the largest mean difference.

DISCUSSION

The research findings revealed statistically significant differences in TGF β expression among all groups. The treatment group that underwent vesicovaginal fistula suturing along with the application of freeze-dried amnion and amnion stem cell seeding exhibited higher TGF β expression compared to both the group treated with only freeze-dried amnion application and the group that underwent fistula suturing alone.

This study is in line with the work conducted who investigated the effectiveness of amnion stem cells in inducing wound healing processes from the epithelial to the urothelial muscle layer in a rat model of hemisystectomy, followed by suturing. Liu et al. examined the immunohistochemical expression of TGF β, as well as the number of muscle layers and fibroblasts within the urothelial layer through histological analysis (Hematoxylin and Eosin-H&E staining) in rat models with wounds extending to

the dermal layer. The research findings showed enhanced epithelialization of the rat's skin layer by the seventh day, along with a significantly increased TGF β expression after amnion stem cell administration compared to the control group without stem cells. The level of fibroblasts was also quantified, revealing an increase in the number of mature fibroblasts histologically. It is suggested that fibroblast cells constitute the dominant layer within the urothelial layer, and TGF β acts as the most potent stimulator capable of synthesizing myofibroblasts from fibroblast differentiation to produce collagen during the proliferative phase of wound healing.[12]

The study conducted highlights that reducing inflammatory conditions can lead to a decrease in the formation of fibrosis or scar tissue, thereby accelerating wound healing. This research focused on the effectiveness of mesenchymal stem cells applied to the injured bladder of mammalian rat models. The study reported a significant increase in TGF β expression within the damaged bladder layers through immunohistochemical analysis ($p < 0.05$), indicating an upregulation that expedited tissue regeneration.[13]

The research findings also indicate that the treatment group, which underwent primary suturing and received freeze-dried amnion without stem cell seeding, exhibited higher TGF β expression compared to the group that only underwent primary suturing. Macroscopically, the surface layer of the vesicovaginal fistula that had been sutured in treatment group 1 showed improved results when compared to the group that underwent suturing alone. A study demonstrated an increase in TGF β expression in oral mucosal ulcers in rats when treated with TGF β growth factor, as opposed to the control group.[14]

Similar findings were obtained from the study by Jepsen et al. on a group of pigs with a vesicovaginal fistula model, where stem cells were injected into the formed vesicovaginal fistula area. This randomized study involved seven pig models with vesicovaginal fistula, wherein the results showed an increase in smooth muscle α-actin content by 98.8% ± 0.6% in immunoreactive cells in the group that received stem cell injections. These findings closely mirror those from a human study, which involved patients with Crohn's Disease and perianal fistulas treated with autologous stem cell injections into the fistula defect. This study, classified as a phase 1 clinical trial, achieved a fistula closure success rate of 82% (27 out of 33).15 Another research effort demonstrated successful wound healing after using freeze-dried amnion membrane in a case of vesicovaginal fistula in a 64-year-old human patient. The study reported positive outcomes from applying an amnion patch to the vesicovaginal fistula suturing performed through an open abdominal surgery approach (transabdominal). By the 7th day post-operation, there were no reports of urinary leakage through the vagina (incontinence), and a cystography examination showed robust epithelial layer healing.[16]

The research outcomes also reveal that the group subjected to fistula suturing and the application of freeze-dried amnion with amnion stem cell seeding exhibited significantly higher expression of type III collagen compared to both the group that received freeze-dried amnion without stem cell seeding and the group that underwent suturing alone. The results of TGF β in this study are consistent with the increased products it generates, namely collagen deposits and other ECM components, serving as biomarkers for successful wound healing. It is well understood that TGF β acts as a key stimulator of collagen production during wound healing processes.[16] Collagen and fibronectin are essential for patching tissue defects, enhancing tensile strength, and preparing the tissue for neovascularization. The primary biological function of collagen is to form an extracellular matrix that strengthens, binds, fills, and maintains the structure of tissues. Without collagen, cellular tissues would become fragile. The organized deposition of collagen matrix is a pivotal indicator of the remodeling or maturation phase.[17]

This study aligns with the research on primary intestinal anastomosis in Wistar rat models with intraperitoneal infection, where the application of Freeze-Dried Amnion Membrane (FDAM) resulted in increased collagen density histopathologically at 400x magnification in the FDAM-treated group compared to the control group. The study reported a significant difference between the groups with a p-value of 0.001 ($p < 0.05$).[18,19] Amnion membrane contains a variety of growth factors such as bFGF, EGF, and TGF-β that stimulate fibroblast growth, neovascularization, and ultimately the formation of collagen deposits. The role of amnion also involves inducing the migration of epithelial cells towards the wound site, thereby triggering cell proliferation and differentiation. Another study

by Robles et al. found similar outcomes. In their research, they reported the successful repair of vesicovaginal fistula in two patients through the application of collagen (Pelvicol) from porcine dermal layers (xenograft) as an interposition during vesicovaginal fistula repair. The study achieved satisfactory repair outcomes within 1-2 weeks post-treatment, and at the 12-month follow-up, optimal results in the urinary vesicle structure were noted from the increased collagen deposition observed through histological analysis.[20] The study also reported similar findings to this research. In their study, they observed an increased TGF β expression in the urinary bladder of rats treated with mesenchymal stem cells along with a scaffold using Bladder Acellular Matrices (BAM) in a rat urinary bladder reconstruction model. The study demonstrated optimal regeneration of the detrusor layer of the urinary bladder through the increased collagen composition. This research concluded that the absence of inflammation reduces scar tissue formation and accelerates wound healing. Conversely, prolonged expression of inflammatory factors is associated with the formation of fibrotic tissue, which worsens the reconstruction of the urinary bladder's layers.[21]

CONCLUSION

The expression of TGF β and type III collagen in New Zealand rabbit models of vesicovaginal fistula, treated with freeze-dried amnion combined with human amnion stem cell seeding, was found to be higher compared to both the group treated solely with freeze-dried amnion and the group that underwent vesicovaginal fistula suturing alone.

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