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Post-Tubularized Incised Plate Urethroplasty Healing: An Experimental Randomized Study in 40 Dogs

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ABSTRACT

INTRODUCTION: The mechanism of urethral healing after tubularized incised plate urethroplasty (TIP) is still debatable. The purpose of the present study was to report post-TIP healing in a randomized-study hypospadias dog model.

METHODS: The hypospadias model was created in 40 male mongrel dogs that were randomly allocated into group 1 and group 2 with 20 dogs in each, and 4 subgroups (1a and 1b; 2a and 2b) with 10 dogs in each. The urethral plate width was reduced to 10 mm and 6 mm in group 1 and group 2, respectively. The urethral plate (UP) was tattooed and bisected by midline longitudinal incision. The length of UP and urethral incision (UI) was 2 cm in subgroups 1a and 2a and 4 cm in subgroups 1b and 2b. Tubularization of UP was done over an 8 Fr feeding tube. After 4 weeks, the phallus was harvested and transverse sections were obtained from the area of UI. They were stained by hematoxylin and eosin (H&E), Masson's trichrome, and fibronectin stains and pathologically examined.

RESULTS: Four cases of proximal urethral fistulae were reported in group 2 (1 in subgroup 2a; 3 in subgroup 2b). Gross inspection exhibited intervening tissues at the site of UI. The size of the intervening tissues between the healing edges of the UI was 0.8 mm (SD = 0.1) in group 1 and 2.5 mm (SD = 0.2) in group 2. Light microscopy revealed creeping of normal-appearing squamous surface epithelium in all specimens. Increased collagen fiber deposition was recorded in group 2 when compared with group 1. Fibronectin was expressed in the subepithelial locations of the urethra in all studied animals. However, dense signals were reported in group 2 in comparison to group 1.

CONCLUSIONS: Healing after TIP occurs by secondary intention with creeping of the surface epithelium. Collagen deposition was increased in cases of UP ≤ 6 mm in width. Post-TIP complications may increase in cases of narrow UP, especially with the neourethra and UI > 2 cm in length.

KEYWORDS: Urethra; Hypospadias; Dog; Healing.

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original study

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INTRODUCTION

The tubularized incised plate urethroplasty (TIP) technique has gained a vast popularity for distal hypospadias repair and has been applied to proximal hypospadias in limited series [1]. The complication rates of TIP range from 3% [2] to 60% [3], with the highest rates in proximal types. Some studies postulated stiffness following TIP repair [3,4]. Also, there has been debate about the mechanism of healing following incision of the urethral plate and the possibility of stricture formation [5].

The authors of some experimental studies have described a few aspects of post-TIP healing [6-8]. Most of these studies had limited animal numbers, used nonrepresentative hypospadias models, and lacked histochemical stains. The authors did not evaluate important factors such as the width of the urethral plate (UP) or the length of the reconstructed neourethra and urethral incision (UI). The present experimental study is an attempt to avoid previous shortcomings and more fully describe post-TIP healing in a dog model.

METHODS

Animals

The study was conducted from March 2006 to August 2008. It included 40 male mongrel dogs ranging in age from 6 months to 1 year and ranging in weight from 18 to 22 kg. The study was conducted at Animal Research House, Assiut University, under regulations of the Animal Research and Care Committee.

Surgical Procedure

Each dog was premedicated with 0.1 mg/kg acepromazine and sedated with 10-12 mg/kg sodium pentobarbital intravenously. Anesthesia was applied with 3% to 5% fluthane (Halothane; Mumbai, India) through an endotracheal tube. The lower abdomen, penis, and urethra were prepared for surgery. The urethra was incised ventrally from the tip of the glans proximally with trimming of the urethra down to 10 mm and 6 mm to create a hypospadias model.

The dogs were randomly allocated into 2 groups: group 1 (n = 20) had a 10 mm UP; group 2 (n = 20) had a 6 mm UP. The urethral plate was tattooed using Indian ink in all dogs. A midline longitudinal incision was done, bisecting the UP. The length of the UP and UI was 2 cm in 10 dogs of each group (subgroups 1a and 2a) and 4 cm in the other 10 animals (subgroups 1b and 2b).

The tubularization of the UP was done over an 8 Fr feeding tube using continuous running 6/0 polyglactin sutures. A second layer cover and fixation of the feeding tube to the glans were performed. A penile block was applied at the end of the procedure using 0.2% bupivicaine.

Both the bio-occlusive dressing and feeding tube were removed 5 days postoperatively under sedation. All dogs were euthanized with 120 mg/kg pentobarbital intravenously 4 weeks postoperatively.

Histopathologic Examination

The phallus was harvested and examined grossly. The distance of the tissue separating the bisected tattoo was measured by using magnified loops (3x).

Histopathologic examination involved 5 transverse sections from the area of the UI. The specimens were fixed in 10% neutral buffered formalin followed by dehydration in a graded series of ethanol. They were then embedded in paraffin. Sections that were approximately 5 μm thick were cut and stained with hematoxylin and eosin (H&E) and Masson's trichrome stains. Immunohistochemical staining was done using murine monoclonal antibodies against fibronectin stain. Tissue sections were mounted on coated slides and processed. All slides of the study were labeled by code numbers and examined by a senior pathologist board. The examiners were blind to the animal group.

RESULTS

No complications related to the procedure were reported in group 1. Four small proximal urethral fistulae were reported in group 2 (1 in subgroup 2a; 3 in subgroup 2b). Gross inspection exhibited intervening tissues at the site of the UI that was marked on its lateral edges by the tattooing reaction. The size of the intervening tissues between the healing edges of the UI was 0.8 mm (SD = 0.1) in group 1 and 2.5 mm (SD = 0.2) in group 2.

Light microscopy examination of cross sections revealed creeping of normal-appearing squamous surface epithelium indistinguishable from unoperated sections in all specimens of group 1 and group 2. Increased collagen fiber deposition in the subepithelium was recorded in group 2 (subgroups 2a and 2b) when compared with group 1 (subgroups 1a and 1b). Excess collagen fibers were detected by H&E (Figure 1) and Masson's trichrome stains in group 2 in comparison to group 1. This was recognized by Masson's trichrome stain in the form of thick dense blue collagen bundles in the subepithelial stroma (Figure 2). Fibronectin histochemical stain was expressed in the subepithelial locations of the urethra in all studied animals. However, dense signal was reported in the subepithelium of group 2 (subgroups 2a and 2b) (Figure 3) in comparison to group 1 (subgroups 1a and 1b) (Figure 4).

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Figure 1. Light Microscopic Examination of Post-TIP Specimen Stained by Hematoxylin and Eosin (×200).
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Specimen revealed creeping of normal surface squamous epithelium, with thick collagen bundles in the subepithelium in group 2.
Abbreviations: L, lumen; E, epithelium

Figure 2. Light Microscopic Examination of Post-TIP Specimen Stained by Masson's Trichrome (×200).
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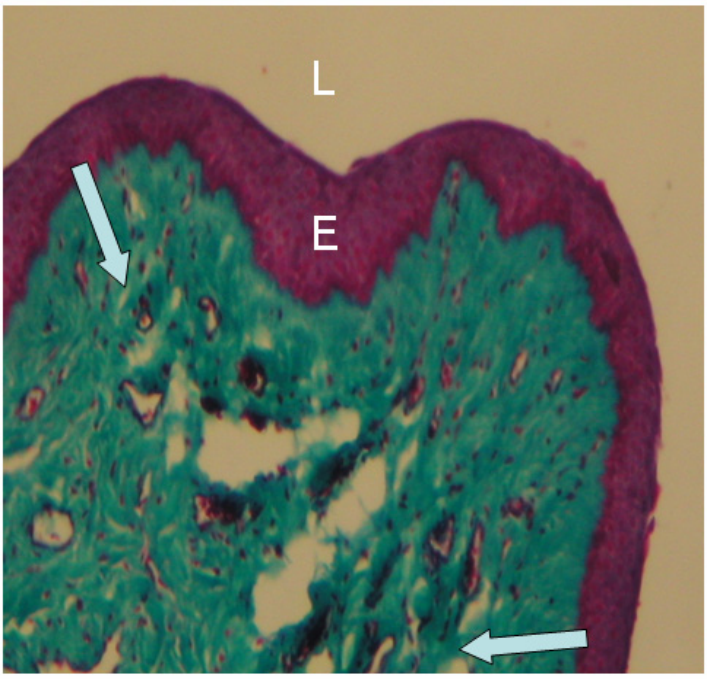


Specimen revealed creeping of normal surface epithelium, with thick dense blue subepithelial collagen bundles between the tattooing marks (arrows) in group 2.
Abbreviations: L, lumen; E, epithelium

DISCUSSION

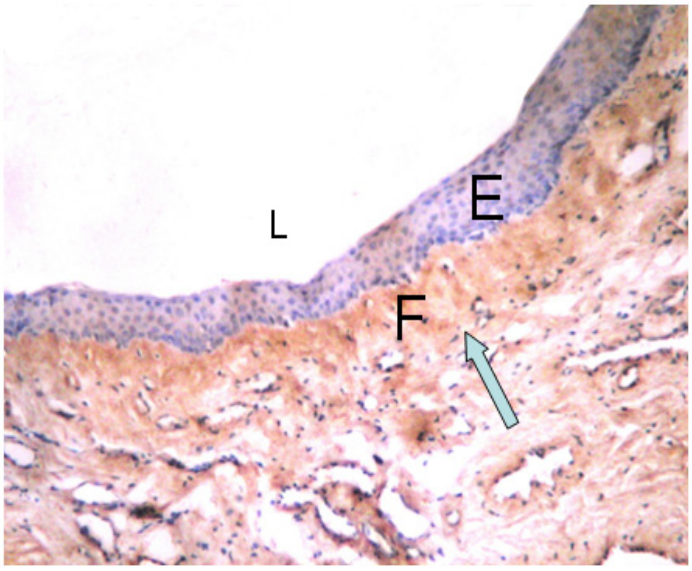
Tubularized incised plate urethroplasty was originally described for treatment of distal hypospadias, but its application for treatment of proximal hypospadias provoked a debate about its outcome [1,3]. Ngyuen et al [9] claimed that TIP is a universal technique for hypospadias repair which includes preservation of UP. Both flat and grooved urethral plates with a width of 4-8 mm reach more than 13 mm after relaxing the incision, and its healing occurs by reepithelialization with no scarring or reduction in its diameter.

The relationship between post-TIP results and both the depth and width of UP was addressed by Holland and Smith [10]. They concluded that patients with shallow and narrow UP are more likely to have complications like urethral fistula or stenosis.

Some authors of clinical and flowmetric studies described the stiffness of reconstructed urethra after TIP [3,4,11]. However, the experimentally stented post-TIP studies in animals did not correlate with published reports [6-8]. Bleustein et al [6] reported urethral healing after incision and tubularization over a catheter during TIP by reepithelialization with normal tissue growth after 21 days in 5 dogs. Lopes et al [7] reported healing by normal reepithelialization without excess collagen or scarring after 21 days in 2 immature pigs. None of the reported animal models included any (or sufficient) excision of the ventral urethra, which could prevent its tubularization even without making the relaxation incision. Therefore, they are not practical representative hypospadias models. Histochemical stains and the impact of the width and length of UI on the post-TIP healing process were not addressed.

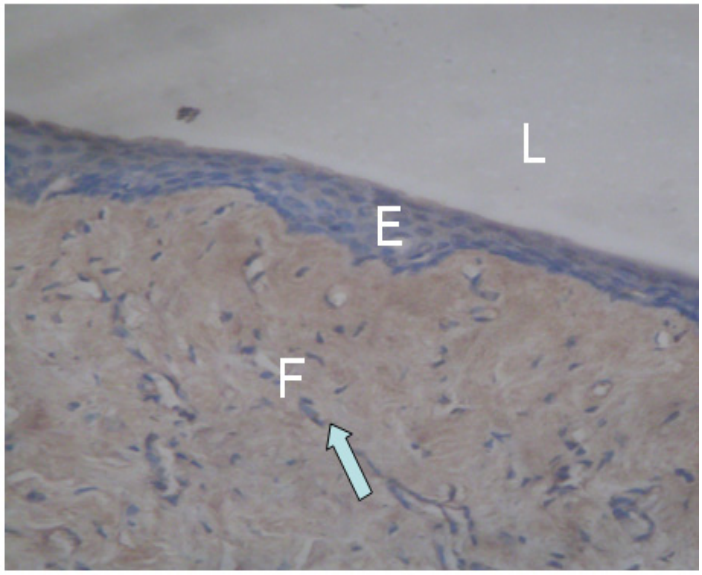
The present authors chose to study post-TIP healing after 4 weeks to pass the 3-week postoperative period proven sufficient for complete healing [6,7]. Wound healing occurs by primary intention when the edges are reapproximated by mechanical means like sutures, while secondary intention occurs when the edges are separated [12,13]. Healing by secondary intention includes creeping of surface epithelium from both edges across the raw area, followed by wound contraction or remodeling to decrease the wound gap and approximate its edges. This

Figure 3. Light Microscopic Examination of Post-TIP Specimen in Group 2 Stained by Fibronectin Stain (×200).
doi: 10.3834/uij.1944-5784.2009.10.07f3



Arrow shows dense signal which is more evident in the subepithelium (darker color) than in layers below the subepithelium. This reflects subepithelial deposition of excessive collagen.
Abbreviations: L, lumen; E, epithelium; F, fibronectin

Figure 4. Light Microscopic Examination of Post-TIP Specimen in Group 1 Stained by Fibronectin Stain (×200).
doi: 10.3834/uij.1944-5784.2009.10.07f4



Arrow shows faint signal in the subepithelium that is indistinguishable from layers below the subepithelium. This reflects deposition of collagen comparable to the normal healing of lower subepithelial layers.
Abbreviations: L, lumen; E, epithelium; F, fibronectin

process occurs by the end of 21 days. Wound contraction should not be confused with scar contraction [14].

Bleustein et al [6] described a 1 mm separation between the healing edges; the present authors reported 0.8 mm (SD = 0.1) in group 1 and 2.5 mm (SD = 0.2) in group 2. This change in distance may be due to the difference in remodeling effect as a result of the difference between the width of the UP in the present study.

Collagen fibers play an important role in the remodeling phase of wound healing. It is logical that an increase in collagen deposition may result in tissue stiffness, especially in distensible organs like the urethra. TIP seems to heal like any gapped wound by secondary intention. When the gap is minute like that in group 1, healing occurs with insignificant collagen deposition. This deposition was previously confused with primary intension, as reported in other studies [6,7]. Lalla et al [15] reported an increase in collagen content with low mechanical quality by chemical analysis in specimens of both TIP and urethral mobilization hypospadias models in rabbits. They concluded that after TIP, some collagen present in the granulation tissue formed during healing may have persisted at follow-up. Lower mechanical quality of the collagen could indicate that the collagen formed after the operation was not fully integrated or circularly aligned in the urethral wall. However, Taneli et al [16] observed no significant difference in the hydroxyproline levels as a measurement of the collagen after TIP in 7 rabbits.

Fibronectins are matrix molecules that act as a scaffold for collagen deposition. They increase in patients with abnormal scarring when compared with normal skin [17]. Strong subepithelial deposition of fibronectins correlate with dense collagen deposition, as was observed by both H&E and Masson's trichrome stains. The present reported finding of increased collagen deposition in the subepithelium in group 2 when compared with group 1 is striking. This supports the postulation that increased intraurethral dynamic flow stress after TIP repair promotes excessive collagen deposition in cases of narrow UP (≤ 6 mm in width).

The present authors found more proximal urethral fistulae in group 2 than in group 1, with a 3-fold increase in subgroup 2b

when compared with subgroup 2a. These fistulae may have been due to increased collagen causing particularly strong urinary flow resistance inside the reconstructed stiff neourethra, especially in the longer model. The same results were reported in clinical studies following TIP repair for proximal hypospadias that could not be attributed to technical factors during the repair [3]. Idzenga et al [18] demonstrated that shortening of an experimental hypospadias tube model led to an increase in flow rate. Healing of the UI during internal urethrotomy of urethral stricture is not comparable to relaxing the incision during TIP. The former is healing of incised scar tissue that is not well vascularized; the latter is healing of well-vascularized tissue [2]. Therefore, the correct term for post-TIP healing is secondary intention and not primary intention as postulated in other studies [6,7].

In spite of the limitations of animal studies and the difference between the UP in hypospadiac infant and animal models [19], animal studies still have their importance for correct understanding of clinical events. Certain points are in need of further study, such as the type of collagen and its relation to time of mature healing. Also, determination of the percentage of different collagen types in relation to other components like elastic and smooth muscle fibers may be beneficial. Finally, objective measurable findings will be superior to subjective observations.

CONCLUSIONS

Healing after TIP occurs by secondary intention with creeping of surface epithelium. Collagen deposition was increased in cases of UP ≤ 6 mm in width. Excessive collagen deposition may cause urethral stiffness. Post-TIP complications such as urethral fistulae may increase in the presence of narrow UP, particularly with neourethra and UI > 2 cm in length.

Conflict of Interest: None declared.

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