

Histopathological Study of Using Single-layer Simple Continuous and Continuous Lembert Suture Patterns for Cystotomy Closure in Rabbits

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Abstract

Two techniques of single-layer closure were studied experimentally by created cystotomy in six male rabbits, they separated randomly into two equal groups (A and B). In animals of either groups, cystotomy was initiated experimentally on the dorsal side of the urinary bladder wall through standard midline ventral laparotomy. In group A, the cystotomy wound was closed by single-layer, full thickness, simple continuous pattern, while in group B, the closure was accomplished by single-layer continuous Lambert pattern, applying polydioxanone PDS (3/0) in the two groups. The effectiveness of cystotomy wound healing and a urine-firm seal of the urinary bladder were examined at days 3, 7 and 14 post-operatively by gross changes. In both groups, the macroscopic observation revealed thickening of the bladder wall with adhesion in both groups, the microscopic evaluation showed optimum healing at the site of cystotomy wound sealing on the 14th day post operation despite a slight to moderate adhesions were noted in both groups. The histological evaluation of the urinary bladder biopsies indicated regular epithelization of the mucosal coat and normal arrangement of the muscularis and serosal layers at the location of cystotomy closure were evident in animals of both groups on the 14th day post operation.

Keywords: Cystotomy, Single-layer Lembert pattern, Rabbits.

دراسة نسجية لاستخدام طبقة واحدة لنماذج خياطة البسيط المستمر ولمبرت المستمر لإغلاق بضع المثانة في الأرانب

الخلاصة

تمت دراسة تقنيتين من الخياطة الجراحية بتقنية الطبقة الواحدة لإغلاق بضع المثانة التجريبي في ستة من ذكور الأرانب، تم تقسيمهم عشوائياً إلى مجموعتين متساويتين (أ و ب). الحيوانات في كلتا المجموعتين خضعت لبضع المثانة التجريبي على الجانب الظهري لجدار المثانة البولية من خلال الفتح الوسطي لجدار البطن. في المجموعة أ، تم عمل الخياطة بواسطة طبقة واحدة ولكل السمك بنمط البسيط المستمر، بينما في المجموعة ب، تم الإغلاق بواسطة نمط لمبرت المستمر وبطبقة واحدة باستخدام خيط بولي دايسكزينون (3/0) في المجموعتين. تم فحص فعالية التئام جرح المثانة والاحكام ضد تسريب البول منها في الأيام 3 و 7 و 14 بعد العملية بواسطة النتائج العيانية والمجهريّة. وفي كلا المجموعتين (أ و ب)، كشفت نتائج الفحص العياني عن سمك جدار المثانة والالتصاقات في كلتا المجموعتين، أظهر الفحص المجهرى الشفاء الأمثل في مكان خياطة جرح المثانة في في المجموعتين وبدون فرق، على الرغم من الالتصاقات القليلة إلى المعتدلة التي لوحظت في كلتا المجموعتين. وظهر التقييم النسجي المرضى لخزعة المثانة البولية، انتظام الظهارة للطبقة المخاطية والترتيب الطبيعي للعضلات والطبقة المصلية في موقع إغلاق جرح المثانة في الحيوانات لكلا المجموعتين في اليوم الرابع عشر بعد العملية.

Introduction

Cystotomy is cutting into the urinary wall, it can be performed to elimination of cystic and urethral calculi, recognition and biopsy of mass lesions, repair of ectopic ureters, or diagnosis of urinary tract infection contrary to treatment (1). Traditionally, cystotomy is performed by conventional laparotomy, which is an incursive operation that can be combined with complications compared to laparoscopy which is minimally invasive, related with a low morbidity and allow good examination of the bladder (2). The most frequent complications of cystotomy are urine leakage, calculus recurrence and adhesions (3). It is well recognized that urine leakage causes unfavorable consequences to wound healing such as respite healing, rise inflammatory reaction and stenosis formation (4). Thus, perfect suturing of cystotomy wounds is very important to give a urine-tight seal to prevent this outcome (5). Some authors suggest that when sutures material used for cystotomy closure passed through a mucous membrane acts as a nidus for cystolithiasis and it may prematurely absorb due to urine contact, therefore a suture material should have least tissue reactivity and resistant to degradation, also it should not induce crystal or stone formation (6). Non-absorbable suture materials, such as Polypropylene and Nylon, although not essential as the tissues of the urinary tract normally heal rapidly, but have been used frequently in urinary surgery and reported to produce an intense inflammatory response when used for ureteral repair (7). The absorbable synthetic sutures (polydioxanone, polyglactin-910, and polyglycolic acid) have all been used strongly (8) whereas chromic gut is familiar with more difficult inflammatory response and less expected time of tensile strength than synthetic absorbable sutures (9). Extended intraluminal exposure to polydioxanone has been noted following urethral repair (10). Polyglycolic acid suture keep tensile strength great than polyglactin-910 in acid urine and the opposite is true in neutral or alkaline urine (11). Several

patterns have been mentioned for the closure of cystotomy incisions. Appositional closure applying a single or double layer simple continuous suture pattern outcome in earlier and more progressive healing than the traditional double inverting suture pattern (12). Some studies proved that single layer cystotomy closure technique has advantage over the double-layer and triple-layer closure techniques through shorten suturing time ,apposition of tissues and better cystotomy wound healing (13). Many factors can lead to delay healing from these microbial contamination in surgical theaters and tools (14).

The purpose of the present study was to evaluate cystotomy wound in rabbits by single-layer continuous Lembert versus full thickness, appositional simple continuous suture patterns.

Materials and methods

Six male rabbits, with average body weight (1-1.5 kg) and 6-8 months age were used in the present study. The animals were treated for internal and external parasites, the animals were divided randomly into two equal groups. In group (A) the cystotomy wound was sutured by single-layer full thickness simple continuous pattern whereas, in group (B), it was sutured by single-layer continuous Lembert pattern, using polydioxanone (3-0). All the rabbits were handled according to the ethical guideline for the animal care of Baghdad University.

The ventral abdominal wall was aseptically prepared for the operation, preanesthetic medication with 0.02 mg/kg, of Acepromazine malate (Vemedim-Vietnam) was administrated intramuscularly, after 10 minutes later, a mixture of 5 mg/kg, of 2% xylazine (Venray-Holland) and 30mg/kg, of 10% ketamine (Alfasan-Holland) was given by intramuscular route (15). The animals were positioned on dorsal recumbence Urine evacuation and bladder emptying was performed by direct aspiration of urine with a sterile 5 ml syringe from the bladder after creation of

celiotomy and exploration of the abdominal cavity. Experimental animals of both groups were subjected to routine midline ventral laparotomy (Fig. 1A). Following abdominal exploration, the U. B. was distinguished, explored and separated from the abdominal viscera with wet sterile laparotomy towels (Fig. 1B). Two stay sutures were fixed on the cranial and caudal ends of the bladder which was then completely evacuated using a 5 ml syringe. A stab incision was achieved by a scalpel on the dorsal bladder wall, then stab incision was extended about 2 cm by a scissor cranially and caudally (Fig. 1C) and the bladder cavity was explored for the appearance of bladder lesions or calculi and flushed with warm saline before closure. The cystotomy wounds were closed by single-layer simple continuous in group A (Fig. 4) and by single layer continuous Lambert pattern in group B (Fig. 1D).

In both groups, cystotomy wounds were sutured with 3/0 polydioxanone. Postoperative concern included monitoring of animals physical status, daily intramuscular injection of systemic antibiotic (penicillin (10.000 IU/kg) and streptomycin (20 mg/ kg) for 3 days. The skin sutures were withdrawn within 10 days post operation.

The macroscopic evaluation was performed to detect the presence of any gross abnormally related to cystotomy closure such as leakage, wound healing complications, and calculi formation within the U.B. Biopsies of 0.5 cm length and width were obtained from the site of cystotomy closure and was wash with normal saline prior to further processing. Then fixed in 10% neutral buffered formalin for 72 hours. Following that, they were exposed to a series of histological preparations prior to sectioning to 5µm in thickness, and staining with hematoxylin and eosin stains (16).

Results and dscusion

Clinical findings: All operated animals in both groups showed depression and restlessness following recovery from the effect of anesthesia

and the urine tinged with blood for the first hour after operation then they started to retain normal physical activity, food intake and urination 1 to 2days post operation. All wounds healed satisfactory and animals showed no complications post-surgery.

Macroscopic findings:In group (A): At the 3rd day post operation the P.D.S. stitches were still clearly visible, with slight adhesion formation between the site of operation and mesentery (Fig. 2A). At the 7th day post operation the bladder wall thickened and congested (Fig. 2B). At the 14th day post operation sever adhesion was seen between the operated site and loop of intestine (Fig.2C).

In group (B): At the 3rd day post operation the P.D.S. stitches were still clearly visible, with no adhesion formation between the site of operation and surroundings (Fig. 2D). At the 7th day post operation the site of suturing not clearly visible, with no adhesion with surroundings (Fig.2E). At the 14th day post operation the site of operation not visible with congested bladder (Fig. 2F).

Histopathological Findings:

Group (A): At 3 days post operation: The sections showed deposition of fibrin with moderate infiltration of with inflammatory cells associated with sever vascular congestion with few fibroblast (Fig. 3A). At 7 days post operation: The sections were showed poor epithelization with loose connective tissue which characterized by marked angiogenesis, with mild infiltration of mononuclear cells (Fig.3B). At 14 days post operation: The field revealed newly regenerated epithelial cells and its core were composed of dense connective tissue, with marked fibroplasia (fibroblast and fibrocytes proliferation) with collagen deposits, angiogenesis with infiltrations (Fig. 3C,D).

Group (B): At 3 days post operation: The sections showed moderate Inflammatory cell infiltration associated with sever vascular congestion with few fibroblasts and fibrin deposition (Fig. 3E). At 7 days post operation: The sections showed poor

epithelization of the mucosal layer, with loose connective tissue which characterized by marked fibroplasia, angiogenesis (Fig.3F,G). At 14 days post operation: The sections showed good epithelization of the mucosal layer with congested blood vessels in lamina propria, with well-organized connective tissue that characterized by producing much of collagen bundles by fibroblast (Fig. 3H,I). The principles of the surgical closure of hollow organs have derived from a historic impression of the importance of the routinely practiced double-layer closure pattern to reach adequate healing and a water tight seal over the single-layer appositional closure pattern to achieve anatomic regeneration, healing minimized, decrease in luminal diameter, and watertight seal (17,18 and 6).

In spite of that, the results of the present study exposed that both patterns of the single-layer suture techniques used for the closure of cystotomy damage were very reliable at the 14th day post operation and the optimal healing at the area of cystotomy closure which was apparent macroscopically and microscopically at the 14th day post operation. The perfect healing at the site of cystotomy closure on the 14th day post operation is in agreement with that of another study done by (19) whom suggested that the major reparative processes of the urinary bladder have been accomplished in 14 days.

No differences were seen regarding the efficiency of the cystotomy closure healing between simple continuous and Lembert pattern concerning the macro and micro evaluation used in the present study. The selection of the single-layer pattern rather than the double-layer pattern for closure of cystotomy wound has resulted in reducing the operative time and the use of minimal amount of suture material and knots that remain at the site of closure.

Similar findings were observed in laparoscopic studies performed in human patients, suffering from bladder lacerations, by single-layer closure pattern using simple

interrupted or continuous sutures through the full thickness of the bladder wall (20,11 and 12). These findings are also supported by experimental studies performed in dogs and stated that healing of cystotomy wound closure obtained by the single-layer appositional pattern is as firm as that obtained by the double-layer continuous inverting suture pattern (21).

Furthermore, they are supported by the successful use of single-layer suture pattern to repair urinary bladder rupture in four postpartum mares (22). The histological examinations of the U. B. biopsies explained that the efficiency of the healing mechanism of the cystotomy wounds was corresponding in both groups. Normal epithelization of the mucosal layer in animals of either group was apparent on the 14th P. O. day associated with slight fibrous connective tissue proliferation, angiogenesis and slight infiltration of inflammatory cells in the lamina propria and serosal layers.

Such a satisfied result can be attributed to suture patterns used for closure of the cystotomy wounds in both groups because the single-layer patterns are proved to be much better than the double-layer pattern for the surgical closure of hollow organs (18). This finding is ascribed to the maturation process which normally takes place at the site of surgical wound healing from the 14th to the 70th P. O. days (23 and 24).

Histopathological changes represented by marked thickening of the serosal layer associated with focal loss of the mesothelial integrity, fibrous connective tissue proliferation and slight infiltration of mononuclear inflammatory cells were evident in the bladder biopsy obtained from the operated site that shows bladder adhesion in both groups. This displeased finding is not surprising in view of the possibility of adhesion formation at the suture line of the cystotomy wound because the conventional surgical suturing often leave dead tissue within the body that may act as a focus for infection and adhesion (25).

Conclusion

In conclusions, cystotomy wound closures by both suturing patterns used in the present study (single-layer simple continuous and Lembert pattern) were found similar in all comparison parameters. It was also concluded that both suturing patterns used in the present study resulted in healing of cystotomy wounds to

a urine-tight level on the 14th post-operative day.

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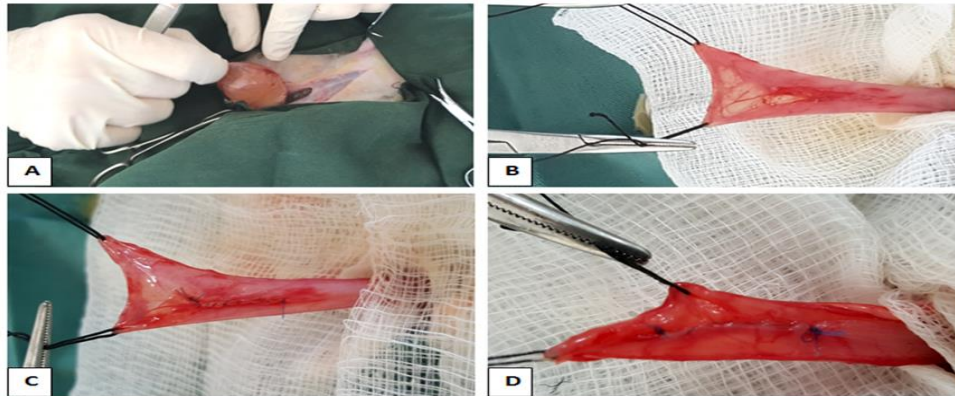


Figure 1: The surgical procedure (A) The urinary bladder is isolated out of the midline ventral laparotomy incision.(B) Two stay sutures are placed and the bladder is incised on its dorsal surface. (C) In group (A), the bladder incision is sutured by single layer of simple continuous suture pattern by PDS (3/0). (D) In group (B), the bladder incision is sutured by single layer of continuous Lembert suture pattern.

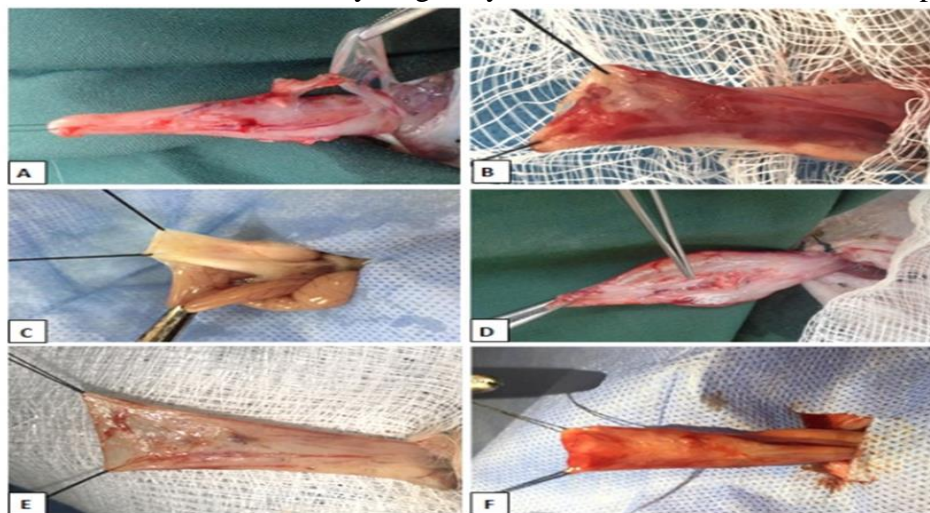


Figure 2: Shows macroscopic appearance (A) in group (A), at 3 days post operation shows, slight adhesion formation between the site of operation and mesentery. (B) in group (A), at 7 days post operation shows, the bladder wall thickened and congested with no visible suture line. (C) in group (A), at 14 days post operation shows, adhesion between the operated site and loop of intestine.(D) in group (B), at 3 days post operation shows, the suture line, with no adhesion formation between the site of operation and surroundings.(E) in group (B), at 7 days post operation shows, the site of suturing not clearly visible, with no adhesion with surroundings.(F) in group (B), at 14 days post operation shows, the site of operation not visible with congested bladder.

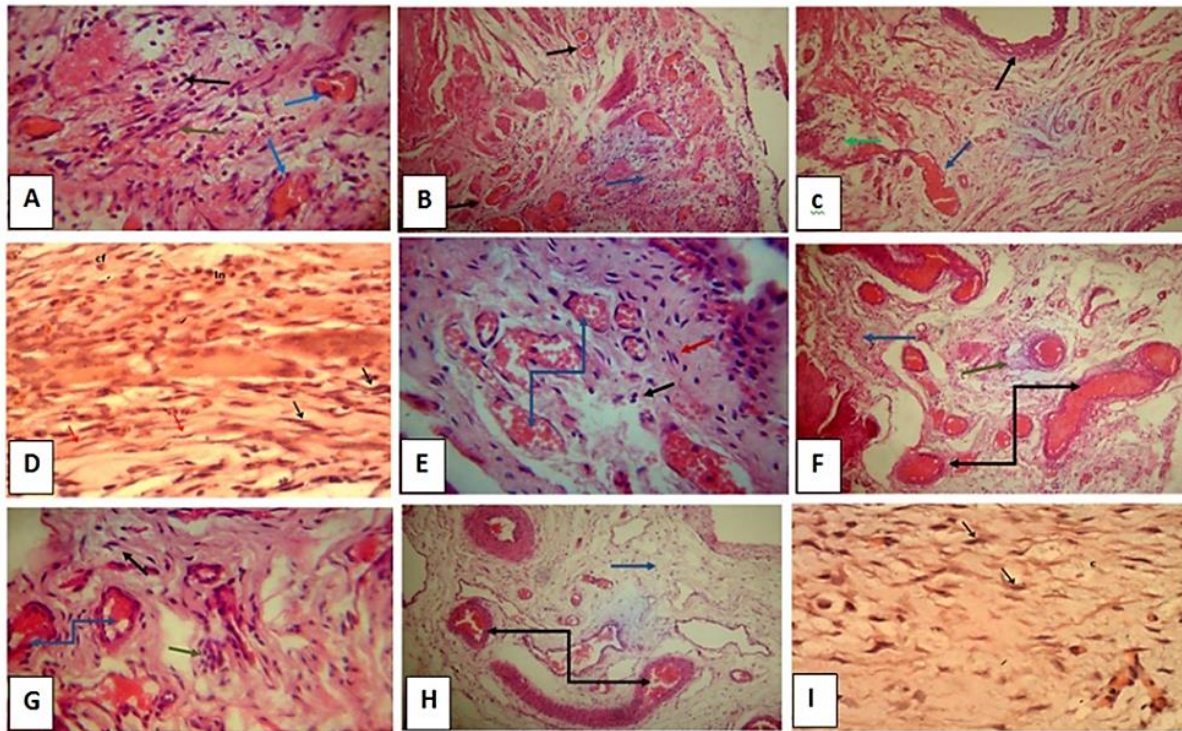


Figure 3: Shows microscopic appearance (A) in group (A) at 3 days post operation shows, deposition of fibrin with moderate infiltration of mononuclear cells (black arrow) associated with sever vascular congestion (blue arrow) with few fibrocyte (green arrow) (H&E X40). (B) in group (A) at 7 days post operation shows, poor epithelization with loose connective tissue which characterized by marked angiogenesis (black arrow), with mild infiltration of mononuclear cells (blue arrow) (H&E X10). (C) in group (A) at 14 days post operation shows, newly regenerated epithelial cells (black arrow) and its core were composed of dense connective tissue, with angiogenesis (blue arrow) with infiltrations of mononuclear cells (green arrow) (H&E X10). (D) in group (A) at 14 days post operation shows, marked fibroplasia (fibroblast and fibrocytes) with collagen deposits, angiogenesis with infiltrations of both polymorphic & mononuclear cells (H&E X40). (E) in group (B) at 3 days post operation shows, moderate infiltration of polymorphic nuclear leukocytes (black arrow) associated with highly vascular (blue arrow) with few fibroblasts and fibrin deposition (red arrow) (H&E X40). (F) in group (B) at 7 days post operation shows, loose connective tissue which characterized by marked fibroplasias (blue arrow), Congested blood vessel (black arrow) with moderate infiltration of mononuclear inflammatory cells (green arrow) (H&E X10). (G) group (B) at 7 days post operation shows, marked fibroplasias (black arrow), angiogenesis (blue arrow) with moderate infiltration of mononuclear inflammatory cells (green arrow) (H&E X40). (H) in group (B) at 14 days post operation shows, good epithelization of the mucosal layer with congested blood vessels (black arrow), with well-organized connective tissue that characterized by producing much of collagen bundles by fibroblast (blue arrow) (H&E X10). (I) group (B) at 14 days post operation shows, well-organized connective tissue that characterized by producing much of collagen bundles by fibroblast (H&E X40).

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