

HISTOPATHOLOGICAL EVALUATION OF TWO SUTURING TECHNIQUES FOR END -TO-END ANASTOMOSIS OF COLON IN DOGS

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ABSTRACT

An experimental study was conducted on 12 healthy adult dogs to determine the best method for anastomosis of colon. An appositional 2 layer (APP-2) suture pattern was compared with the inverting 2 layer (INV-2) suture pattern for end-to-end anastomosis. Vicryl[®] (size 3-0) was used for anastomosis. At day 10 after surgery, all anastomotic lines were apposed but the mucosa did not cover the lumen of the anastomosis with APP-2 layer suture pattern. The specimens closed with INV-2 layer suture pattern showed more pronounced inversion of all layers and the cut ends of the mucosa and submucosa at the tip of fold were apposed by a thick plug of fibrous connective tissue. At day 20, the mucosa covered the internal surface and bridged the narrow defect with a single layer of epithelial cells with APP-2 layer suture pattern. The specimens closed with INV-2 layer suture pattern showed slight inversion of the serosa and smooth muscle caused moderate thickening of the wall. Histopathological differences between samples collected at 30 days were associated mostly with the maturation process. The current study showed the superiority of the APP-2 layer suture pattern for colonic end-to-end anastomosis in terms of better apposition of the anastomotic layers and better anastomotic healing over the INV-2 layer suture pattern.

Key words: Suturing techniques, end-to-end anastomosis, colon, dogs.

INTRODUCTION

Diseases of gastrointestinal tract requiring surgical intervention are fairly common in dogs (Tangner, 1982). Resection and anastomosis are usually required in cases of intussusception, megacolon, neoplasia, obstruction or trauma. Complications associated with intestinal resection and anastomosis are the major cause of death in animals requiring abdominal surgery (White, 1985). Complications occur mainly due to lack of strict asepsis, poor surgical technique, improper suture material and poor suturing methods. Commonly encountered problems for intestinal anastomosis are the frequency of breakdown and leakage of intestinal contents in the terminal colon as compared to other intestinal segments (Nichols *et al.*, 1973). Passage of solid faeces through a lumen, as well as problems in the bowel wall (increased bacterial concentrations, poor blood supply, strong muscular activity), have been cited as reasons for poor healing (Hawely, 1973; Jiborn *et al.*, 1980; Keller and Horniey, 1985). An end-to-end intestinal anastomosis is a simple and physiologically compatible method for intestinal repair as compared with side-to-side intestinal anastomosis. Controversies have continued, however, with regard to the preferred technique for intestinal closure. In order to devise and evaluate a technique, which is simple and promise to cover any situation, an

experimental model was designed to compare double layer appositional technique with double layer inversion technique for anastomosis of colon in dogs.

MATERIALS AND METHODS

Experimental animals

Twelve healthy mongrel dogs of both sexes, various ages (10 ± 2 months) and weights (mean 20 ± 5 kg) were randomly allotted to group-A and group-B, with 6 animals in each group. The dogs were kept at Indoor Patient Ward, Department of Surgery and Obstetrics, Sindh Agriculture University, Tandojam in isolation and allowed to adopt the surroundings (14 days) prior to experimentation to rule out the possibility of any latent infection. During this period they were dewormed with Anthalmin[®] (Levamisole hydrochloride 7.5% w/v, Reveex Spain) at a dose of 5mg/kg-body weight administered intramuscularly. They were vaccinated against rabies, canine adeno virus, canine parvo virus, leptospirosis and canine distemper with Hexadog[®] (Merial Co. Karachi, Pakistan; 1 ml/dog) at least 14 days prior to operation.

One day before surgery, each dog was medicated with 10-20 ml of liquid paraffin and 2 to 5 tablets of dulcolax[®] (Bisacodyl B.P 5mg/Tab, Merk Marker Pvt. Limited, Quetta). Meat and bread were with-held for 24

hours before surgery but a continuous access to water was allowed.

Anaesthesia

The dogs were premedicated with acetylpromazine 0.04 mg/kg body weight intramuscularly 10-20 minutes before induction of anaesthesia. The dogs were anaesthetized with 5 per cent solution of Thiopental sodium to effect. Anaesthesia was maintained with incremental doses of thiopentone, when required.

Surgical procedure

The standard caudal celiotomy and resection procedures were performed (Griener and Christie, 1975). Appositional two layer (APP-2) suture pattern was used in dogs of group-A. The first layer comprised of simple interrupted sutures and the 2nd layer was of cushioning suture pattern (Griener and Christie, 1975; Hanson *et al.*, 1988; Kachiwal and Kalhoro, 2000).

Inverted two layer (INV-2) pattern was used in dogs of group-B. The first layer was a continuous Connell and the second layer was that of continuous Cushioning pattern, using 3/0 vicryl suture (Larsen and Blellenger, 1974; Hanson *et al.*, 1988; Kachiwal and Kalhoro, 2000). Water and milk were provided *ad libitum* on post-operative day 1 and a soft diet on post-operative day 3.

Observations and necropsy findings

Monitoring of the animals consisted of recording the physiological parameters daily up to post-operative day 7

Postmortem examination of the anastomotic sites and abdominal viscera were performed at 10th, 20th, and 30th day after surgery. Dehiscence, leakage from anastomotic site, break down/loss of suture line, adhesion formation between anastomotic site and visceral organs or peritoneum, if any were recorded as described by Hanson *et al.* (1988). Twenty-four segments of colon (6 of both patterns and 12 controls) were opened longitudinally and then tissues were fixed in neutral-buffered 10% formalin for 48 hours. Two sections from each segment containing the anastomotic site were made in a transverse plane across the sutured incision lines. Two sections from each control were also made in a transverse plane. Formalin-fixed tissues were processed and sectioned at 6 μ m and stained with hematoxylin and eosin. The anastomotic site was examined histo-pathologically for apposition of tissue planes, degree of inflammation, presence of granulation and proliferation across the defect in each of the sections selected for sectioning and staining. Evaluation of sections was performed without prior knowledge of the postoperative period to which each section belonged.

RESULTS AND DISCUSSION

In the present study, all the anastomotic techniques were clinically successful. None of the dogs died and all of them returned to normal defaecation, urination, respiration and pulse within 24 hours after surgery.

Post operative changes in temperature, pulse rates, respiration rates, duration for the passage of first faeces and urine, normal intake of food and defaecation were recorded from day 1 to day 10. However, these were not significantly different among groups. Kachiwal and Kalhoro (2000) and Hanson *et al.* (1988) had also recorded similar observations while comparing appositional single layer, inverting two layer and appositional two layer end-to-end intestinal anastomotic techniques for colon in dogs and horses, respectively.

During this study, no clinical evidence of peritonitis, dehiscence, leakage from anastomotic site, break down/loss of suture line in any dog were noticed. This is in agreement with the findings of Athar *et al.* (1996) who compared double layer inverting technique with simple interrupted suture technique and Gambee sutures in dogs. This is also in agreement with the findings of Kachiwal and Kalhoro (2000) in dogs and Hanson *et al.* (1988) in horses, who compared double layer inverting technique with appositional 2 layer technique.

Average adhesions were 35% at 10th day after surgery with APP-2, where as INV-2 had 0% adhesions. Adhesions were significantly higher ($P < 0.01$) in APP-2 than INV-2 at 10th day after surgery (Table-1). Most of the adhesions of the INV-2 anastomoses were between the anastomotic line and the adjacent mesentery, whereas the APP-2 adhesions were between adjacent loops of colon. Most adhesions were observed within the first 20th days after surgery. Adhesion average was 55% with APP-

Table 1: Average adhesions to the intestinal anastomotic sites expressed as percentage of astomotic circumference

Days after surgery	Group-A	Group-B
10	35*	0
20	55*	40
30	7.5*	6
Over all mean	32.5+ 9.10	15.33 + 4.94

* = Significant difference between group-A and group-B at $P < 0.05$

** Significant difference between group-A and group-B at $P < 0.01$

2 pattern and 40% with INV-2 pattern. Adhesions were minimal with both patterns evaluated at 30th day after surgery. Average adhesions of 7.5% and 6% with APP-2 and INV-2 respectively were observed at 30th day. Adhesions were significantly higher ($P < 0.05$) with APP-2 than INV-2 at 20th and 30th days after surgery (Table-1). Kachiwal and Kalhor (2000) in dogs and Hanson *et al.* (1988) in horses had also made similar observations while comparing appositional two layer, inverting two layer and appositional single layer end-to-end intestinal anastomotic techniques for colon. Athar *et al.* (1996) also observed $79.2 \pm 18.8\%$ adhesions with double layer inverting suture technique.

At day 10 after surgery, all anastomotic lines were apposed but the mucosa did not bridge the anastomosis. The inversion formed a ridge protruding into the lumen around most of the circumference with APP-2 layer suture pattern. The mucosal and serosal veins adjacent to the incision were thrombosed. The sutures were surrounded by pyogranulomatous inflammation, immature granulation tissues with vascular buds and thickening of submucosa and serosa was apparent in some specimens. A thick serosa and patchy adhesions closed the anastomotic defect externally. This layer was composed of tissues ranging from immature granulation tissues consisting of proliferation fibroblasts and capillary buds to maturing granulation tissue with collagen deposition to dense fibrous connective tissue. The mucosa did not cover the lumen of the anastomosis. Similar change had occurred in the submucosa but the maturation process lagged behind that of the serosa with APP-2 layer suture pattern (Plate-1). The inversion was more pronounced in the specimens closed with INV-2 layer suture pattern. The cut ends of the mucosa and submucosa at the tip of fold were apposed by thick plug of fibrous connective tissue. The inverted serosal surfaces were held firmly together by fibrous connective tissue (Plate-2). Hanson *et al.* (1988) also recorded similar findings in horses after 10 days of surgery.

At day 20, the mucosa covered the internal surface and bridged the narrow defect with single layer epithelial cells in sections sutured with APP-2 layer suture pattern. The serosa and submucosa were thick and composed of dense, well-differentiated fibroblasts associated with collagen fibers and moderate numbers of capillaries (neovascularization). These changes were associated with multifocal granulomas characterized by central areas with birefringent material (suture material), which were surrounded by epithelioid macrophages, multinucleated giant cells, moderate



Plate 1: Tissue section through anastomotic site of the APP-2 from a dog at day 10. Note a moderate tissue inversion and complete bridging.



Plate 2: Tissue section of INV-2 pattern from a dog at day 10. Note an excessive inversion of the tissue layers

numbers of neutrophils and lymphocytes with fewer plasma cells. The center of some granulomas was composed of amorphous basophilic to eosinophilic material suggestive of mineralization and remnants of suture material. The fibrosis and neovascularization extended to the submucosa and mucosa. The surrounding muscular layers near the anastomotic site showed thickening characterized by increase in the

number of smooth muscle cells. The adjacent mucosa showed multifocal increase in the numbers of lymphocytes and plasma cells with rare neutrophils and macrophages in the lamina propria, associated with mild fibrous connective tissue proliferation (Plate-3). In specimens closed with INV-2 layer suture pattern, a slight inversion of the serosa and smooth muscle caused moderate thickening of the wall. The inverted serosal surfaces were held firmly together by mature fibrous connective tissue (Plate-4).

At day 30 after surgery, the specimens closed with APP-2 layer suture pattern showed mild multifocal increase in the numbers of lymphocytes and plasma cells in the lamina propria (Plate-5). The mucosa covered internal surface in all sections closed with INV-2 layer suture pattern, bridged the narrow defect with a single layer of epithelial cells. A band of mature fibrous connective tissue joined inverted smooth muscle bundles. A thin rim of epithelial macrophages and fibrocytes surrounded sutures in this part of the wall. The serosa and submucosa were thick and composed of dense, well



Plate 3: Tissue section of APP-2 pattern from a dog at day 20. Note a tissue inversion with apposition better than that in the INV-2 Pattern.



Plate 5: Tissue section from APP-2 pattern from a dog at day 30. Note a precise apposition of the tissue layers.



Plate 4: Tissue section of INV-2 pattern from a dog at day 20. Note an excessive and uneven inversion of the tissue layers.



Plate 6: Tissue section of INV-2 pattern from a dog at day 30. Note an excessive inversion.

vascularized connective tissue (Plate-6). Hanson *et al.* (1988) in horses observed after 28 days of surgery that the mucosa had bridged the narrow defect with a single layer of epithelial cells. A band of maturing fibrous connective tissue joined inverted smooth muscle bundles.

The current study showed the superiority of the APP-2 layer suture pattern in terms of better apposition of the anastomotic tissues and better anastomotic healing over the INV-2 layer suture pattern at 10, 20 and 30 days after surgery.

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