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The effect of different suture materials on the safety of colon anastomosis in an experimental peritonitis model

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Abstract. – BACKGROUND: The aim of this experimental study was to compare the safety of different suture materials in a left colonic anastomosis in the presence of peritonitis.

MATERIALS AND METHODS: Twenty-one male Wistar albino rats were randomly divided into three groups. First, left colonic injuries were created in all groups for the peritonitis model. After 24 hours, coated polyglactin 910 and silk suture were used in Group I rats, polydioxanone and silk suture were used in Group II rats, and coated polyglactin 910 plus antibacterial suture and silk suture were used in Group III rats during colonic anastomosis. Tissue hydroxyproline, anastomotic bursting pressure, and histopathologic findings on the anastomosis line were evaluated on the 10th postoperative day by performing a relaparatomy.

RESULTS: The mean bursting pressure values were 198 ± 11.37, 220 ± 17.7, and 244 ± 9.52 in Groups I, II, and III, respectively (Group I vs. II, $p < 0.035$; I vs III, $p < 0.002$; and II vs III, $p < 0.021$). The mean hydroxyproline levels were 1.21 ± 0.58, 1.47 ± 0.44, and 2.11 ± 0.32 in Groups I, II, and III, respectively (Group I vs II, $p < 0.338$; I vs III, $p < 0.011$; and II vs III, $p < 0.025$). When histopathologic findings of the groups were compared, the healing score of the intestinal tissue was higher in Group III than in Group I ($p < 0.015$), whereas there were no statistically significant differences among Groups I vs II and II vs III ($p < 0.081$ and $p < 0.095$, respectively).

CONCLUSION: Antibacterial suture usage increased anastomosis safety in the presence of peritonitis in resection and primary anastomosis.

Key Words:
Colon anastomosis, Antibacterial suture, Peritonitis.

Introduction

Gastrointestinal anastomoses are among the most commonly performed procedures in general surgery clinics[1]. Gastrointestinal anastomotic leakage is one of the major postoperative complications of gastrointestinal surgery[2]. Within the gastrointestinal system, anastomotic leakage is most frequently observed in the colon, and the risk increases through the distal colon. A lack of collateral connections for arterial nourishment, rich bacterial flora, and high collagenase enzyme activity facilitate the occurrence of leakage after surgical interventions. Studies pertaining to the healing of colon anastomosis have attracted the attention of colorectal surgeons for many years[3].

Performing a gastrointestinal anastomosis in the presence of peritoneal infection is an important issue of debate[4]. In various experimental studies, anastomotic leakage in the presence of peritonitis has been shown to occur. Therefore, in general surgery clinics, primary anastomosis is avoided and multistep procedures are preferred in cases of a contaminated abdomen during elective and emergent procedures involving left colon interventions. The reasons are as follows: failure of wound healing in a contaminated area, high risk for anastomotic leakage, and increased morbidity and mortality[5-7]. Colon anastomoses can be performed in different ways (e.g., single layer-double layer, inverting-everting, continuous-interrupted suture, end-to-end, end-to-side, side-to-side, manual or with stapler, and anastomosis with a biofragmentable anastomotic ring) using various suture materials. The most important principles to follow while performing anastomosis include supplying adequate circulation to the anastomosis line and good surgical technique[8].

In the present experimental study, different suture materials were compared in terms of anastomosis safety in left colon anastomosis in the presence of peritonitis.

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Materials and Methods

Protocol

This study was conducted at Firat University, School of Medicine, Experimental Animal Raising and Research Laboratory following approval from the local Ethics Committee. All experimental manipulations were undertaken in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Animals

Twenty-one male Wistar albino rats, 11 to 12 weeks of age and weighing 180 to 240 g, were acclimatized for 1 week before the experiments. The animals were kept in individual cages, housed at constant room temperature, and given standard rat chow. Only water was provided in the 12 h preceding the experiments.

Experimental Groups

The rats were randomly divided into three groups of seven rats each. In Group I, coated polyglactin 910 (Vicryl; Ethicon, Sommerville, NJ, USA) and silk suture were used for colon anastomosis of the rats. In Group II, polydioxanone (PDS; Ethicon, Sommerville, NJ, USA) and silk suture were used. In Group III, coated polyglactin 910 plus antibacterial suture (Vicryl Plus; Ethicon, Sommerville, NJ, USA) and silk suture were used.

Experimental Design

All rats were anesthetized by a combination of 5 mg/kg Xylazine (Rompun; Bayer, Istanbul, Turkey) and 30 mg/kg ketamine hydrochloride (Ketalar; Parke-Davis, Istanbul, Turkey). All animals breathed spontaneously throughout the procedures. The mid-abdominal area was shaved and prepared with povidone iodine as an antiseptic and covered with sterile drapes by leaving the operation site uncovered. The abdomen was accessed through an approximately 4-cm vertical incision after passing through the cutaneous and subcutaneous tissues, linea alba, and peritoneum. The left colon was cut full-thickness 2 to 3 cm above the peritoneal reflection. The feces within the lumen were spread onto the border of the wound to provide absolute fecal peritonitis. Thereafter, the abdomen was closed by continuous suturing with 3/0 silk in two layers (fascia and skin). One day later, the abdomen was reopened under general anesthesia. After the abdomen was washed with saline, the rats were divided into three groups prior to the colon anastomosis. For the colon anastomosis, the rats in Group I underwent full-layer continuous suturing with 5/0 vicryl for closure of the first layer, and seromuscular Lambert suturing with 5/0 silk for closure of the second layer. The rats in Group II had full-layer continuous suturing with 5/0 PDS closure of the first layer, and seromuscular Lambert suturing with 5/0 silk for closure of the second layer. The rats in Group III had full-layer continuous suturing with 5/0 coated Vicryl plus antibacterial suture for closure of the first layer, and seromuscular Lambert suturing with 5/0 silk for closure of the second layer. After the anastomoses was completed, the abdomen was closed by continuous suturing with 3/0 silk in two layers (fascia and skin). The subjects were kept under conditions of stable temperature and humidity at Firat University, School of Medicine, Experimental Animal Raising and Research Laboratory until the day of surgery, as well as the day of the relaparotomy (10^{th} postoperative day). During this period, all rats were given a normal diet. On the 10^{th} postoperative day, relaparotomies were performed on all rats following general anesthesia to evaluate anastomotic improvement. The rats were subsequently evaluated by the same surgeon, who had no information about the groups in terms of complications, such as anastomotic leakage, abscess, and fistulas.

Measuring the Bursting Pressure of the Anastomosis Site

Anastomotic bursting pressures were calculated *in vivo* using the following procedure. A catheter was inserted through the anus and advanced 2 to 3 cm proximally so that the tip of the catheter lay at the level of the anastomosis. The peritoneal cavity was filled with saline. The colon was tied with 2/0 silk at 2 cm above and below the suture line. With both ends tied and a catheter inside, the segment of the colon was filled with saline colored with methylene blue at a rate of 4 mL/min using an infusion pump (Abbott LC 5000 infuser, Chicago, IL, USA). During the infusion, pressures were monitored (Petas KMA 375 S/N 0013, Turkey) by a pressure transducer (Abbott Single Transpact, Park, IL, USA). The pressure value was taken while blue intracolonic flued leaked out into the peritoneal cavity and was recorded as the bursting pressure.

Obtaining the Samples

A 2-cm colon segment, including the anastomosis line, was resected and then dissected throughout the colon lumen and washed with normal saline to remove intestinal contents. One-third of this tissue

was placed into vials containing 10% formaldehyde for later histopathologic examination. The remaining two-thirds of the tissue was wrapped with aluminum foil and stored in a biochemical laboratory to determine the level of hydroxyproline.

Measuring Hydroxyproline Level

Two-thirds of the 2-cm segment of the colon, including the line of anastomosis, was washed with distilled water, dried with a blotter, sectioned into small pieces during tissue homogenization, and kept frozen at –80°C until the day of the test. The hydroxyproline level was determined by modifying the method described by Woessner[9]. During the procedure, OH-P standard, chloramine-T, P-dimethyl amino benzaldehyde, perchloric acid, isopropanol, Na acetate $3H_2O$, Na citrate $5.5H_2O$, 12 N HCL, and 1 mM HCL were used. Reagents were added to the sample and blank in order, vortexed, and incubated in a water bath at 60°C for 25 minutes. Optical density was measured at 558 nm, and the concentration of hydroxyproline was calculated by comparing it with the blank. Results are expressed as mg/g dry tissue.

Histopathological Examination

Tissue samples obtained from the anastomotic site were embedded into paraffin blocks following routine histochemical procedures. Four- to five-micron-thick sections were stained with hematoxylin-eosin and examined under a light microscope. The degree of wound healing at the line of anastomosis was graded on a scale of I to IV: grade I, fibrinopurulent exudate; grade II, granulation tissue < 25%; grade III, granulation tissue between 25% and 75%; grade IV, granulation tissue > 75% or collagen fibers < 25%; and grade V, collagen fibers > 25%[10].

Statistical Analysis

Data were analyzed using SPSS 15.0 for Windows (SPSS, Inc., Chicago, IL, USA). The Kruskal-Wallis test was used to determine whether there was a difference among the groups. A Mann-Whitney's U test was used to determine differences between two groups. A p-value of < 0.05 was considered to be statistically significant.

Results

A total of 21 male Wistar albino rats with body weights ranging from 180 to 240 g were used in this experimental study. No statistically significant differences in weight or age were detected among the rats in all three groups after randomization. During the study, no mortality was detected in any of the groups, and no complications, such as wound breakdown, intraabdominal abscess, gangrenous lesion and incisional hernia, were observed. No anastomotic separation or gross anastomotic leakage was detected in any of the rats included in the abdominal exploration performed on day 10.

Measuring the Bursting Pressure of the Anastomosis Site

The highest bursting pressure value was observed in Group III, which was statistically significant compared with the other two groups ($p < 0.002$ for I vs III and $p < 0.021$ for II vs III). The bursting pressure was higher in Group II than in Group I, and the difference was statistically significant ($p < 0.035$). The mean bursting pressure and statistical analysis of all groups are summarized in Tables I and II, respectively. The statistical differences among all groups are also shown in Figure 1.

Tissue Hydroxyproline Level

Mean tissue hydroxyproline values were higher in Group III than in the other two groups (Table I). This high level of hydroxyproline was statistically significant when compared with Groups I ($p < 0.011$) and II ($p < 0.025$). The hydroxyproline value was higher in Group II than in Group I. However, the difference was not statistically significant ($p < 0.338$) (Table II). Statistically significant differences among all groups are shown in Figure 2.

Table I. Comparison of the mean±standard deviation values of bursting pressure, tissue hydroxyproline level, and wound healing score of the groups.

Parameters	Group-I (mean ± SD)	Group-II (mean ± SD)	Group-III (mean ± SD)
Bursting pressure (mmHg)	198 ± 11.37	220 ± 17.7	244 ± 9.52
Hydroxyproline (mg/g dry tissue)	1.21 ± 0.58	1.47 ± 0.44	2.11 ± 0.32
Wound healing score	3.43 ± 0.53	4.00 ± 0.57	4.57 ± 0.78

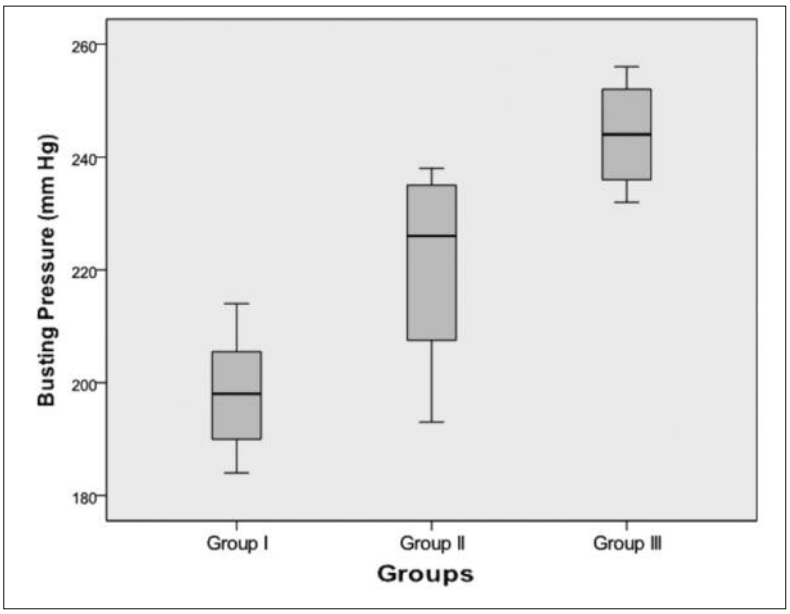

Figure 1. Anastomosis site bursting pressure levels of the study groups.

Wound Healing Score

The highest mean wound healing score was observed in Group III compared with the other two groups. This difference was statistically significant when compared with Group I ($p < 0.015$), whereas no statistically significant difference was observed between Groups II and III ($p < 0.095$). When Groups I and II were compared, the wound healing score was higher in Group II. However, the difference was not statistically significant ($p < 0.081$). The mean wound healing score of the three groups and statistical analysis are summarized in Tables I and II, respectively.

Discussion

Anastomotic leakage is a leading complication affecting surgeons practicing colorectal surgery. Anastomotic leakages that develop after resection and primary anastomosis performed especially because of colorectal cancers may suppress the patient's immune system. This immunosuppression could result in much more serious mortality and morbidity, which is an unpleasant reality. Because the number of microorganisms within the colon lumen is higher, as compared with other parts of the gastrointestinal system, the frequency of leakage from colon anastomosis has been observed to be higher than that from anastomoses performed in other locations of the gastrointestinal system in terms of morbidity and mortality[3]. In addition, the risk of anastomotic leakage in the left colon is higher when compared with the other parts of the colon because of less arterial feeding, higher stool density, and larger numbers of microorganisms in the left colon.

Many factors have been shown to have a profound effect on wound healing and colonic anastomosis. Systemic and local factors play a role in anastomotic wound healing. Anemia, hypovolemia, low arterial pO_2, neutropenia, malnutrition, vitamin and zinc deficiency, high-dose corticosteroids, and uremia are some of the systemic factors that reduce wound healing. Local factors consist of infections, intestinal contents, prophylactic antibiotics, suturing techniques, radiation, mesenteric vascular occlusion, and suture materials[11-14].

Table II. Statistical evaluation of bursting pressure, hydroxyproline, and histopathologic wound healing score of the groups.

Parameters	Group-I vs II	Group-I vs III	Group-II vs III
Bursting pressure (mmHg)	0.035*	0.002*	0.021*
Hydroxyproline (mg/g dry tissue)	0.338	0.011*	0.025*
Wound healing score	0.081	0.015*	0.095

*$p < 0.05$ is significant for the Mann Whitney U-test.

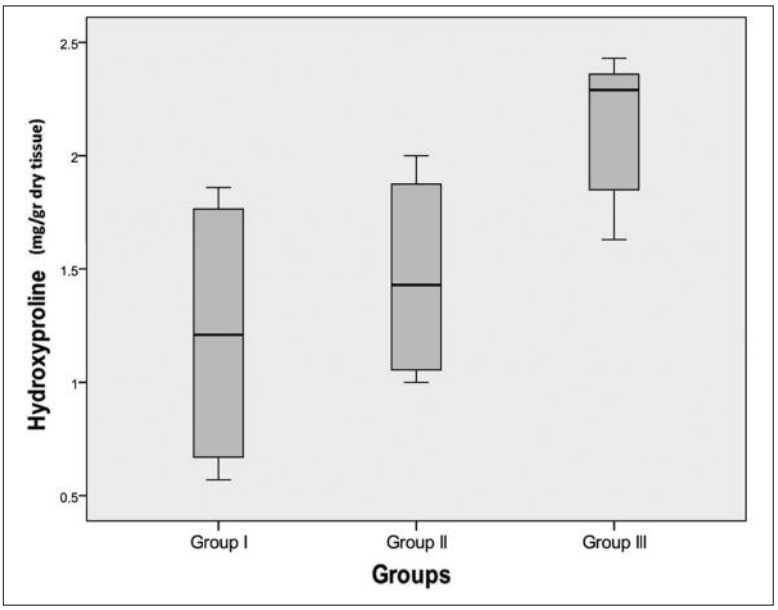

Figure 2. Anastomosis site tissue hydroxyproline levels of the study groups.

Foreign bodies in wounds are known to negatively affect wound healing. The most frequently encountered foreign bodies in wounds are suture materials. Therefore, a good suture material must cause minimal tissue reaction and should be resistant against bacterial colonization[15]. This feature is closely related to suture material being absorbable or nonabsorbable and monofilament or multifilament. The most frequently used suture materials in colorectal anastamosis are absorbable monofilament polydioxanone and poliglecaprone, absorbable multifilament polyglactin, and nonabsorbable monofilament silk suture materials. In addition to these materials, several studies on three recently introduced antibacterial suture materials coated with triclosan have been published in the literature. These are multiflament polyglactin 910 plus antibacterial suture (Vicryl Plus), absorbable monofilament polydioxanone plus antibacterial suture (PDS Plus), and absorbable monofilament poliglecaprone plus antibacterial suture (Monocryl Plus). Triclosan is adsorbed to all three of these products by different means during sterilization. Among these three suture materials, some intestinal anastomosis were performed only with Vicryl Plus antibacterial suture material, whereas no intestinal anastomoses were performed with the other two suture materials[16]. In this study, we compared Vicryl Plus suture material with two different non-antibacterial suture materials.

The development of an infection, which has the most negative effect on healing, is directly associated with the degree of bacterial adherence to the suture material. The characteristics of suture materials differ with regard to bacterial adherence. The potential effect of suture material on an infection can be reduced by coverage with antimicrobial agents. Because antimicrobial suture material can markedly reduce the number of bacteria, its use has been suggested in clean or clean-contaminated wounds in which other suture materials can potentiate the infection. In many studies, it has been determined that bacteria can move within multifilament suture material due to its capillarity and fluid absorption properties, and the bacterial transport rate is associated with the degree of this property[17].

Andersen et al[18] determined that the antibacterial chemical structure of polyglactin 910 has a considerable effect on infection. It has been reported that the adherence of *Staphylococcus aureus* and *Escherichia coli* to polydioxanone is due to its monofilament and chemical structure. In addition, bacterial adherence is related to the type of bacteria and the duration of contact, as well as the structure of the suture[19]. When the structural characteristics of suture materials are compared, polyglactin 910 is more favorable than polydioxanone in terms of durability, resistance to tension, and rare breaks during knot-tying[20]. When the resistance against friction is examined, monofilament polypropylene and monofilament nylon provide the most reliable knot among nonabsorbable suture materials. It has been observed that among absorbable suture materials, braided sutures are more reliable than monofilaments. Moreover, monofilament synthetic suture materials can easily become untied[21].

Intraperitoneal sepsis is one of the major obstacles in surgery. The optimal approach to left

colonic disease complicated by perforation and subsequent peritonitis is still under debate[22]. The negative effects of intra-abdominal infections on the improvement of a colon anastomosis have been demonstrated in clinical and experimental studies[22-24]. In an infection model developed by Ahrendt et al[25] in which the cecum of rats was tied and perforated, resulting infection led to a decrease in anastomotic bursting pressure and collagen concentration.

Multi-stage procedures are generally accepted in cases of generalized fecal peritonitis. However, there is a recent trend toward one-stage resection and anastomosis in selected cases in parallel with the use of broad-spectrum antibiotics, preoperative patient preparation, increased importance of postoperative patient care, advances in surgical techniques and intensive care conditions, and a better understanding of the wound healing mechanism. Hence, new methods increasing the safety of colonic anastomosis performed in peritonitis medium are required.

Coated polyglactin 910 suture (Vicryl; Ethicon) is the most commonly used suture material in the world. Because the suture knot is believed to be the principal site of bacterial colonization in the wound, polyglactin 910 sutures coated with triclosan (Vicryl Plus; Ethicon) were developed to imbue the suture material with antibacterial activity against the most common putative pathogens that cause surgical site infection (SSI). The active component in polyglactin 910 suture coated with triclosan is triclosan itself, a broad-spectrum antiseptic agent. In experimental studies using a guinea pig model, Storch et al[27] found that polyglactin 910 antibacterial suture material reduced bacterial colonization 30.5-fold compared with the standard polyglactin 910 suture materials. Gomez et al[16] conducted research on two animal models for evaluating the effectiveness of antibacterial suture in abdominal surgery. The results of these studies showed that the antibacterial suture material microbiologically prevented the colonization of bacteria, clinically regulated the inflammatory response, and allowed for tissue healing, even in an infectious environment. Fleck et al[27] retrospectively examined 479 cases in which they performed cardiac surgery. They showed that none of the 103 patients in whom antibacterial suture was used developed wound infection, while 24 of 376 patients in whom normal suture material was used developed wound infection. In studies comparing classical polyglactin 910 suture materials with antibacterial polyglactin 910 suture material in pediatric patients subjected to a variety of surgical procedures, Ford et al[28] did not find a significant difference between two groups in terms of wound healing parameters with the exception of pain. The results obtained in this study statistically show that the anastomotic bursting pressure and tissue hydroxyproline levels for the group in which coated polyglactin 910 plus antibacterial suture was used were higher than those in the other two groups.

Mechanical, biochemical, and histopathologic methods are used to determine the degree of improvement in intestinal anastomoses[29,30]. As a mechanical method, bursting pressures of intestinal segments, including the anastomosis site, are measured. The tissue hydroxyproline level, which is an indicator of the tissue collagen level, is determined via biochemical methods. Histopathologic methods are performed to examine the improvement stages of anastomotic wounds[29]. In our study, we also used anastomosis bursting pressures, tissue hydroxyproline levels, and wound healing score methods together to demonstrate the safety of anastomosis.

Conclusions

Despite advances in modern medicine and surgical techniques, anastomotic colon leakages are still an important cause of serious morbidity and mortality. In general, surgeons prefer to perform two-step procedures instead of primary anastomosis when encountering a dirty abdomen during abdominal surgery. This is disadvantageous in terms of patient comfort and cost. Many factors play a role in the occurrence of anastomotic leakages. In the present study, it was considered that the use of antibacterial sutures would increase the safety of anastomosis by reducing the harmful effects of an infected abdomen, and the mortality and morbidity rates in intestinal anastomoses would thus be decreased. Based on the outcomes of the present study, it was suggested that antibacterial suture can be used as an alternative method for the safety of primary anastomosis in dirty abdomens. However, further experimental and clinical studies are needed for clinical use.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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