

Splenic Autotransplantation is Safe in Colorectal Surgery

Kolorektal Cerrahide Dalak Ototransplantasyonu Güvenlidir

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ÖZET

Amaç: Kolorektal cerrahide en ciddi komplikasyon anastomoz kaçağıdır. Dalak kolon yaralanma birlikteliği %10 olarak bildirilmektedir. Elektif kolon cerrahisi esnasında iyatrojenik dalak yaralanma oranı %1,4 ve splenektomi oranı %0,3' dür. Splenektomi eklenen kolon cerrahisi sonrası karın içi enfeksiyon oranları 5 kat artmaktadır. Bu çalışmada splenektominin kolon anastomoz yara iyileşmesine etkileri ve eş zamanlı dalak ototransplantasyonunun güvenilirliği araştırılmıştır. **Yöntemler:** Toplam 30 rat üç eşit gruba ayrıldı, hepsinde inen kolonda rezeksiyon ve anastomoz uygulandı. Grup II'de işleme splenektomi eklenirken, grup III'te splenektomiyi takiben dalak ototransplantasyonu yapıldı. 14. gün ratlar sakrifiye edildi. Gruplarda mortalite, adezyon, histolojik ve mekanik yara iyileşme skorları ve periton kültür sonuçları karşılaştırıldı. Ek olarak ototransplantasyon grubunda ekilen dalak dokusu

ABSTRACT

Objective: The most serious complication of colorectal surgery is anastomotic leak. The rate of combined spleen-colon injuries is 10%. In elective colon surgery, iatrogenic splenic injury is seen as 1.4% and 0.3% of patients may require splenectomy. After colonic surgery, intra abdominal infection rate increases five-fold when splenectomy is added. In our study, we aimed to evaluate the effects of splenectomy on wound healing in colonic anastomosis and if simultaneous splenic autotransplantation can be implemented safely. **Methods:** 30 rats were divided in to three equal groups and resection-anastomosis was performed in the descending colon in all rats. Splenectomy was added in group II and autotransplantation of spleen in group III. On the 14th day, rats were sacrificed. Mortality, adhesion scores, histological and mechanical healing, peritoneal culture results were examined. In addition, the transplanted splenic tissue was evaluated histopathologically.

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histolojik olarak incelendi.

Bulgular: Grup II ve III'te mortalite %20 iken grup I'de mortalite izlenmedi. Grup III'te ekilen dalak dokularının canlılığı histopatolojik olarak gösterildi. Patlama basınçları ve histolojik yara iyileşme skorları değerlendirildiğinde, istatistiksel fark olmasa da en iyi değerler Grup I'de saptanırken, bu değerleri sırasıyla ototransplantasyon ve splenektomi grupları izledi ($p>0.05$). Ortalama adezyon skorları en düşük splenektomi grubunda bulundu ($p>0.05$). Grup I'de periton kültürleri negatifken, grup II ve grup III'te sırasıyla %37.5 ve %12.5 üreme tespit edildi.

Sonuç: Splenektomi yara iyileşmesini kötü etkilemiyor görünse de olası antibakteriyel etkileri ve immünolojik faydaları sebebiyle kolorektal cerrahi esnasında splenik ototransplantasyon güvenle yapılabilir.

Anahtar Kelimeler: Dalak ototransplantasyonu, Kolorektal cerrahi, Dalak yaralanması, Yara iyileşmesi

Results: There was no mortality in group I. Mortality was 20% in group II and III. Histopathologic evaluation revealed viable splenic tissue in group III. When bursting pressures and histologic wound healing scores were analyzed, despite no statistically significant difference in terms of bursting pressure, the highest values were obtained in control group followed by splenic autotransplantation and splenectomy groups ($p>0.05$). Mean adhesion scores were lower in splenectomy group ($p>0.05$). The cultures were negative in group I, 37.5% and 12.5% in group II and group III, respectively.

Conclusion: Although, splenectomy does not adversely affect wound healing, the spleen should be protected for possible antibacterial effects and immunologic benefits. Simultaneous splenic autotransplantation can be performed safely in colorectal surgery.

Key words: Splenic autotransplantation, Colorectal surgery, Splenic injury, Wound healing

Introduction

The leading cause of morbidity and mortality after colorectal surgery is anastomotic leakage and peritonitis. Especially in emergency interventions, concomitant pathologies distorting general condition such as peritonitis and sepsis increase the rate of complications.¹ The rate of infection and mortality were found to be 25% and 3.6% in the patients with colon injuries respectively. In multiple regression analysis, it has been indicated that, splenic injury negatively affected these rates.² The incidence of combined spleen-colon injuries has been reported to be 10% after blunt abdominal traumas.³ The belief of "splenic autotransplantation is relatively contraindicated if there is a concomitant colon injury" is dominant in surgeons. Mettke et al. have investigated 46.682 patients who underwent surgery for colorectal cancer in respect of iatrogenic splenic injury, and found that injury and splenectomy rates were 1.4% and 0.3% respectively.⁴ Blackwood *et al.* have identified the rate of intraabdominal sepsis requiring reoperation as 5.7% after splenectomy and 8.9% after colon injury. This rate was 46.7% in combined spleen-colon injury if splenectomy was required. Ultimately, the idea that protection of the spleen is an indication rather than a

contraindication gained value in spleen injuries accompanying colon injuries.⁵ In our study, we aimed to demonstrate whether splenic omental autotransplantation is safe in colonic anastomosis.

Materials and Methods

This experimental study was held in the animal laboratory of the Ministry of Health Ankara Education and Research Hospital. The study was started after documented approval of the hospital's ethical committee for animal experimentation.

A total of 30 male Wistar-Albino rats, 16-18 weeks of age and weighing between 160-180 g. were given single dose of 50 mg/kg intramuscular ceftriaxone (Forsef iv, Bilim Pharmaceutical Industry and Trade Co., Istanbul, Turkey) preoperatively after 6 hours of fasting from the standard rat diet. Anesthesia was provided by intramuscular injection of 75 mg ketamine HCl and 5 mg xylazine HCl (Rompun) per kilogram bodyweight. After shaving the abdomens, the abdominal midline was disinfected with 70% alcohol and a 5-cm standard midline incision was made. Colonic distension was provided by giving 2 ml of 0.9% saline with a catheter inserted into



Figure 1. Splenic fragments prepared for the transplantation in the omentum.

the anal canal. The colon was perforated 4 cm proximal from anus with a scissor. Thus, peritoneal contamination was provided.

Subsequently, 30 rats were divided into 3 groups according to the surgical technique:

Group I (n=10): Resection and anastomosis

Group II (n=10): Resection, anastomosis and splenectomy

Group III (n=10): Resection, anastomosis, splenectomy and omental splenic autotransplantation

After the resection of the perforated area with 1 cm margins, the anastomosis was performed in continuous fashion with 6/0 polyglactin (Vicryl, Ethicon Endo-Surgery, Cincinnati, USA). The abdomen was filled



Figure 2. The spleen slices were attached in an omental pouch created by sutures.

with saline, air was given through the catheter which was previously inserted for air-bubble test. Abdomen was irrigated with 20 cc warm 0.9% saline and 2 ml of saline was left intraperitoneally before closure. Laparotomy was closed by a continuous suture technique with 3/0 polyglactin (Vicryl, Ethicon Endo-Surgery, Cincinnati, USA) suture. Splenectomy was added for group II. Splenic vein and artery were ligated by using 4/0 polyglactin (Vicryl, Ethicon Endo-Surgery, Cincinnati, USA) suture. In group III, spleen was cut into 2 mm slices after splenectomy (Figure 1). The slices including 50% of total splenic tissue were transplanted in the omentum with 4/0 polyglactin (Vicryl, Ethicon Endo-Surgery, Cincinnati, USA) suture (Figure 2). Spleen was measured by a sterile ruler before slicing to define the amount for autotransplantation. All operations were performed by a single surgeon. All rats were fed with standard rat diet postoperatively. Autopsy was carried out on rats which were died before the 14th postoperative day. During autopsy peritoneal fluid samples were obtained for microbiological examination. All of the living rats were sacrificed after sedation by giving 10 ml intracardiac air at the 14th postoperative day. Re-laparotomies were performed with right paramedian incision in order to observe adhesions. Peritoneal samples were also collected for microbiological examination for all rats. The abdominal adhesions were evaluated by the adhesion score scale described by Bothin et al (Table 1).⁶ The colon was divided 1.5 cm proximal and distal of the anastomosis for the measurement of bursting pressure. This segment was removed together with the surrounding

Table 1. Adhesion Scoring Scale.⁶

Description	Score
No adherence	0
A single adherence between two organs or between an organ and the abdominal wall	I
Two adhesions between organs or one organ and the abdominal wall	II
More than two adhesions between the organs or a massive generalized adherence of the intestine with no adherence to abdominal wall	III
Generalized adhesions between the organs and the abdominal wall or massive adherence among all organs	IV

adhesions and the organs, to prevent anastomosis from possible damages. The colon was cleaned from fecal content by washing with saline before the measurements. Following biomechanical measurement, the colonic segments were stained with hematoxylin and eosin then scored by a single pathologist. The Verhofstadt scoring system was used to evaluate histological parameters such as necrosis, polymorphonuclear cell (PMN), lymphocytes, macrophages infiltration, edema, mucosal epithelium and sub mucosal muscle. During this evaluation, the amount of necrosis at anastomotic site was shown none (0 points), some patches (two points), or massive (three points). For cellular infiltration (Polymorphonuclear cells, macrophages, and lymphocytes) none or normal number was accepted as zero points, slight increase as one point, marked infiltration as two points and finally massive infiltration as three points. To show the amount of edema, the ratio of the intestinal wall thickness at the anastomotic site to non-anastomotic site was used. If equal or less, level of edema was accepted as none (zero points), some (1-1.5* normal thickness; one point), marked (1.5-2* normal thickness; two points), or severe (>2* normal thickness; three points). Mucosal healing was expressed as normal if restored glandular epithelium was shown (zero points). The healing points were as following, intact mucosa with cubic epithelium but without glands (one point), mucosa only partially covered by cubic epithelium (two points), or mucosa completely devoid of epithelial coverage (three points). Submucosal-muscular repair was assessed with fibroblast stretching and bridging the anastomotic wound in terms of good (zero points), average (one point), poor (two points), or no (three points).⁷

Data analysis was performed in SPSS 20 program. Kruskal-Wallis test was used to examine the difference between the groups in terms of wound healing score, adhesion score and bursting pressure. When statistically significant values were found, paired comparisons were performed by using the Mann-Whitney U test. A p value of <0.05 was considered statistically significant.

Results

While there was no mortality in the control group, two rats died from each group II and group III. From group II, one rat died due to intraabdominal hemorrhage at the second day after surgery and the other rat died as a result of anastomotic leakage at the 7th. In group III, one rat died at the 5th postoperative day and another one died on the 8th day. In the rat which died at the 5th day, a colonic anastomotic leakage was detected. But in the rat which died at the 8th day, despite anastomosis was macroscopically normal, generalized peritonitis was detected and cause of death is recorded as micro perforation.

The abdominal cultures obtained were negative in group I. In group II, in one rat *Escherichia coli* and in two rats *Bacteroides fragilis* was grown. Finally, an abscess was detected in the neighborhood of splenic autotransplantation pouch in one rat from group III and the culture of the abscess revealed *Escherichia coli*. While the cultures in group I were negative, 37.5% and 12.5% growth were observed in group II and group III, respectively. The adhesion scores and bursting pressures were analyzed in groups. In the statistical analysis, there was no significant difference between the groups in terms of adhesion scores (p=0.237) and bursting pressures (p=0.152).

Table 2. Comparison of Groups' Scores.

	Group I	Group II	Group III	Total	p
Adhesion Score	2+/-0.471	1.375+/-0.916	1.750+/-1.281	1.730+/-0.919	0.237
Necrosis Score	0	0.125+/-0.353	0	0.385+/-0.196	0.325
PMN cells Score	1+/-0.942	1+/-1.069	0.625+/-0.744	0.884+/-0.908	0.671
Lymphocytes Score	1.2+/-0.421	1.125+/-0.624	1.375+/-0.571	1.230+/-0.514	0.637
Macrophages Score	1.1+/-0.994	1.125+/-1.125	0.500+/-0.534	0.923+/-0.934	0.365
Edama Score	0.900+/-0.576	0.625+/-0.517	1.000+/-1.195	0.692+/-0.549	0.310
Mucosal Epithelium Score	0.600+/-0.843	1.250+/-1.281	1.500+/-1.195	0.923+/-1.092	0.503
Submucosal Muscle Score	1.200+/-1.135	1.750+/-1.488	1.500+/-1.603	1.461+/-1.363	0.804
Bursting Pressure (mmHg)	238.100+/-56.220	201.875+/-46.859	194.125+/-10301	213.423+/-46.774	0.152

When the wound healing scores were examined, scores did not show a significant difference between groups ($p=0.945$). The groups mean healing scores and bursting pressure are summarized in Table 2.

Splenic tissues collected by omentectomy were examined histopathologically and viable splenic tissues including severe inflammation and local necrosis were detected in all auto transplanted rats.

Discussion

Whereas the majority of blunt trauma/traumatic colon injuries were fatal until the First World War, this rate declined to 70% with the advances in surgery in the middle of the twentieth century.⁸ Unlike primary repair used in the early period, during the Second World War, mostly the surgeries with colostomy were performed and mortality declined to 30%. This rate was decreased to 10 to 15% with the use of antibiotics, fluid replacement and blood transfusions.⁹ Today, mortality of colon injuries and post-operative complication rate are 5% and 15-50%, respectively.^{10,11}

When the limited number of studies in the literature are examined, the rate of splenic injuries accompanying the colon injuries is 10-22%.^{3,12} There are also risks of splenic injury in elective colorectal surgery. In a large retrospective study, 46682 colorectal cancer surgery patients were examined; injuries and splenectomy were found to be as 1.4%, 0.3%, respectively. The same researchers observed morbidity and mortality significantly lower in patients who have undergone spleen repair than those with splenectomy.⁴ For this type of injuries, splenorrhaphy is usually unsuccessful.¹³

Surgeons have been looking for alternatives in the surgical treatment of sepsis seen in splenectomized patients.^{15,16} With the development of surgical technique and abdominal imaging, splenorrhaphy and follow-up without surgery were accepted and applied routinely.¹⁷ In a study, 58 patients with splenic injury, 90 patients with colon injury and 13 patients with combined colosplenic injury were examined, intra-abdominal sepsis rate requiring reoperation in splenectomy group was 5.7%, the same rate was found to be as 8.9% in colon repair group and 46.7% in combined injury. These results reinforce the idea of protecting spleen after injuries including both spleen and colon.⁵

However, the splenic autotransplantation is recommended

for splenic injuries that cannot be treated conservatively. In the case of spleen injuries concomitant with colon injuries, the idea of avascular splenic implants will act like an agar for microorganisms is widely accepted. Moore et al. examined splenic autotransplantation in 43 patients with isolated spleen injury and in 23 patients with combined colo-splenic injuries, postoperative infectious complications were seen in only one patient. Consequently, it was reported that peritoneal contamination is not a contraindication to perform spleen autotransplantation. In our study, we perforated the descending colon at the beginning of the procedure to see the effect of peritoneal contamination on transplanted splenic tissue and whether if it was safe in septic abdominal cavity.

In daily practice the commonly used method for splenic autotransplantation is the transplantation of splenic tissue in pouches created in the omentum.^{18,19} Other methods are the placement of splenic slices in to the preperitoneal subfascial region or adhering them to the liver.^{20,21} Linuma et al. transplanted 25, 50, 100, 200 and 300 mg splenic tissue in the omental pouches, intramuscular field or intraperitoneally and found that transplantation of 50% of the spleen in the omental pouch is the most effective transplanting location.²² It was also reported that neovascularization in transplanted spleen started in the 3rd day after transplantation and the blood supply of fragments was provided as centripetal from the splenic, short gastric, mesenteric and gastroepiploic arteries.²³ In our study, more than 50% of splenic tissue after splenectomy was divided into 2 mm slices and implanted in the omentum. In the examination performed at the 14th postoperative day, histopathologically intact splenic tissue was detected in all rats.

There are different experimental methods for determining if transplanted spleen has a function or not. Intravenous pneumococcal bacterial clearance²⁴ and intraperitoneal pneumococcal inoculation²⁵ are some of these methods. Mycoplasma or Haemophilus influenzae clearances are also used in these examinations.^{26,27} Marques *et al.* has reported a protective effect of splenic transplantation on Escherichia coli sepsis in rats.²⁸ However, the protected spleen cannot achieve its former functional capacity.²⁹ In a splenectomized patient, the effectiveness of splenic autotransplantation must be revealed by different methods for ethical reasons because relaparotomy or bacterial

clearance measurements are not possible to show splenic function.³⁰ Patel *et al.* used platelet count, peripheral blood smear, levels of IgM and C3 and also scintigraphy and reported that, the platelet count, IgM and C3 levels returned to normal values, Howell-Jolly bodies and target cells disappeared on peripheral blood smear and the presence of functional splenic tissue in the scintigraphy performed 8 weeks after procedure.³¹ The aim of our study was not to evaluate the hematological effects of transplanted splenic tissue. What we wanted was to determine whether omental transplantation can be done safely in colorectal surgery. Therefore, transplanted splenic tissue was only examined histopathologically. The complications of splenic autotransplantation are very rare. Intestinal obstruction,^{32,33} and development of abscess,^{14,34} have been reported. In our study, the spleen fragments, which were inoculated to the omentum, were examined histopathologically and severe inflammation and scattered necrotic areas were detected. However, these findings did not cause a decline in anastomotic healing or an increase in adhesions. The most important factors that cause abdominal adhesions are previous operations, foreign bodies and necrotic materials. Intraabdominal infections, abscesses and fistulas increase adhesion formation.³⁵ In our study, there was no significant difference between the adhesion scores. The highest mean adhesion score was obtained in group I. This was followed by group III. The splenectomy group showed the lowest adhesion scores. This was probably secondary to reduced immune response in splenectomy. In our study, auto transplanted splenic parts were not found to be as a risk factor for adhesion formation.

Although wound healing in colorectal surgery has been studied in various different conditions, the effect of splenectomy to the healing of colon anastomosis has not been presented in an adequate level. In the literature, we could not find any other study which examined the interaction of splenic autotransplantation and colonic anastomosis.

In a small number of case reports, it was reported that, chronic wounds closed spontaneously in patients who underwent splenectomy.^{36,37} Some publications are also available indicating that splenectomy has long term adverse effects in wound healing.³⁸ Werber *et al.* found a severe reduction in fibroblastic activity and wound

healing in the skin incisions one month after splenectomy.³⁹ In our study, wound healing scores and bursting pressures were used as an indicator of wound healing. Although the difference between groups was not statistically significant, the highest bursting pressure values were obtained in the control group. It was followed by splenic autotransplantation and splenectomy group, respectively.

In histopathology, there were no significant difference between necrosis, polymorphonuclear cells, lymphocytes, macrophages, edema, mucosal epithelium and submucosal muscle measurements. It has been concluded by Naycı *et al.* that splenectomy did not affect the healing in colonic anastomosis. In the same study, septic conditions were found to be adversely affecting anastomotic healing and this effect of sepsis reduced after splenectomy. The authors discussed that these result were secondary to the impaired maturation of T lymphocytes which have adverse effects on wound healing.⁴⁰ In our study, splenectomy caused a decrease in the colon bursting pressure. Higher bursting pressure values were obtained in the splenic autotransplantation group. We could not detect any retardation in wound healing in 3 splenectomized rats with positive abdominal cultures. This was compatible with the findings of Naycı *et al.* In the splenectomy group, we detected lower adhesion scores and this may also be caused by T cells immaturation.

In conclusion, omental splenic autotransplantation can be performed safely in colorectal surgery requiring splenectomy. Although splenectomy did not seem to be affecting wound healing adversely, we believe that the spleen should be protected due to the postoperative lower infection risk and the possible infectious and immunological benefits.

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