EXPERIMENTAL STUDY

Comparison of "primary repair" and "placing a drain without repair" methods in duodenum perforations

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ABSTRACT

BACKGROUND: Duodenal ulcer perforation is a serious condition. A number of methods have been defined and used in surgical treatment. In this study, it was aimed to compare the effectiveness of "primary repair" and "drain placement without repair" methods in duodenal perforations using an animal model.

METHODS: Three equivalent groups of ten rats each were formed. Perforation was created in the duodenum in the first (primary repair/sutured group) and the second group (drain placement without repair/sutureless drainage group). In the first group, the perforation was repaired with sutures. In the second group, only a drain was placed in the abdomen without sutures. In the third group (control group), only laparotomy was performed. Neutrophil count, sedimentation, serum C-reactive protein (CRP), serum total antioxidant capacity (TAC), serum total thiol, serum native thiol, and serum myeloperoxidase (MPO) analyses were performed on animal subjects in the pre-operative period and on the post-operative 1st and 7th days. Histological and immunohistochemical (transforming growth factor-beta 1 [TGF-β1]) analyzes were performed. Blood analysis, histological, and immunohistochemical findings obtained from the groups were compared statistically.

RESULTS: There was no significant difference between the first and second groups, except for the TAC on the post-operative 7th day and MPO values on the post-operative 1st day ($P>0.05$). Although tissue healing was more pronounced in the second group than in the first group, there was no significant difference between the groups ($P>0.05$). TGF-β1 immunoreactivity observed in the second group was found to be significantly higher than in the first group ($P<0.05$).

CONCLUSION: We think that the sutureless drainage method is as effective as the primary repair method in the treatment of duodenal ulcer perforation and can be safely applied as an alternative to the primary repair method. However, further studies are needed to fully determine the efficacy of the sutureless drainage method.

Keywords: Duodenal perforation; duodenal perforation drainage treatment; duodenal ulcer; peptic ulcer; suturing duodenal perforation.

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INTRODUCTION

Peptic ulcer disease is frequently encountered in the stomach and proximal duodenum. Complications such as bleeding, penetration, perforation and gastrointestinal tract strictures, and gastric cancers can be seen in peptic ulcers.[1]

The incidence of gastroduodenal peptic ulcer perforation varies between 7 and 10/100.000.[2] The surgical method to be used in the treatment of perforated gastroduodenal ulcers is still under discussion. However, the condition of local peritonitis, the size of the defect, the general conditions of the patient, and the experience of the surgeon affect the treatment methods. Treatment of perforated gastroduodenal ulcers can range from non-surgical treatment to simple closure, gastrectomy, and vagotomy. Closing the perforation hole by suturing is the most commonly used treatment.[3]

Today, the perforated gastroduodenal ulcer is repaired by suturing the perforated area, and then proton-pump inhibitor therapy is started. If helicobacter pylori are detected in the sample taken during control endoscopy, combined antibiotic therapy is given. Duodenal perforation cases are less common after the widespread use of proton-pump inhibitor drugs. Partial resection, selective, and non-selective vagotomy methods applied in the past were limited to resistant cases.[4]

In cases of duodenal ulcer perforation, it is seen that the omentum, also called the abdominal policeman, is in the perforation area and usually closes the perforation hole with the omental plug. Actually, the perforation is repaired by the omentum. With this logic, we hypothesized that instead of suturing the perforation hole closed by the omentum, evacuating the contamination in the abdomen through a drain would be sufficient for healing.

Our study, in which animal subjects were used, it was aimed to compare and evaluate the efficacy of the primary repair method, which is frequently used in duodenal perforation, and the method in which drainage is applied only by placing a drain on the area without using sutures.

MATERIALS AND METHODS

Animal Subjects

The study was performed on a total of 30 Wistar albino male rats (250–300 g and 12 weeks old) with the approval of the Inonu University Faculty of Medicine Animal Experiments Local Ethics Committee (2021/3–4). Animals were obtained from Inonu University Medical Faculty Experimental Animal Production Center. During the experiment, the rats were housed at a constant temperature (22±2°C) and humidity (50±10%) with a light-dark cycle (12:12 h), fed with standard rodent chow, and allowed free access to water.

Three equivalent groups of ten rats each were formed. The first group was arranged as the primary repair group (sutured group), the second group as the drain placement without repair group (sutureless drainage group), and the third group as the control group.

Anesthesia and Post-operative Analgesia

Before surgery, animals were administered intraperitoneally for anesthesia by combining ketamine 75 mg/kg and xylazine 8 mg/kg.

For post-surgical analgesia, two doses of buprenorphine 0.1 mg/kg intramuscularly were administered to all animals post-operatively at 12-h intervals.

Surgical Technique

All rats that underwent surgery were subjected to skin shaving under anesthesia, the abdominal wall was wiped with 10% polyvinylpyrrolidone-iodine antiseptic solution and disinfected, and laparotomy was performed with a median incision.

A laparotomy was performed in the first and second groups under anesthesia, and a perforation of approximately 3 mm was created on the anterior wall of the first part of the duodenum with the tip of a 15 numbered scalpel (Fig. 1). After duodenal perforation was created in both groups, it was waited for 10 min for the duodenal contents to spontaneously flow into the abdomen and to contaminate the peritoneum. In the first group, the perforation was closed by a single suture using 4/0 vicryl. In the second group, the perforation was not closed with sutures, the perforation was left open. Then, in both groups, duodenal contents were cleaned from the area, the area was washed with 0.9% NaCl solution, and a drain was placed in the area for drainage. A Nelaton catheter [3.33 mm (10 Ch)] was used as drains, these drains were fixed to the skin with 3/0 silk suture material. Only laparotomy was performed under anesthesia on the rats in the third group. The abdomen was closed using 3/0 polypropylene sutures in the abdominal wall and 3/0 silk sutures on the skin in all three groups.

The rats in all groups were followed for 30 days, while morbidity and mortality were recorded. For blood analyzes from rats in all groups, 1 cc of blood was taken from the jugular vein on pre-operative and post-operative 1st and 7th days. Neutrophil count, sedimentation, serum C-reactive protein (CRP), serum total antioxidant capacity (TAC), serum total thiol, serum native thiol, and serum myeloperoxidase (MPO) levels were analyzed in peripheral smear from blood taken from rats in all groups. Neutrophil count, sedimentation, and CRP are markers of inflammation. MPO is one of the inflammatory markers associated with the severity of tissue damage. TAC, total, and native thiol are antioxidant markers that occur in the organism against oxidative stress occurring in peritonitis in duodenal perforation. TAC is a parameter that shows the degree of protection of the organism against oxidative stress and oxidative tissue damage caused by antioxidants in the blood.

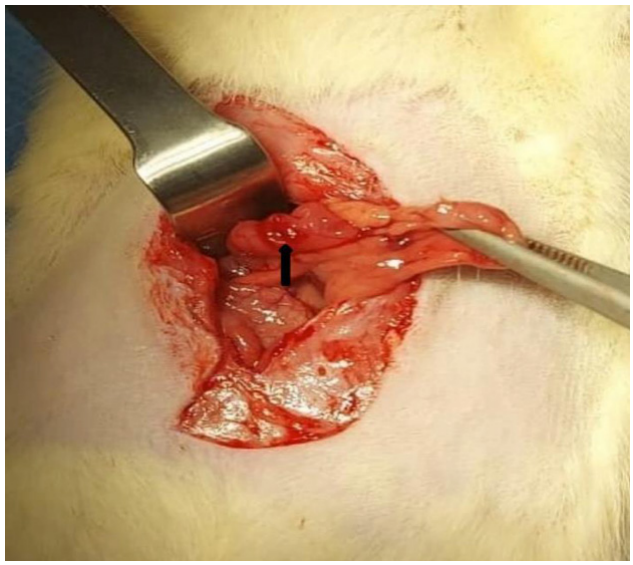

Figure 1. Duodenal perforation cre ated is marked with the arrow

In the first and second groups, Nelaton catheters were taken on the 2nd post-operative day. On the 30th post-operative day, the animals were euthanized by administering a high-dose anesthetic (4 times the anesthetic dose of Ketamine and Xylazine), and the animals were sacrificed. Samples were taken from the duodenum and surrounding tissues for histological and immunohistochemical analyzes. Stained preparations were examined with a Leica DFC-280 research microscope using the Leica QWin Image Analysis System.

Histological examinations were performed to detect tissue repair and healing. The semi-quantitative scoring system given in Table 1 was used for histological evaluation, and each tissue section was scored according to this scoring system. This histological scoring was made according to the presence or absence of cellular invasion, granulation tissue formation, vascularity, and regeneration in the duodenal layers.[5]

For immunohistochemical analyzes, transforming growth factor-beta 1 (TGF-β1) values, an important cytokine that plays essential roles in cell proliferation, differentiation, immune response, angiogenesis, and tissue repair, were examined. Immunohistochemical evaluation was scored semi-quantitatively based on the extent (0: none, 1: 1-25%, 2: 26-50%, 3: 51-75%, and 4: 76-100%) and severity (0: none, +1: mild, +2: moderate, and +3: severe) of immunoreactivity. The total staining (H) score was obtained by calculating extent x severity.[6]

H score=(extent of immunoreactivity) X (severity of immunoreactivity)

Analyses were performed using the Leica QWin Image Analysis System with a Leica DFC-280 research microscope.

Obtained biochemical, histological, and immunohistochemical analysis results were statistically compared and evaluated within the groups.

At all stages of our work, we acted in accordance with the criteria of the "Declaration of Helsinki."

Statistical Analysis

The variables used in the study were summarized as median (min-max.) and mean±standard deviation. The conformity of the variables to the normal distribution was examined with the Shapiro–Wilk test. In terms of variables, the Mann–Whitney U-test was used to see if there was a statistically significant difference between the two groups. In terms of variables, the Kruskal–Wallis H test was used to see if there was a statistically significant difference between more than two groups. The Conover test was used for pairwise comparison (post hoc) of the groups. The correlation between variables was examined with Spearman's rank correlation coefficient. p≤0.05 was accepted as the statistical significance level. "Kruskal–Wallis" (1) web-based application developed by İnönü University Faculty of Medicine, Department of Biostatistics and Medical Informatics and IBM SPSS Statistics 26.0 Package Program were used in the analyses.

RESULTS

The study was started with a total of 30 rats, ten rats in each group. In the study, one rat in the control group died during the surgical intervention and the study was completed with 29 rats.

The results of the neutrophil count, sedimentation, serum

Table 1. Histological scoring

Scoring	Histological score criteria
1–3	No cell accumulation, granulation tissue, or epithelial migration.
4–6	Thin, immature granulation tissue with a predominance of inflammatory cells but few accumulations of fibroblasts, capillaries, or collagen. Mild regeneration of duodenal layers.
7–9	Moderately thick granulation tissue with more fibroblasts, collagen deposition, and extensive neovascularization compared to inflammatory cells. Moderate regeneration of duodenal layers.
10–12	Thick, vascular granulation tissue dominated by fibroblasts and dense collagen deposition. Completed regeneration in duodenal layers.

Table 2. Blood values in all groups and their comparison

	Pre-operative	1st group	2nd group	Control group	p value
Neutrophil count	0.812a	8.562c	8c (6.25–11.25)	4.25b (1.625–5.5)	<0.001
(Post-operative 1st day)	(0.375–1.375)	(6.5–10.375)			
Neutrophil count		6.625c	5.467c	3.125b	<0.001
(Post-operative 7th day)		(1.625–8.375)	(3.571–6.533)	(1.125–4.75)	
Sedimentation	0.5a (0.4–0.6)	1.05b (1–2.1)	1b (0.7–1.8)	1.05b (0.3–2)	<0.001
(Post-operative 1st day)					
Sedimentation		0.55a	0.5a (0.3–1.3)	0.5a (0.4–0.8)	0.5601
(Post-operative 7th day)		(0.4–0.9)			
CRP (ng/mL)	0.634a	0.725ab	0.957b	0.795b	0.0158
(Post-operative 1st day)	(0.517–0.655)	(0.49–1.07)	(0.621–1.232)	(0.568–1.007)	
CRP (ng/mL)		0.741a	0.604a	0.596a	0.2586
(Post-operative 7th day)		(0.558–1.174)	(0.442–1.106)	(0.364–1.151)	
TAC (mmol/L)	1.37a	1.154b	1.295b	1.264b	0.0018
(Post-operative 1st day)	(1.32–1.568)	(1.028–1.468)	(1.119–1.37)	(1.009–1.396)	
TAC (mmol/L)		1.232b	1.119c	1.389a	<0.001
(Post-operative 7th day)		(1.063–1.352)	(0.943–1.267)	(1.217–1.43)	
Total thiol (umol/L)	354.667a	241.333b	271.333ab	334.667ab	0.0290
(Post-operative 1st day)	(301.333–424.667)	(194.667–361.333)	(198–438)	(208–378)	
Total thiol (umol/L)		264.667b	251.333b	331.333a	<0.001
(Post-operative 7th day)		(201.333–318)	(154.667–308)	(244.667–368)	
Native thiol (umol/L)	217.5 a	142.5b	157.5ab	198ab	0,0153
(Post-operative 1st day)	(167.5–265)	(132.5–210)	(142.5–270)	(105–232.5)	
Native thiol (umol/L)		161.25bc	140c	172.5b	<0.001
(Post-operative 7th day)		(132.5–187.5)	(115–180)	(115–217.5)	
MPO (U/g protein)	72.42a	175.015c	257.896b	292.899b	<0.001
(Post-operative 1st day)	(45.061–97.365)	(101.388–267.954)	(227.721–317.843)	(217.26–346.811)	
MPO (U/g protein)		191.511b	186.28b	214.846b	<0.001
(Post-operative 7th day)		(127.942–233.353)	(132.77–246.228)	(119.895–291.289)	

*There is a statistically significant difference in group categories that do not contain the same letter. **: Variables are summarized as "median (min.-max.)"

CRP, serum TAC, serum total thiol, serum native thiol, and serum MPO analyzed in all groups on pre-operative and post-operative 1st and 7th days are given in Table 2.

No statistically significant difference was found between the blood values obtained between the first and second groups, except for the TAC values on the 7th post-operative day and the MPO values on the post-operative 1st day. While TAC values on the post-operative 7th day in the first group were higher than in the second group, MPO values on the 1st post-operative day in the second group were higher than those in the first group.

In the evaluations made for sections with hematoxylin-eosin staining, cellular invasion in the perforation area, granulation tissue formation, vascularity, and regeneration in the duodenal layers were examined. Since duodenal perforation was not performed in the control group, this group was not evaluated (Fig. 2a). In the first group, it was noted that the duodenal layers, especially the mucosa and submucosa, were regenerated, but in some samples, the regeneration was not completed in the muscular layer, and infiltrative cells were also observed in this area and the presence of dense granulation tissue (Fig. 2b). Similar to the first group, in the second group, regeneration was completed in the duodenal layers in most samples, but granulation tissue continued to exist around the regenerated muscular layer in a few samples (Fig. 2c). In terms of histological evaluations, tissue healing was more prominent in the second group compared to the first group, but this difference between the groups was not statistically significant ($p>0.05$).

In the evaluations made in terms of TGF-β1 immunoreac- tiv-

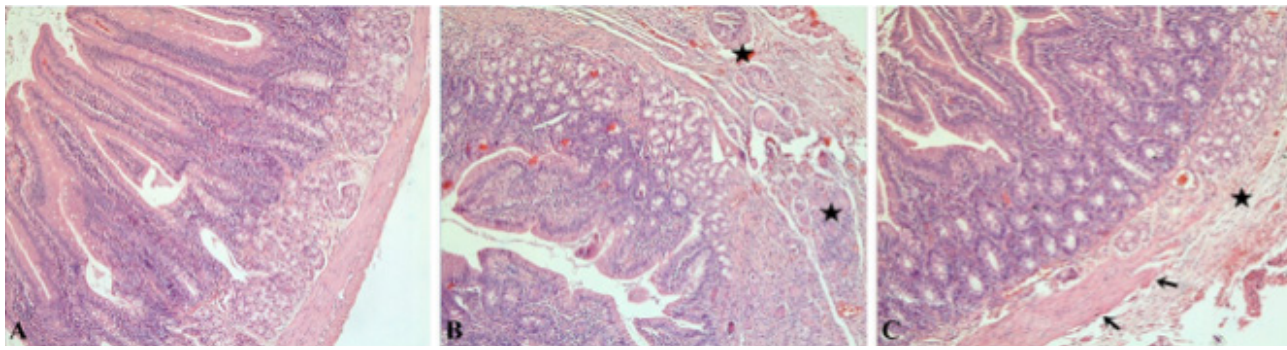

Figure 2. Normal histological structure of the duodenum is observed in the control group (a). In the first group (b), significant regeneration was observed in the mucosal and submucosal layers, while dense granulation tissue (asterisk) was observed in the developing muscular layer. In the second group (c), there was granulation tissue (asterisk) in the muscular layer with regeneration in the mucosal and submucosal layers. In addition, regeneration (arrows) was observed in the muscle tissue. H-E ×10.

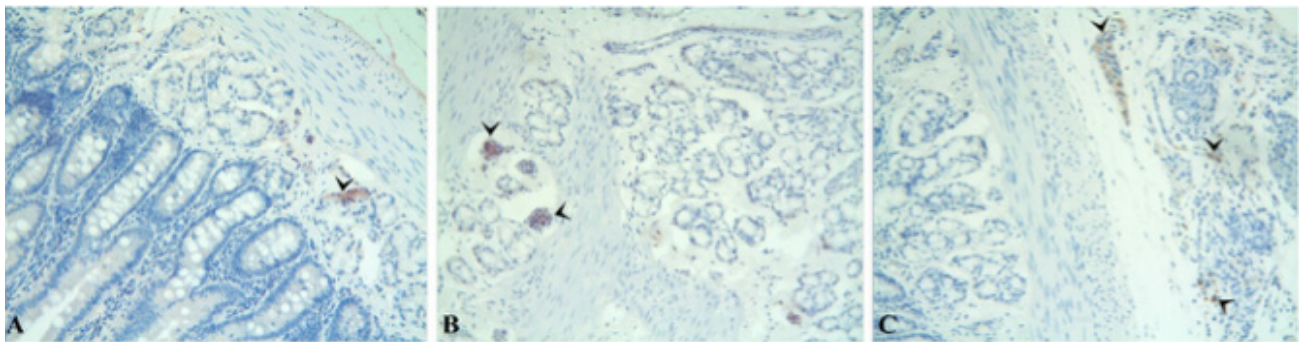

Figure 3. TGF-β1 immunoreactivity in control (a), first (b), and second (c) groups (arrowheads). TGF-β1 immunohistochemistry ×20

Table 3. Histopathological and TGF-β1 immunoreactivity evaluation results

	Control group	1. group	2. group	p value
Histological score**	–	8 (3-12)	12 (7-12)	0.057
H score (TGF-β1)**	0a (0-4)	0a (0-3)	1b (0-4)	<0.001

**Variables are summarized as "median (min.-max.)"; *There is a statistically significant difference in group categories that do not contain the same letter

ity in the perforation area, it was determined that the immunoreactivity observed in the second group was statistically significantly higher than the first group (Fig. 3) ($p<0.05$). Histological and TGF-β1 immunoreactivity evaluation results of the groups are given in Table 3.

DISCUSSION

A perforated peptic ulcer is a condition that requires urgent surgical intervention. Peptic ulcer perforations are considered an important cause of death, especially in elderly patients.[7,8] Perforated appendicitis is in the first place and peptic ulcer perforation is in the second place in abdominal organ perforations that require urgent surgical intervention. The incidence of duodenal ulcer perforation is 15 times that of gastric ulcer perforation and is more common in males.[9]

Perforation is seen in 2–10% of patients with peptic ulcer.[10] The lifetime prevalence of perforation in patients with a peptic ulcer is as high as 5%. Therefore, many surgical treatment methods have been reported for the treatment of perforation.[11,12] Perforation has mortality rates ranging from 6.2% to 27%.[13,14] Perforation accounts for 70% of deaths in patients with peptic ulcers.[15] Peptic ulcer perforation remains a surgical disease and requires urgent surgical intervention.[16]

Primary repair has been one of the most frequently used methods by surgeons in duodenal ulcer perforations due to the ease of surgical technique and advances in medical treatment.[17] The treatment of duodenal perforation aims to prevent duodenal leakage and associated peritonitis. However, post-operative complications such as fistula formation and duodenal separation often occur, as it is difficult to suture or resect a portion of the duodenum.[18] However, the use of sutureless repair methods in duodenal peptic ulcer perforation with a minimally invasive approach may facilitate surgical intervention and shorten the operation time.[19]

Since the closure of the perforation hole with sutures requires surgical skill and takes a long time, some sutureless

methods have been proposed.[20] In perforated duodenal ulcers, the perforation hole was repaired by laparoscopic and endoscopic methods using omentum, fibrin spray, ligamentum teres hepatis, gelatin sponge, and synthetic grafts.[21-24]

Lau et al. reported that fibrin glue can be used as a sutureless repair method in duodenal perforations in the laparoscopic or endoscopic way.[20,23] In the repair of duodenal perforations, Mouret et al. used an omental patch with a fibrin plug, and Sim et al. used a fibrin patch (TachoComb).[25,26] These techniques have less wound infection, mortality, post-operative adhesion, and incisional hernia and are also less costly. The disadvantage of these techniques is the higher rate of reoperation.[27]

Many studies have been conducted in which the omentum is used to close the perforation hole. Wang et al. stated that the sutureless omental patch method is as safe and effective as the sutured omental patch method in the laparoscopic repair of duodenal ulcer perforation.[16] In perforated peptic ulcer surgery, the most widely used and accepted treatment method in many centers is the simple closure of the perforation, with or without an omental patch.[3,28]

In the treatment of duodenal ulcer perforation, some repair methods have been applied to animal subjects and results have been published. For example, in the study of Yakan et al., the method of using primary sutures and the methods of using "expanded polytetrafluoroethylene" (ePTFE) grafts in perforation repair were compared and it was found that the macroscopic and microscopic results of the ePTFE grafting method were similar to the primary suture results.[5] In another study, classical surgical techniques and sutureless repair techniques with adhesion barriers such as DuraSeal or fibrin glue were compared in experimental duodenal perforation in rat subjects. Here, burst pressure and tissue hydroxyproline level were checked and a histopathological examination was performed. It has been observed that sutureless repair techniques using DuraSeal or fibrin glue are not superior to conventional (sutured) repair techniques.[19]

We did not find any equivalent study in our literature review. The main motivation for our study was to observe whether there would be a difference between morbidity and mortality when we removed the perforation contents from the peritoneal area with drainage and lavage, with or without primary repairing the perforation.

Mortality and morbidity in peptic ulcer perforation are caused by chemical burns and peritonitis is caused by digestive system contents. In the case of peritonitis, the values of inflammation markers increase and there is activation of oxidative stress and phospholipases.[29]

In cases of duodenal ulcer perforation, leukocyte count, neutrophil ratio, and CRP values increase significantly due to inflammation or infection.[30,31] In our study, there was a similarity between primary repair and sutureless drainage groups in terms of neutrophil count. The neutrophil count in both groups was significantly increased compared to the control group. However, CRP values in primary repair, sutureless drainage, and control groups were similar and there was no difference between them. We attribute this situation to the absence of advanced peritonitis in the perforated groups.

Increases in MPO activity are also seen in cases of peritonitis due to any reason.[32] In all three groups, MPO values increased in the post-operative period compared to the pre-operative period. While the MPO value in the sutureless drainage and control group on the post-operative 1st day was significantly higher than the primary repair group, the MPO values on the post-operative 7th day were the similar in all three groups.

TGF-β1 levels are a parameter associated with fibrosis, cell proliferation, and adhesion development after surgery.[33] TGF-β1 values in our study were also found to be significantly higher in the sutureless drainage group than in the primary repair group. These results show us that cell proliferation and fibrosis development are higher in the sutureless drainage method. In histological examination, there was no significant difference between the primary repair method and the sutureless drainage method in terms of tissue healing.

In this study, since there are important similarities between the blood analysis and histological findings obtained from the primary repair method and the sutureless drainage method, the sutureless drainage method can be safely used in the treatment of duodenal ulcer perforation. By applying sutureless drainage method, surgical intervention will be facilitated, and suture-related complications and operation times will be reduced, which will reduce morbidity and mortality in patients.

Since we used healthy young rats in our study and we performed the intervention 10 min after the perforation was created, the cases to be selected in the human model should be cases within the first 6 h, there are no additional comorbid factors, the patients should be under 50 years old, and the patients should not have undergone previous abdominal surgery. The situations mentioned above in the real human model are the limitations of our study.

Conclusion

There was a similarity between the primary repair and sutureless drainage groups in terms of blood analysis, except for TAC values on the seventh post-operative day and MPO values on the 1st post-operative day. On histological examination, tissue healing was identical in the sutureless drainage group and the primary repair group. TGF-β1 immunoreactivity observed in the sutureless drainage group was found to be significantly higher than in the primary repair group.

According to the findings of our study, the sutureless drainage method in the treatment of duodenal ulcer perforation is as effective as the primary repair method and can be used as an alternative to the primary repair method. Despite these results, we have obtained, we think that further studies are needed, especially with human subjects, as well as studies with animal subjects, to fully demonstrate the effectiveness of this sutureless drainage method.

Ethics Committee Approval: This study was approved by the Inonu University Medical Faculty Animal Experiment Ethics Committee (Date: 11.02.2021, Decision No: 2021/3-4).

Peer-review: Externally peer-reviewed.

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Conflict of Interest: None declared.

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DENEYSEL ÇALIŞMA - *ÖZ*

Duodenum perforasyonlarında "primer tamir" ile "tamir yapmadan dren konulması" yöntemlerinin karşılaştırılması

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AMAÇ: Duodenal ülser perforasyonu ciddi bir durumdur. Cerrahi tedavide bir takım yöntemler tanımlanmış ve kullanılmıştır. Bu çalışmada, duodenal perforasyonlarda "primer tamir" ve "tamir yapmadan dren konulması" yöntemlerinin etkinliklerinin, hayvan modeli kullanılarak karşılaştırılması amaçlandı.

GEREÇ VE YÖNTEM: Her biri on sıçandan oluşan üç eşdeğer grup oluşturuldu. Birinci (primer tamir / sütürlü grup) ve ikinci grupta (tamir yapmadan dren konulması / sütürsüz drenaj grubu) duodenumda perforasyon oluşturuldu. Birinci grupta perforasyon sütürle tamir edildi. İkinci grupta ise perforasyon sütürle tamir edilmeden, karın içine sadece dren yerleştirildi. Üçüncü gruba (kontrol grubu) sadece laparotomi uygulandı. Hayvan deneklere preoperatif dönemde, postoperatif 1. ve 7. günlerde nötrofil sayısı, sedimantasyon, serum C-reaktif protein (CRP), serum total antioksidan kapasitesi (TAK), serum total tiyol, serum nativ tiyol ve serum miyeloperoksidaz analizleri yapıldı. Histolojik ve immünohistokimyasal (transforming growth factor beta-1 [TGF-β1])) analizleri yapıldı. Gruplardan elde edilen kan analizleri, histolojik ve immunohistokimyasal bulgular istatistiksel olarak karşılaştırıldı.

BULGULAR: Postoperatif 7. gün TAK ve postoperatif 1. gün MPO değerleri dışında birinci ve ikinci grup arasında anlamlı fark yoktu ($p>0.05$). İkinci grupta doku iyileşmesi birinci gruba göre daha belirgin olmasına rağmen gruplar arasında anlamlı fark yoktu ($p>0,05$). İkinci grupta izlenen TGF-β1 immünreaktivitesi birinci gruba göre anlamlı olarak yüksek bulundu ($p<0.05$).

TARTIŞMA: Sütürsüz drenaj yönteminin duodenal ülser perforasyonu tedavisinde primer tamir yöntemi kadar etkili olduğunu ve primer onarım yöntemine alternatif olarak güvenle uygulanabileceğini düşünüyoruz. Ancak sütürsüz drenaj yönteminin etkinliğinin tam olarak belirlenebilmesi için daha ileri çalışmalara ihtiyaç vardır.

Anahtar sözcükler: Duodenal perforasyon; duodenal perforasyon drenaj tedavisi; duodenal perforasyonun dikilmesi; duodenal ülser; peptik ülser.

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