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ORIGINAL ARTICLE
ORİJİNAL ARAŞTIRMA

Effect of Different Bacterial Contamination on Experimental Adhesive Intestinal Obstruction in Rats

Sıçanlarda Farklı Bakteriyel Kontaminasyonun Deneysel Adeziv Barsak Obstrüksiyonuna Etkisi

Gökhan Demirtaş[1], Doğuş Çalışkan[2], Pınar Celepli[3], Sema Hücümenoğlu[3], Hüseyin Tuğrul Tiryaki[1]

[1]Ankara City Hospital, Childrens' Hospital, Department of Pediatric Urology, Ankara, Turkey
[2]Ankara Training and Research Hospital, Department of Pediatric Surgery, Ankara, Turkey
[3]Ankara Training and Research Hospital, Department of Pathology, Ankara, Turkey

ABSTRACT

Aim: Postoperative peritoneal adhesions (PPA) cause pain, intestinal obstruction and infertility after abdominal surgery and to date there is no shown pathogenesis or definitive treatment. Intestinal flora and its effect on infection is one of the most serious factors that influence the morbidity during intraabdominal surgeries. Different microorganisms found in intestinal flora or added ones as hospital flora might be the reason of the inflammatory processes and cause PPA formationThere are a lot of tegorizedstudies showing intraabdominal infections cause PPA but there is no study comparing the effect of different bacterial strains on PPA formation. That is why we designed this study using the most common microorganisms isolated in intraabdominal infections and hospital flora. In our study, investigating the impact of different bacterial strains on the nascency and degree of PPA in adhesion formation in rats.

Material and Method: In this this experimental study, subjects were divided into five groups, each one obtaining 12 rats. Groups were categorized as; *E. coli* group, *Klebsiella* group, *Bacteriodes fragilis* group, Sham and Control groups. after the scarification on the 14th day, re abdominal exploration was performed. The results were exmained according to the previously mentioned microscopic-macroscopic classifications.

Conclusion: Microorganisms have been found to have an important role in PPA formation in the experimentally created adhesion model. But there was no significant difference between bacterial strains on PPA formation.

Keywords: Postoperative peritoneal adhesions (PPAs), bacteria, adhesion in rats

ÖZ

Amaç: Postoperatif peritoneal adezyonlar (PPA) abdominal cerrahi sonrası ağrı, intestinal obstrüksiyon ve infertiliteye neden olabilir. Bugüne kadar kanıtlanmış bir patogenezi veya kesin tedavisi yoktur Karın içi girişimlerde en önemli noktalardan biri barsak folarası ve enfeksiyona etkisidir. Florada bulunan veya sıklıkla hastane florası olarak eklenen farklı mikroorganizmaların inflamatuvar süreçlere ve PPA oluşumuna neden olabileceği düşünülmüştür. Karın içi enfeksiyonların PPA'ya neden olduğunu gösteren birçok çalışma vardır. Ancak farklı bakteri suşlarının PPA oluşumu üzerindeki etkisini karşılaştıran bir çalışma yoktur. Bu nedenle çalışmayı intraabdominal enfeksiyonlarda en sık izole edilen mikroorganizmalar ve hastane florası kullanarak tasarladık. Farklı bakteri suşlarının (*Klebsiella* spp, *E. coli* spp, anaerob) sıçanlarda PPA oluşumu ve adezyon derecesi üzerine etkisini araştırmayı amaçladık.

Gereç ve Yöntem: Denekler 12 rattan oluşan beş gruba ayrıldı. Gruplar *E. coli*, *Klebsiella*, *Bacteriodes fragilis*, Sham ve Kontrol grupları olarak adlandırıldı. Sıçanlar 14. Günde sakrifiye edildi ve relaparatomi uygulandı. Sonuçlar daha önce belirlenen sınıflamalara göre makroskopik ve mikroskopik olarak değerlendirldi

Bulgular: Bakteriyel enfeksiyonlu gruplar sham ve control grupları ile karşılaştırıldığında, PPA'da hem mikroskopik hem de makroskopik olarak anlamlı artış gözlemlendi

Sonuç: Çalışmamızda kullanılan mikoorganizmaların PPA oluşumunda rol oynadığı deneysel olarak oluşturulan adezyon modeli ile gösterilmiştir. Ancak bakteri suşları arasında PPA oluşumu üzerinde anlamlı bir fark yoktur

Anahtar Kelimeler: Postoperatif peritoneal adezyon (PPA), bakteri, sıçanlarda adezyon

Corresponding Author: Gökhan Demirtaş
Address: Ankara City Hospital, Childrens' Hospital, Department of Pediatric Urology
Universiteler Boulvard, 1604. Street, 06800, Çankaya/Ankara/ Turkey
E-mail: drgokhandemirtas@gmail.com

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INTRODUCTION

PPAs may lead many clinical problems such as intestinal obstruction, severe abdominal pain, intestinal dysfunction and infertility (1). In Pediatric Surgery Clinics, PPAs have an important place in terms of morbidity and hospitalization. In Western countries, PPAs are the most common cause of intestinal obstructions (1). Following abdominal surgery, approximately two-thirds of patients develop PPA, but symptoms are observed in only one-fifth of the patients. Adhesion-induced intestinal obstruction is most common in the pediatric age group. Eight percent of newborns undergoing laparotomy undergo relaparotomy in the future (2). Adhesive obstruction can occur at any time in one-third of patients within one year after the first surgery, and in the remaining one at any time within a long period of 20 years (2,3). Although our knowledge about PPA is gradually increasing, PPA continues to be a problem for surgeons from different disciplines. Many materials and different techniques have been tried to prevent peritoneal adhesions but have not been fully successful to date. Despite all these mechanisms of occurrence, the fact that PPA does not develop at the same level in every patient is a sign that host factors are also important. Intestinal flora and its effect on infection formation are very important in intraabdominal surgical procedures. Different microorganisms found in flora or often added as hospital flora can lead to different levels of inflammatory processes in individuals, causing PPA formation (4).

There are not enough studies in the literature showing how different microorganism presence affects PPA. In our study, we aimed to evaluate whether there is a difference in the formation of peritoneal adhesion in rats contaminated with three different microorganisms that can be found in the gastrointestinal tract.

MATERIAL AND METHOD

After receiving institutional Animal Experiments Ethics Committee approval (2017-42). Before the study, all rats were weighe done by one, their weights were recorded and 60 Wistar-Albino mixed rats, each weighing approximately 200-300 g, wereused in thestudy. In this study, rats were divided into 5 groups, 12 rats each.These groups are;

- Group 1: The group transmitted with *E. coli*,
- Group 2: The group infected with *Klebsiella* spp.
- Group 3: Anaerobic strain (*Bacteriodes fragilis*) infected group,
- Group 4: Control group,
- Group 5: The Sham group.

All rats to be used in the study were kept in the same laboratory environment for 1 week before the experiment. All rats were fed with standard pellet feed and water and were monitored in metabolic cages in standard laboratory conditions (day/night=12/12 hours, temperature 21±2°C, humidity 50%) in isolated environment.

Surgical procedures were applied in sterile atmosphere. Intraabdominal ketamine (Ketalar®, Parke Davis and Co. Inc., 50 mg/kg) and xylazine (Rompun®, Bayer 5 mg/kg) were given as an anaesthetic agent. For the rats to be normothermic (37°C), the temperature of the environment was maintained with a heating lamp. After the abdominal surface was washed and shaved with 10% poviiodin, and sterile covering, laparotomy was performed with an aseptic surgical technique and approximately 3 cm midline incision.

After examing that there was no adhesion in the abdomen, the cecum was observed. As a well-defined adhesion model in all rats; after the parietal area of the cecum was deserosalized and abrasion was formed with dry gauze on the the cecum's antimezenteric surface (4). This treat was continued until focal petechial bleeding was seen on serosal surfaces. Subsequently, the standard *E. coli* spp. coded ATCC 25922, standard *Klebsiella* spp. strains obtained from the Department of Microbiology of Ankara University Faculty of Medicine, the standard *Klebsiella* spp. strains coded ATCC 22914, and the standard anerob (*Bacteriodes fragilis* spp) strain from the Ministry of Health Refik Saydam Hygiene Institute; 1×10^4 'Colony Forming Unit' (CFU) was applied to the pre-determined groups under the supervision of a specialist by the microbiology specialist at the University of Health Sciences Ankara Hospital SAUM Clinical Microbiology Laboratory. (**Figure 1**) To create the sham group, the abdominal walls of 12 rats, which were found to have no adhesion following a 3 cm midline incision after anaesthesia, were continuously covered with 3/0 vicryl and their skin was individually followed by 3/0 silk sutures. In the control group, adhesion model was applied to 12 rats without any drug or bacterial strain. Then all the rats were followed.

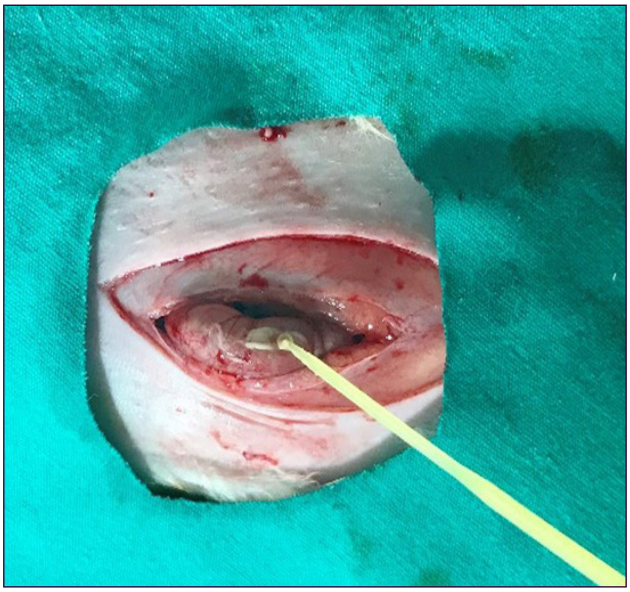

Figure 1. Bacteria planting process in the abdomen)

On the fourteenth postoperative day, all rats were weighed again, and their weights were recorded again. In the post-operative period, 4 rats were lost in 2 different groups due to surgery or anaesthesia. In accordance with the Helsinki contract, all rats were sacrificed on the fourteenth day with a high dose of ether. Then, maximum visibility was achieved by making U incision (it extends from the right epigastric region to below the umbilicus and from there to the left epigastric region) on the abdomen of the subjects. Adhesions were interpreted quantitatively with the classification defined by Nair et al. (5) The evaluation was carried out by two separate persons in accordance with the classification previously described and double-blind (**Table 1**).

Table 1: 'Nair' macroscopic adhesion classification

	Grade	
No adhesion	Grade 0	No adhesion
Adverse adhesion	Grade 1	One band between organs or between the organ and the abdominal wall
Pronounced adhesion	Grade 2	Two band structures; bands between organs and bands between organs and abdominal wall
Pronounced adhesion	Grade 3	Adhesion of intestinal loops between organs or between the organ and the abdominal wall, with no more than two adhesive band
Severe adhesion	Grade 4	Viscera adheres to the abdominal wall directly

During the exploration for adhesions the bands were resected together with the affected organs and only the parietal peritoneum was resected in those who did not have adhesions. The pathological specimens were fixed in 10% formol. The preparations were embedded in paraffin blocks. Five micrometers thick sections were taken on the slide and stained with Hematoxylin-Eosin and examined by light microscopy. Histopathological examination was interpreted in OLYMPUS brand, BX51TF model ×4, ×10, ×20, ×40 lenses. After histopathological evaluation, the preparations were exposed to microscopic grading as defined by Zühlke (6) (**Table 2**). While evaluating the findings statistically, IBM SPSS (Statistical Package for the Social Sciences, version 22.0; SPSS Inc., Chicago, IL) program was used in the study. When comparing the data, Pearson chi-square test was used.

Table 2: The grading system of microscopic adhesion (Zühlke classification)

Grade 0	Normal Findings
Grade 1	Mild connective tissue, fibrin stuctures, thin fibrils of reticulin
Grade 2	Connective tissue consisting of diffuse cells and capillaries, and small amounts of collagen fibers
Grade 3	Thickened connective tissue, decreased cell count, decreased elastic and smooth muscle fibers, increased vasculature
Grade 4	Former granulation tissue, poorly differentiated serosal layers, and cell-poor structure

RESULTS

Macroscopic findings of rats according to Nair classification are shown in **Table 3**. In addition, macroscopic views after sacrification are available in **Figure 2** . According to the microscopic adhesion grading system (Zuhlke) the structural changes in the samples of the intestinal wall were evaluated (**Table 4**).

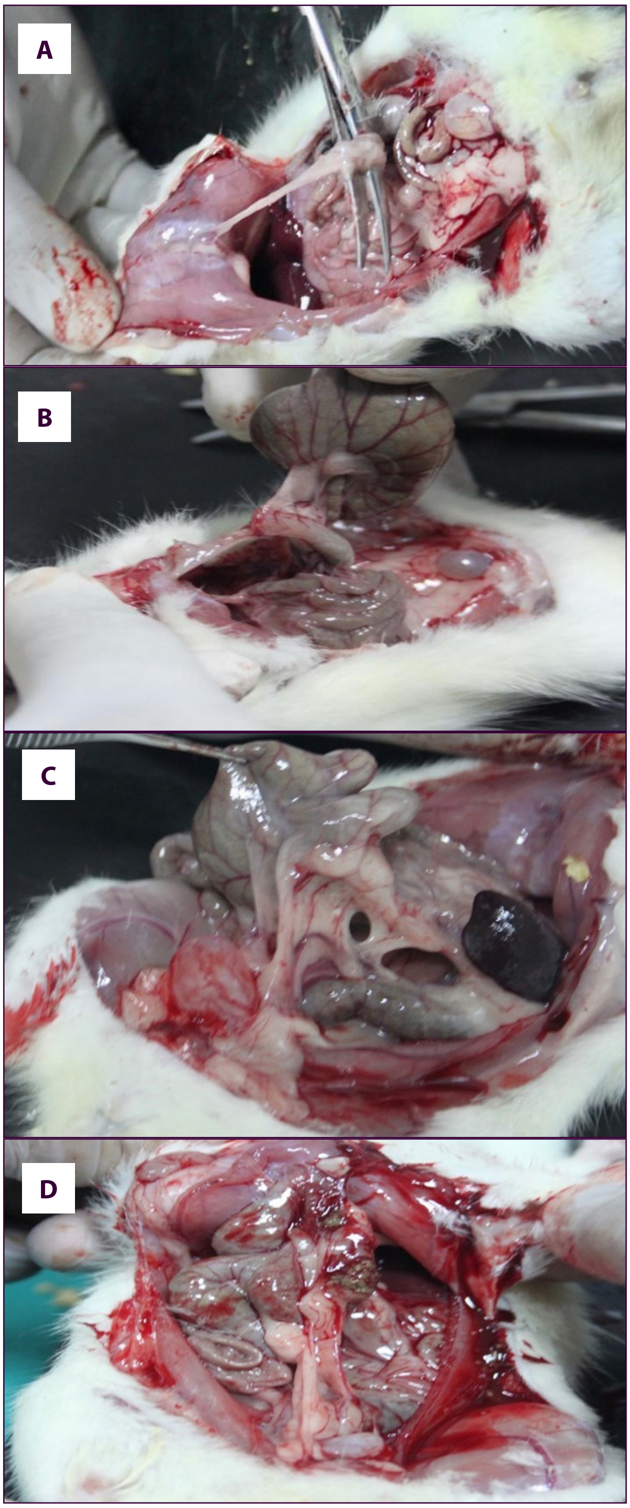

Figure 2. A Macroscopic Grade 1 image, B Macroscopic Grade 2 image, C Macroscopic Grade 3 image, D Macroscopic Grade 4 image

Table 3: Macroscopic adhesion grading by groups

Rats	*E. coli* (Grade)	*Klebsiella* (Grade)	Anaerob (Grade)	Sham (Grade)	Control (Grade)
1.	2	3	4	1	0
2.	2	3	4	4	4
3.	4	4	Ex	0	0
4.	4	3	3	0	0
5.	4	4	3	0	0
6.	3	0	3	1	0
7.	3	4	4	2	0
8.	0	3	4	1	0
9.	1	2	4	1	1
10.	4	3	Ex	1	0
11.	3	Ex	4	1	1
12.	4	Ex	4	1	0

Table 4 Histopathological classification (microscopic adhesion grading) according to groups:

Rats	*E. coli* (Grade)	*Klebsiella* (Grade)	Anaerob (Grade)	Sham (Grade)	Control (Grade)
1.	3	3	2	2	2
2.	3	3	3	4	4
3.	3	3	Ex	1	2
4.	3	3	3	0	1
5.	3	4	3	0	1
6.	4	2	3	1	1
7.	3	3	3	1	1
8.	3	3	4	0	1
9.	3	2	3	1	3
10.	4	4	Ex	2	2
11.	3	Ex	3	1	4
12.	4	Ex	3	1	1

When control, sham and *E. coli* groups are compared; significant difference was found both on Nair classification and Zuhlke classification ($p=0.001$ according to Pearson chi-square test). When the control, sham and *Klebsiella* groups are compared; there was a significant difference on both Nair classification ($p<0.001$ according to Pearson chi-square test) and Zuhlke classification ($p=0.005$ according to Pearson chi-square test). When control, sham and *Bacteriodes fragilis* groups are compared; there was a significant difference both on Nair classification and Zuhlke classification ($p<0.001$ according to Pearson chi-square test). When *E. coli*, *Klebsiella* and Bacteriodes groups are compared; there was no statistically significant difference ($p=0.525$ according to Pearson chi-square test).

DISCUSSION

PPA is the one of the most serious cause of long-dated morbidity (7). Therefore, efforts on preventing PPAs are increasing day by day in the recent literature. PPAs may provoke recurrent abdominal pain, intestinal obstructions, and infertility (2). There is a repeated need for outpatient or inpatient treatment. Some patients even must undergo surgery. This situation is reflected as a serious burden on health expenditures as well as the additional morbidity brought to the patients. A process that deactivate PPA formation will prevent repetitive surgeries and the morbidity and financial burden it brings (8).

The widely accepted idea is that careful surgical technique can limit postoperative adhesions. However, increased surgical trauma, unnecessary and excessive manipulations, foreign body and necrotic tissues not being removed from the surgical area and minimally invasive procedures are the reasons causing an increase in PPA formation (9). However, the inflammatory process that develops due to infection or bacterial exposure is an important reason that increases the formation of PPA (10,11).

According to the findings obtained from the results of the study, PPA formation in infected groups was significantly higher than in the control and sham groups. However, information on the effect differences of different microbiological agents on PPAs could not be obtained. Considering that there may be different microorganisms in individuals and each surgery room has its own flora; It was concluded that PPA can be monitored in different degrees and incidence. In our study; Three different bacterial strains were used: *E. coli* spp, *Klebsiella* spp. and *Bacteriodes fragilis* spp. Serious PPA was observed in all groups, and both microscopic and macroscopic differences were found in PPA formation when compared to control and sham groups. However, there was no significant difference between the 3 bacterial groups in terms of PPA formation and severity. Thus, it was found that the infection itself is an important factor in PPA formation, but it has no effect on the degree of adhesion of different bacterial groups. Therefore, we think that the use of antibiotics for the dominant flora before the procedure will significantly decrease the formation of PPA. Also, we can reduce the rate of PPA with surgery to minimize tissue damage with methods that will prevent infection and bacterial translocation.

CONCLUSION

Microorganisms have been found to act a considerable role in PPA construction. But there is no significant difference was noted between *E. coli*, *Klebsiella* and Bacteriodes groups.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by University of Health Sciences Ankara Hospital SAUM Experimental Animals Laboratory (Protocol: 2017-42).

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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