Effects of intra-abdominal pressure increase on intestinal ischemia and bacterial translocation in experimental sepsis model

Ugur Kesici, MD, Sevgi Kesici, MD, Erdal Polat, MD, Birol Agca, MD, Ulku A. Turkmen, MD, Deniz Ozcan, MD, Musa K. Sari, MD.

ABSTRACT

الأهداف: التحقق من مدى فعالية العلاج بالمنظار لتشخيص وعلاج التهاب الصفاق الحاد الناتج لإنتانات داخل البطن تحت الضغط بمقدار 8 مم زئبقي لمدة ساعة واحدة.

الطريقة: أجريت هذه الدراسة بمختبر معهد جامعة اسطنبول للأبحاث المخبرية الطبية خلال الفترة من أبريل إلى مايو 2009م، اسطنبول، تركيا. وقد ضمت هذه الدراسة 32 جرذ أنثى من نوع ألبينو ويستر تقدر أوزانهم مابين 20±250 جرام قسموا إلى 4 مجموعات. تم إصابة الجرذان بالإنتان داخل البطن وذلك بحقن داخل الصفاق بجرعة مقدارها ملل واحد(10^9) من الإشريكية القولونية وأصيبت كذلك باسترواح الصفاق وذلك بنفخ ثاني أكسيد الكربون تحت درجة ضغط 8 مم زئبقي لمدة ساعة واحدة. تم تقسيم المجموعات إلى المجموعة الأولى التي أعطيت ملل واحد من المحلول الملحي المتعادل، والمجموعة الثانية أعطيت ملل واحد من المحلول الملحي وأصيبت باسترواح الصفاق. أصيبت المجموعة الثالثة بالإشريكية القولونية، أما المجموعة الرابعة فقد أصيبت بالإشريكية القولونية مع استرواح الصفاق. تم تحليل البيانات باستخدام برنامج الحزمة الإحصائية (SPSS) النسخة 15 لنظام الويندز.

النتائج: وجدنا ارتفاع في درجة الحرارة ومعدل كريات الدم البيضاء في المجموعتين الثالثة والرابعة مقارنة بالمجموعة الأولى والثانية (p=0.001). تم تحديد معدل إعادة إنتاج سلالة الإشريكية القولونية بمقدار 0% في المجموعة الأولى والثانية و100% في المجموعة الثالثة والرابعة.

خاتمة: في هذه الدراسة تمت الإصابة باسترواح الصفاق عند درجة ضغط 8 مم زئبقي لمدة ساعة في حالة انتان داخل البطن المستحدث حيث تكمن أهمية العلاج. تعد طريقة المنظار مع الضغط القليل آمنة في التشخيص والمعالجة.

Objectives: To investigate the safety of laparoscopic intervention for diagnosis and treatment at 8 mm Hg pressure in one-hour period on acute peritonitis related intra-abdominal sepsis model.

Methods: In this study, we included 32 female Wistar-Albino rats, weighing 250 ± 20 g, and divided them into 4 groups. This study was conducted in Istanbul University Experimental Medical Research Institution (DETAE) laboratory from April to May 2009. Intra-abdominal sepsis was created with intraperitoneal (i.p.) one mL (10^9 CFU/mL) *Escherichia coli* (*E. coli*) injection, and pneumoperitoneum was formed with CO_2 insufflation at 8 mm Hg pressure for one hour i.p. The rats were administered with: Group 1 - one mL i.p. isotonic saline; Group 2 - one mL i.p. isotonic saline + pneumoperitoneum; Group 3 - i.p *E. coli*; and Group 4 - i.p. *E.coli* + pneumoperitoneum. Data were analyzed using the Statistical Package for Social Sciences version 15 for Windows (SPSS Inc, Chicago, IL, USA).

Results: Fever and leukocyte values were considered high in Groups 3 and 4 compared with Groups 1 and 2 (p=0.001). The administered reproduction ratio of the *E. coli* strain was determined as 0% in Groups 1 and 2, and 100% in Groups 3 and 4.

Conclusion: In this study, as pneumoperitoneum was formed for one hour at 8 mm Hg pressure, in case of intra-abdominal derived sepsis where emergency intervention is needed, we consider that laparoscopic approaches with low pressure may be used safely for diagnosis and treatment.

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From the Department of General Surgery (Kesici U), Akcaabat State Hospital, the Department of Anaesthesiology and Reanimation (Kesici S), Numune Training and Research Hospital, Ministry of Health, Trabzon, and Department of Microbiology and Clinical Microbiology (Polat), Cerrahpasa Faculty of Medicine, University of Istanbul, and the Third Department of General Surgery (Agca, Sari), the Department of Anesthesiology and Reanimation (Turkmen), the Department of Pathology (Ozcan), Okmeydani Training and Research Hospital, Ministry of Health, Istanbul, Turkey.

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Address correspondence and reprint request to: Dr. Ugur Kesici, Department of General Surgery, Akcaabat State Hospital, Ministry of Health, Trabzon, Turkey. Tel. +90 (462) 2213085. Fax. +90 (462) 2277779. E-mail: ugurkesici77@mynet.com

According to the well-accepted statement in the International Abdominal Compartment Syndrome (ACS) Consensus Definitions Conference held in 2004, intra-abdominal pressure (IAP) is a "steady state pressure".[1] Normal IAP is equal to the atmospheric pressure, or slightly below this in all living beings with lung respiration. The IAP level above the defined level is identified as intra-abdominal hypertension (IAH). The ACS is characterized with acute and pathologic increase of IAP, and if not treated, it is a clinical syndrome that leads to death.[2,3] The IAH is defined as 12 mm Hg or more, above the IAP in the IAH evaluation algorithm as declared by the World Society of the Abdominal Compartment Syndrome (WSACS) in 2007.[4] There are various classifications showing the effects of IAP in the organs. The classification declared by WSACS in 2007 is used nowadays.[4] Blood flow in all venter organs significantly decreases with the increase of IAP. When IAP practically exceeds 10 mm Hg, it is accepted that there are changes in the physiological parameters and organ functions.[3,5,6] However, there are studies denoting that organ functions are affected at pressures below 10 mm Hg.[7-9] Bacterial translocation is the permeation of endoluminal bacteria, as a result of destroyed intestinal obstruction to systemic circulation and tissues. Bacterial translocation is most frequently formed with destroyed intestinal obstruction function induced by intestinal ischemia. Bacterial translocation in IAP is formed primarily in the mesenteric lymph nodes. Peritoneal surface is normally sterile. The most reproducing bacterium is *Escherichia coli* (*E. coli*). It is believed that as a result of bacterial translocation, intestinal induced bacteria or endotoxin is provocative, or an intensifier of existing septic state, and thus, contribute to the development of multiple organ failure.[10,11] When compared with the laparotomy benefits obtained from laparoscopic results in many respects, they are considered better, especially in terms of homeostasis. The objective of this study is to investigate the safety of laparoscopic intervention for the diagnosis and treatment at 8 mm Hg pressure in one-hour period on acute peritonitis related intra-abdominal sepsis model.

Methods. This study was conducted in Istanbul University Experimental Medical Research Institution (DETAE) laboratory from April to May 2009 on rats obtained from Istanbul University DETAE after an approval was granted by the Istanbul University Animal Experiments Local Ethics Committee. A total number of 32 Wistar-Albino female rats were used in the study. A 10 mg/kg intraperitoneal (ip), one dose ketamine hydrochloride (HCL, 50 mg/ml,) (Ketalar® Flakon, Eczacibasi Medicine and Commerce, A.C. Istanbul, Turkey), and 5 mg/kg subcutaneous (sc) xylazine hydrochloride (23.32 mg/ml Xylazine [Rompun® Flakon, Bayer Turkish Chemistry Industry Ltd. Co., Istanbul, Turkey]) anesthesia were administered to rats in a suitable environment after creating operating room conditions. All operations on rats were carried out under anesthesia. During the operations all rats were treated humanely according to the Guide for the Care and Use of Laboratory Animals. Four groups were formed with 8 rats in each group with a randomization method, such as: Group 1 - one ml i.p. saline; Group 2 - one ml i.p. saline + pneumoperitoneum; Group 3 - one ml i.p. *E. coli*; and Group 4 - one ml i.p. *E. coli* + pneumoperitoneum. Intra-abdominal sepsis was developed with injection of i.p. one mL (10^9 CFU/mL) *E. coli* 1104512. However, pneumoperitoneum was formed after the abdominal front wall disinfection with the aid of an 18 G angiocut (BD Neoflon,® Helsingborg, Sweden), and one hour carbon dioxide (CO_2) insufflation at 8 mm Hg pressure. Insufflator pressure was controlled at 10-minute intervals. Rectal temperature and leukocyte values of all the rats were controlled 2 hours after i.p. injection. A 0.5-0.8 ml blood was obtained for leukocyte measurement near the tail point with the aid of a 26 G angiocut (BD Neoflon,® Helsingborg, Sweden). Pneumoperitoneum was administered after the rectal temperature values were checked, and blood sample was obtained for leukocyte measurement. Thoracotomy and laparotomy was administered after 6-8 hours abdomen and thorax front wall surgical area disinfection. A 4-6 ml blood was taken by intracardiac puncture from all rats, and placed into a BACTEC culture tube (manufacturer, city, country) for microbiological investigation. A 2-gram tissue sample was obtained from the liver and spleen, and placed into sterile culture plates for microbiological investigation. Approximately 3-4 cm tissue sample from the terminal ileum level was obtained, and placed into a 10% formaldehyde containing plates for histopathologic investigation.

Microbiological method. Tissue culture samples obtained to investigate for bacterial translocation were transferred in a general reproductive media, and kept in an oven at 37°C for 24-48 hours. Endo and chocolate agar passages were obtained from the broth medium, where reproduction occurred in the first 24-48 hours. Broth mediums with no reproduction after 48 hours were evaluated as "no bacteria reproduced". Samples obtained from bacteria colonies that were reproduced in

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endo mediums, and fell off one-by-one, and evaluated with API 20E test, and *E. coli* 1104512 was determined as the reproduced bacteria. Besides *E. coli* 1104512 strain applied to develop intra-abdominal sepsis, we investigated whether there was *E. coli* strain, bacteroides fragilis, *Enterobacter*, *Enterococcus*, *Pseudomonas*, and *Staphylococcus* reproduction. Blood sampled for blood culture was inoculated into a BACTEC hemoculture tubes, and kept at 37°C in an oven for 48, 72, 96, and 120 hours. At the end of this period, the samples injected by sterile injectors from hemoculture bottles were inoculated into the endo and chocolate agar medium with reduction method. These mediums were kept in an oven at 37°C for 24-48 hours. The mediums without reproductions were considered as negative. Gram straining was performed in different colonies that fell separately in mediums with reproduction.

Biochemical method. White blood cell (WBC) count. To determine WBC count in all the rats, a 0.5-0.8 mL blood was added into an ethylenediaminetetraacetic acid (EDTA) containing tube, and was measured with a blood count device (Abacus, Diatron, Wien, Austria). The blood sample, rotated for 5 minutes in the mixer was transferred to the device after the sample information was entered into a device record menu (Measurement using full automated method). Meanwhile, results were reported after generating blood cells and leukocyte histogram by obtaining with automatic probe 20 microL pipettes from the sample and "impedance" method in 60 seconds.

Pathologic method. Incisions prepared for the histopathologic analysis were stained with hematoxylin-eosin, and were examined in light microscope with 10 and 40 times magnification, and were evaluated with Modified Chiu point scoring system (**Table 1**).[12] All samples were examined by the same pathologist.

The Statistical Package for Social Sciences version 15 for Windows (SPSS Inc, Chicago, IL, USA) was used for statistical analysis. Illustrative statistical methods (frequency, percentage, mean, standard deviation) were used while evaluating the data. As distribution according to groups was small, the non-parametric statistical method was preferred. In case of 2 or more groups in comparing quantitative data, Kruskal-Wallis test was used when comparing parameters between groups, and Mann-Whitney U test was used when determining the group causing difference. Findings were evaluated in 95% confidence interval, and significance was considered at $p<0.05$ level.

Results.

Histopathological findings. Rats were divided into 2 according to the modified Chiu scoring system as: 0 - for ischemia not seen; and 1 and above - for ischemia seen rats. Cases with 2-5 score were not seen in the histological evaluation. Intestinal ischemia ratios are presented in **Table 2**. Although intestinal ischemia ratio in Groups 3 and 4 was higher than the intestinal ischemia ratio in Groups 1 and 2, a statistically significant difference was not found ($p>0.05$).

Microbiological findings. If there was reproduction in any of the tissue and blood sample, it was assessed as "reproduction +". We determined that the bacteria are *E. coli* 1104512 strain with API 20E test according to their Gram (+) and Gram (-) features. No bacteria other than *E. coli* 1104512 strain was reproduced in any of the microbiological evaluations. In other words, bacterial translocation was not determined in any of the groups. Reproduction ratios in Groups 3 and 4 were significantly higher than reproduction ratios in Groups 1 and 2 ($p=0.000$). Reproduction results were presented in **Table 3 and Table 4**.

Fever. Fever was not observed in any of the rats in Groups 1 or 2, but it was observed in all of the rats in Groups 3 and 4 (**Table 5**). We did not determine

Table 1 - Modified Chui Point Scoring System.

Score	Histopathological findings
0	Normal
1	Desquamation and necrosis in one third of villuses
2	Progressive desquamation at the center of villus
3	Desquamation at lower one third of villuses and necrosis of crypt cells
4	Necrosis of one third of crypt cells
5	Complete loss of basal crypt

Table 2 - Comparison of intestinal ischemia ratios of subjects with regard to groups.

Groups	Ischemia (+)	Ischemia (-)	Ischemia (+) (%)	*P*-value
1	0	8	(0)	
2	0	8	(0)	
3	2	6	(25)	
4	2	6	(25)	0.219

Table 3 - Number of *Escherichia coli* reproduced in the tissue and blood samples of groups.

Groups n=8	Liver	Spleen	MLN (%)	Blood
1	0	0	(0)	0
2	0	0	(0)	0
3	6	7	(5)	8
4	7	7	(6)	8

MLN - mesenterium lymph node

Table 4 - Comparison of the reproduction ratios of *Escherichia coli* in the tested subjects with regard to groups.

Groups	Reproduction (+)	Reproduction (-)	Reproduction (+) (%)	*P*-value
1	0	8	(0)	
2	0	8	(0)	
3	8	0	(100)	
4	8	0	(100)	0.000

Table 5 - Fever (°C) level distribution of subjects with regard to groups.

Groups	n	Mean ± standard deviation	*P*-value
1	8	36.33 ± 0.24	
2	8	36.26 ± 0.23	
3	8	39.20 ± 0.35	
4	8	39.21 ± 0.26	0.001

Table 6 - Distribution of leukocyte measurements of subjects with regard to groups.

Groups	n	Mean ± standard deviation	*P*-value
1	8	7018.75 ± 358.75	
2	8	7162.50 ± 462.72	
3	8	16987.50 ± 628.92	
4	8	17181.25 ± 842.59	0.001

any statistically significant difference between rectal temperature values of Groups 3 and 4 ($p>0.05$). We detected that the rectal temperature values of Groups 3 and 4 were significantly higher compared with Groups 1 and 2 ($p=0.001$).

Leukocyte. While no leukocyte was detected in the rats in Groups 1 and 2, leukocyte was detected in Groups 3 and 4. We acknowledge that this was related to the *E. coli* induced sepsis (**Table 6**).

Discussion. Even if it is agreed that practically there are changes in the physiological parameters and organ functions at pressures above 10 mm Hg, there are studies showing that there are changes in organ functions at pressures below 10 mm Hg.[7-9] Small increases in the intra-abdominal pressure may affect organ functions according to Malbrain.[7] Dessol et al[8] proved hepatic microcirculation decreased extremely at intra-abdominal pressures above 8 mm Hg in their experimental studies. Kologlu et al[9] concluded that even pressures of 4-6 mm Hg affected the healing of intestine anastomosis negatively, and this was probably related to the decrease in intra-abdominal perfusion. Contrary to these studies, Ozmen et al in their studies in 2002[13] defended that in case of pneumoperitoneum at 12 mm Hg pressure, there was no change in the microcirculation of the viscera, and there was no negative effect on the intestine perfusion. In sepsis model performed on rats by Youssef et al,[14] they reported that changes on the liver were more apparent in the pneumoperitoneum performed group for 30 minutes, when compared with the laparotomy performed group. In another study on the chemical peritonitis model in rats, bacterial translocation was identified explicitly in the 15 mm Hg pressure administered group for one hour.[15] In a study carried out by Pitombo et al[16] in rats, they stated that bacterial dissemination seen on the liver was more apparent in the 20 mm Hg pressure administered group, when compared with the 10 mm Hg pressure administered group.

Although Ozmen et al[13] concluded that the intestine perfusion was not affected negatively at 12 mm Hg pressure in their study conducted in 2002, they had determined bacterial translocation at a lower pressure (10 mm Hg) at sepsis presence in their study conducted in 1999.[17] When the results of these studies were compared, the intestine perfusion was affected at a lower pressure in the presence of sepsis. In a compilation performed by Karantonis et al,[18] various studies were reported directing surgeons to use laparoscopy in abdomen derived sepsis or peritonitis. Besides, they concluded that more studies were needed to strengthen the role of pneumoperitoneum on abdomen derived sepsis. Based on these discussions, we also investigated in our study the effects of intra-abdominal pressure increase on intestinal ischemia and bacterial translocation in the experimental intra-abdominal infection model. We aimed to present whether pneumoperitoneum applied in sepsis, at especially low pressure causes intestinal ischemia and bacterial translocation. We concluded in our study, that 8 mm Hg average pressure applied to rats for one hour did not cause intestinal ischemia and bacterial translocation. Ozmen et al[17] emphasized in the experimental intra-abdominal infection models they produced in 1999, in case of peritonitis CO_2 insufflation caused bacterial translocation, and thus, laparoscopic approaches should be applied with care under these circumstances.

Contrary to the study conducted at 10 mm Hg pressure by Ozmen et al,[17] and based on these results obtained, we concluded that in the case of intra-abdominal infection, laparoscopic approaches could be applied safely for both diagnosis and treatment at low pressures (8 mm Hg). Strobel et al,[19] in severe acute pancreatitis with infected necrosis model performed on rats in 2006, reported that there were no differences between cytokine levels, bacterial translocation, and organ complications when the one hour 8 mm Hg

pressure administered group was compared with the laparotomy administered group, and there was less trauma with laparoscopy. The results of this study[19] support the results obtained in our study. We consider that this study must be supported by more extensive experimental models and further clinical studies.

This study was limited to one hour of pneumoperitoneum treatment. Research of the effects of longer periods of pneumoperitoneum treatment was not an objective of this study. Besides, we consider that one hour treatment is an optimum time period to present the objective of this study as in the case of intra-abdominal sepsis, long term laparoscopic interventions are not convenient for clinical practices.

In conclusion, in acute peritonitis related intra-abdominal sepsis, laparoscopic interventions at 8 mm Hg pressure for one hour can be used with safety in diagnosis and treatment.

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