

Effects of laparoscopy and laparotomy on stress and hemodynamics in experimental sepsis model in rats

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Abstract

Aim: Laparoscopic abdominal surgery leads to reduced inflammatory response compared with laparotomy. However, there is controversial approaches are present in abdominal diseases complicated by diffuse or localized peritonitis.

Materials and Methods: Experimental cecal ligation and puncture method is used in the study. A total of 24 rats were used in the study. We divided all rats randomly four groups: control, sepsis, laparotomy and laparoscopy. After sepsis model was applied, we took blood samples and lung and liver samples as well. In plasma samples we determined 8-OHdG, 3-Nitrotyrosine, HNE, PGF-2 α via ELISA methods. The tissue samples were analyzed since the amount of blood samples were inadequate in all subjects. We measured MPO activity to assess the neutrophil infiltration rates.

Results: 3-NT mean values in laparotomy and laparoscopy groups were found to be almost the same while the difference between sepsis and laparoscopy groups was statistically significant ($p=0.015$) and decreased in the group undergoing laparoscopy. MPO levels obtained in rats underwent laparoscopy in liver tissue were found to be lower when compared with control and laparotomy groups and the difference between them was statistically significant. Also, MPO levels was found higher in sepsis laparotomy model as compared sepsis laparoscopy models.

Conclusion: Laparoscopy is accepted as golden standard in several elective procedures, since it is less stressful. Our results indicate that laparoscopy is superior to laparotomy in terms of inflammatory response in sepsis and should be preferred in septic patients.

Keywords: Laparoscopy; laparotomy; oxidative stress; sepsis

INTRODUCTION

Laparoscopic surgery is a less invasive compared to laparotomy and may provide some advantages such as decreased blood loss, postoperative pain, hospital stay, and complication rates (1-6). Stress and immunological responses are the result of any surgical interventions and the severity of the responses are thought to be proportional to the induced trauma in the body (7). The surgical effect leads to impaired immune function and infectious complications, which may lead to greater morbidity and postoperative complications (8-10).

Septicemia and septic shock develop due to the presence of microorganisms or toxic products in the bloodstream. The host reaction against the antigen comprises the sequence of events. Systemic inflammatory response syndrome (SIRS) leads to multiple organ dysfunction syndrome (MODS). Fever, shivering, tachycardia, tachypnea, and changes in consciousness can be seen in septicemia. The clinic syndrome is termed as septic shock or septic syndrome when hypotension and hypoperfusion are

included (11). Sepsis originating from an abdominal origin of infection may be referred to as intra-abdominal sepsis. Intraabdominal sepsis usually begins as peritonitis. In the case of peritonitis, peritoneal inflammation is present and peritoneal fluid increases due to fibrin-rich, leukocyte polymorphonuclear cells containing transition.

In recent years, minimally invasive techniques have begun to gain the most interest. Laparoscopy is one of the techniques that allow surgeons to perform operations in the abdominal region. Laparoscopy is accepted as golden standard in several elective procedures, since it is less stressful. (12-16). Laparoscopic surgery has the advantage that it enables early mobilization and causes less postoperative morbidity. In addition to this, there are benefits such as less bleeding and postoperative pain as well as less hospital stay (1,3,6,17). An inflammatory response develops after each surgery, depending on the magnitude of the trauma. An increase in infectious complications may occur secondary to this inflammatory response (8-10).

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In this study, we evaluated the effects of laparotomy and laparoscopy on cecal pellet sepsis model in which we created by using cecal ligation in rats.

MATERIALS and METHODS

In our study, cecal pellet model was used and cecal ligation was applied. A total of 24 Sprague-Dawley 350-400 gr rats were used in the study. We randomly divided rats into four groups; control, sepsis, laparotomy (LT) and laparoscopy (LC) group. Blood, lung and liver tissues of the rats were collected following sepsis. In the plasma samples, 8-OHdG (8-hydroxydeoxyguanosine), 3-Nitrotyrosine, HNE (4-Hydroxynoneal), 8-iso-Prostaglandin F2a (PGF-2a) molecules were determined by ELISA method. Also, NO (nitric oxide) molecule was detected in serum samples to evaluate the endothelial function and to detect inflammation. Myeloperoxidase (MPO) levels were determined in lung and liver tissue homogenates as an indicator of neutrophil inflammation.

Cecal ligation model

The cecal ligation model is an intra-abdominal sepsis model developed to investigate pathophysiology, treatment and systemic consequences of abdominal sepsis. Cecum is ligated and punctured with a needle in cecal ligation and puncture (CLP) model. CLP model results in septic shock and death after 24-48 hours (18). It is associated with polymicrobial sepsis, bacteremia, early hyperdynamic phase, and late hypodynamic phase. The mortality rate and the time interval to death can be modulated by the size of the needle (ie. as the needle size increases, the mortality rate increases).

Statistical analysis

Statistical calculations were made using SPSS (International Business Machines) statistical package (version 25). The distribution of numerical data in each group was evaluated by Shapiro Wilk's test. Levene test was used to evaluate the distribution of homogeneity of the groups. For comparison of multiple groups, F values (indicative of homogenization of distribution) or Welch values (indicative of non-homogeneity of distribution) were used for statistics. The Dunnett T3 method was used for the Welch-Test, and the Tukey HSD method was used for the F-Test in the comparison of binary groups. All tests were evaluated according to $\alpha = 0.05$.

RESULTS

The difference between the mean values between the control group and the other three groups was found to be statistically different in all parameters while it was not statistically significant (Table 1). The 3-NT molecule is an indicator of the effects of the peroxynitrite molecule (ONOOH) and thus cellular damage and oxidative stress. In our study, the mean values of LT and LC groups were found to be almost the same. The molecule is one of the best predictors of peroxidation of lipids in oxidative stress. However, the difference between sepsis and LC groups was statistically significant ($p = 0.015$) and decreased in the group undergoing laparoscopy (Table 1).

Table 1. 8-OHdG, 3-NT, 4-HNE and PGF-2 levels in plasma samples

Group	8-OHdG (ng/mL)		3-NT (ng/mL)		4-HNE (ng/mL)		PGF-2a (pg/mL)	
	Ort. D.	SD	Ort. D.	SD	Ort. D.	SD	Ort. D.	SD
Control	2.431	0.75 ^a	5.71	1.23 ^e	2.747	0.74 ⁱ	9.489	2.91 ^m
Sepsis	14.76	2.23 ^b	24.24	4.31 ^f	17.38	1.62 ^j	74.22	9.03 ⁿ
LT	21.83	4.99 ^c	21.37	3.47 ^g	16.09	2.24 ^k	67.22	12.07 ^o
LC	18.39	4.44 ^d	21.16	4.37 ^h	14.14	1.83 ^l	56.79	9.65 ^p

MPO levels in lung tissue were significantly lower in the control group compared to sepsis, LT, and LC groups, and the difference was statistically significant (Table 2). In addition, the mean MPO values obtained from the sepsis and laparoscopy group were found to be lower than the laparotomy group, and the difference was statistically significant. Furthermore, when the values obtained from liver tissue were compared, the difference between the control group and sepsis and LT group was found to be statistically significant. MPO levels obtained in rats underwent laparoscopy in liver tissue were found to be lower when compared with control and laparotomy groups and the difference between them was statistically significant (Table 2).

Table 2. MPO Levels in lung and liver tissues

Group	Lung MPO (U/g wet tissue)		Liver MPO (U/g wet tissue)	
	Ort. D.	SD	Ort. D. ± SD	
Control	114.44	82.83 ^a	71.07	18.9 ^e
Sepsis	1157.9	1167.4 ^b	147.96	31.96 ^f
LT	1360.1	878.8 ^c	143.8	28.12 ^g
LC	511.05	161.07 ^d	52.43	9.11 ^h

Although the NO levels of the control group were significantly increased compared to the sepsis group, the difference was not significant due to the large standard deviation (Table 3). Furthermore, NO levels in LT and LC groups were lower than the sepsis group, but there was no significant difference.

Table 3. NO Levels in serum samples

Group	NO (mol/L) Ort. D. ± SD
Control	14.58 14.54
Sepsis	35.05 27.45
LT	20.41 23.75
LC	19.67 12.98

DISCUSSION

Multiple organ dysfunction (MODS) is the most common cause of mortality and morbidity in intensive care units. In sepsis, renal and respiratory functions are affected in the case of MODS. Sepsis is a complex syndrome characterized

by a pro-inflammatory and anti-inflammatory response to pathogens. In the progression of sepsis, the production of reactive oxygen species (ROS) and the disruption of the balance between cellular antioxidants that break them down is very important. The deterioration of this balance has provided new clues for the creation of new treatment methods. Pro-inflammatory effects of ROS, including endothelial damage, the formation of chemotactic factors, migration of neutrophils, release of cytokines and mitochondrial insufficiency, all lead to an increase in free radicals and ultimately to oxidant-antioxidant balance. Laparoscopy is advantageous compared to laparotomy since it causes less trauma and inflammatory and neuroendocrine response (19).

Laparoscopic sepsis has been reported to cause less trauma and cause mild inflammation and neuroendocrine response. In response to systemic peritoneal, numerous mediator molecules are synthesized or regulated by the liver. However, the advantage of laparoscopic surgery over conventional surgery or mini-laparotomy remains unclear. However, it is not possible to say that the minimally invasive technique has no advantages compared to mini-laparotomy. Karantonis et al. found that the accumulation of ED-1 positive monocytes in the liver parenchyma of the full laparotomy and the accumulation of monocytes in the liver compared with other groups (19). Both mini and full-laparotomy have been reported to cause an increase in ED-2 positive cells in Kupffer cells. Laparoscopy, on the other hand, has been proposed to completely inhibit the growth of the macrophage population and maintain the order of normal cells. Postoperative regulation of the immunologic pathway is different due to the activation of immunocompetent cells and accumulation in the periportal area during laparotomy. Since the last two decades, surgeons have preferred laparoscopic cholecystectomy instead of conventional open cholecystectomy (OC). Laparoscopic cholecystectomy compared to open cholecystectomy has shown that the proliferation of T lymphocytes against various mitogens is detected by in vitro trials, resulting in less cell-mediated immune response reduction (20).

In the same study, laparoscopic and open cholecystectomy were performed randomly in the same group and blood results were found to be higher in the group with open cholecystectomy (IL-6, TNF- α , and IL-1 β) than the laparoscopic group. The data obtained showed that the laparoscopic method preserves the immune functions and the peritoneal immune response is better in the formation of sepsis compared to the open laparotomic method. It has been indicated that CO₂ may reduce the ability to respond to peritoneal contamination by disrupting macrophage physiology (20). The level of cell-mediated immune response is found to be decreased in laparoscopic cholecystectomy (21). In a study performed porcine models, both LT and LC groups were found to cause cellular immunosuppression. The severity and

duration of this impairment were found to be less in laparoscopy group (22).

When the effects of mini-laparotomy, laparotomy and laparoscopy on the hepatic macrophage populations were compared, the following results were obtained. Increased ED-1-positive monocytes (new monocytes) in the liver parenchymal and hepatic monocyte uptake were found in laparotomy group. ED-2-positive cell (Kupffer cells) increase was found in both mini and full laparotomy groups. Furthermore, no increase in hepatic macrophage population was found in laparoscopy group while normal cell placement was maintained in laparoscopy group. Postoperative regulatory immunological pathways are different from those occurring after sepsis (23).

In a study comparing patients underwent LT or LC due to benign conditions similar results were obtained. The activity of B-cells and natural killer cells was not affected in only the LT group, whereas the number of HLA-DR expressing monocytes showed a long-term reduction. Postoperative cell-mediated immune dysfunction was shown to be increased LT group. In a prospective study, assessing the serum cytokine levels in LC or OC patients, there was a significant difference in terms of IL-6 levels. IL-1 beta, IL-10, TNF-alpha, and cortisol levels were not significantly different ($p > 0.05$). Cytokine concentrations were higher in the OC group than in the LC group (24). The data mentioned above indicates that laparoscopy helps to protect the immune functions in a better way compared to laparotomy.

Pro-inflammatory cytokines released during inflammation also cause the release of (ROS). TNF- α leads to increase in NO and MPO levels subsequently in the respiratory burn. In our study, laparotomy (LT) and laparoscopy (LC) were performed in a sepsis model. Blood samples, liver and lung tissue samples were obtained after rats were sacrificed. Although we planned to evaluate MPO in leucocytes in the first place, MPO levels were studied in lung and liver tissues, which are the two most affected organs in sepsis, due to inadequate volume of blood samples.

When our results were evaluated, it was found that ROS which is related with DNA damage, were statistically decreased in laparoscopic group compared to the laparotomy group. However, when the 4-HN and PGF-2 α molecules were investigated, it was observed that the mean values of these two molecules are lower in LC group while the difference was not statistically significant. The average values of the 3-NT molecule, which indicates nitrotyrosine production (nitrated tyrosine residues) and the peroxynitrite molecule formed by the interaction of NO and superoxide radical were almost the same in both groups. Serum NO levels were almost the same in LT and LC groups and their standard deviations were very high in our study. In other words, the sensitivity of the NO molecule in these groups is quite low. Since the formation of the 3-NT molecule is related to the NO molecule, it is expected

that the mean values of this molecule are almost the same in both groups. MPO levels, one of the best indicators of inflammation, were detected in lung and liver tissue and found to be decreased in the LC group compared to the LT group.

CONCLUSION

When all these data are considered, we can say that our study is original. Although the data we obtained are new and there are very few publications on this subject, our findings are in accordance with the literature. Nevertheless, the study of patient blood samples will increase the accuracy of the data.

Conflict of interest: The authors declare that they have no competing interest.

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Ethical approval: The study is approved by the ethics committee of the Adnan Menderes University (64583101/2017/060).

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