Original Article

Carbon Dioxide Pneumoperitoneum May Alter Ovarian Apoptosis: An Experimental Study

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Abstract

Objectives: The aim of this study was to evaluate ovarian immunohistochemical CD95 expression in a rabbit carbon dioxide pneumoperitoneum model.

Materials and Methods: The study group including seven rabbits was subjected to intra-abdominal pressure (IAP) (12 mmHg); the control group was not subjected to IAP (the sham group, $n = 7$). At the end of the experiment, ovariectomy was performed. Immunohistochemical stained histologic specimen of the ovary with CD95 was evaluated. Based on the degree of cytoplasmic or membranous staining for CD95 from 0 (none) to 3 (severe), a microscopic apoptosis scoring system was used.

Results: Statistically significantly higher apoptosis scores in ovarian surface epithelial cells (2.57 ± 0.53, vs. 1.14 ± 0.38, $P = 0.002$, Mann–Whitney *U*-test, respectively), follicular epithelial cells (2.85 ± 0.38, vs. 1.85 ± 0.38, $P = 0.002$, Mann–Whitney *U*-test, respectively), and stromal cells (2.71 ± 0.49, vs. 1.29 ± 0.49, $P = 0.002$, Mann–Whitney *U*-test, respectively) were observed in pneumoperitoneum group, compared with no-pneumoperitoneum group.

Conclusion: Even at safe IAP (12 mmHg) for an acceptable operation time period, there was a significant increase in apoptosis of ovarian cells.

Keywords: Apoptosis, gynecologic endoscopy, ovary, pneumoperitoneum

Introduction

Laparoscopy has been used in gynecologic surgery for 30 years. Laparoscopic surgery is more preferable than laparotomy, and minimally invasive procedures such as robotic surgery are more trendy today. The shorter hospital stays, less pain, and smaller surgical incision make the gynecologic endoscopic surgery more preferable to other surgical approaches. However, there are some complications of laparoscopy. In particular, complications associated with high intra-abdominal pressure (IAP) due to carbon dioxide (CO_2) pneumoperitoneum are not well described.[1]

As clearly described in previous studies, laparoscopy caused ischemia and reperfusion injury in intra-abdominal organs by altering organ blood perfusion. Previous studies have reported the effect of high IAP on intra-abdominal blood flow to empty and solid organs.[2,3] In our previous study, it was shown that pneumoperitoneum caused biochemical and histological damage by causing significant oxidative stress in ovaries, even at safe IAP levels.[4] Nowadays, researchers mostly focus on the effect of laparoscopy on apoptosis. CO_2 pneumoperitoneum causes substantial oxidative stress. This oxidative stress induced intra-abdominal organ apoptosis and also resulted in cell injury.[5]

In many studies, the effect of CO_2 on intra/extra-abdominal organs (including gastrointestinal, urinary, pulmonary, and genital system) was evaluated in experimental pneumoperitoneum models. For the reduction of oxidative

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stress, the keeping of IAP at 8–12 mmHg was determined to be the most appropriate pressure level in which the organs were minimally affected. Under 12 mmHg, IAP may be accepted as a safe level.[3,6-10]

The exhaustive literature review was failed to reveal any studies investigating or reporting the harmful effect of CO_2 pneumoperitoneum on ovarian apoptosis. The objective of this experimental study was to investigate the effect of CO_2 pneumoperitoneum on apoptosis in ovaries.

Materials and Methods

CO_2 pneumoperitoneum experimental model which was well described in our previous study[4] was used. Following totally 60 min pneumoperitoneum (IAP of 12 mmHg) and 10 min of reperfusion period in the study group (n = 7) and the same period without pneumoperitoneum in the control group (n = 7) under anesthesia, ovaries were excised and examined for the degree of apoptosis.The approval (2007/23) from Karadeniz Technical University Animal Care and Ethics Committee was obtained for this study.

Rabbit ovary was bigger and less fragile than rat ovary. Since CO_2 pneumoperitoneum during laparoscopy may have the ability to increase the fragility induced ovarian damage, we prefer to use rabbit model instead of rat ovary.

Adult female rabbits were used. All rabbits were at least 6-month-old and randomly selected for study or control groups. The median weight of rabbit was 2200 g. The animals were kept under standard laboratory conditions at room temperature. Animals were feeded with a standard laboratory diet and had free access to food and water.

The animals were anesthetized with an IM injection of 30 mg/kg ketamine (Ketalar; Parke-Davis, Istanbul, Turkey) and 10 mg/kg 2% xylazine hydrochloride solution (Rompun; Bayer, Leverkusen, Germany) and were allowed to breathe spontaneously (not intubated) during the experiments. The animals were oxygenated with an oxygen mask with a volume of 1–1.5 L/min throughout the experiment. The rabbits were placed on the operating table in a supine position, and the abdomen was shaved and disinfected with polyvidone iodine. In both groups, after a midline abdominal incision was made, a 5-mm trocar (Karl Storz, Tuttlingen, Germany) was placed caudal to the sternum. After placement of the 5-mm trocar, the abdomen was closed with an interrupted suture technique. For controlling the IAP during pneumoperitoneum, an electronic electric gas insufflator (Karl Storz) was used. All animals were anesthetized for a total of 80 min: 10 min preinsufflation (laparotomy, trocar placement, and closure of the abdomen were performed during this interval), 60 min of pneumoperitoneum, and 10 min of reperfusion. The subsequent pneumoperitoneum versus no-pneumoperitoneum experiment was performed after 10 min of anesthesia. After a midline abdominal incision was made, the ovaries were localized, and the right ovary was excised for histologic studies and fixed in formaldehyde (10%) solution for histopathologic examination.

Routine ovarian specimen preparation with hematoxylin and eosin (H and E) staining was used, and tissue sections (5 mm thick) were stained with the CD95 immunohistochemical staining method (NCL-Fas-310 mouse monoclonal antibody, Sitogen, Istanbul, Turkey) for apoptosis evaluation.[11] Membranous staining in ovarian cells was used as the positive control. For the evaluation of CD95, comprehensive scoring made use of the method of Shibakita *et al*.[12]

Initially, all H and E stained slights were examined for the presence of corpus luteum. The absence of corpus luteum was confirmed in all specimens. Immunohistochemical staining of fas (CD95) in the ovarian surface, follicle, and stromal cells was evaluated. Each specimen was scored with a scale ranging from 0 to 3 (0; none, 1; mild, 2; moderate, and 3; severe) based on cytoplasmic or membranous staining.

Statistical analysis

Apoptotic scores were expressed as mean ± standard deviation. Statistical comparisons among groups were performed using the Mann–Whitney *U*-test with Bonferroni correction as *post hoc* test. Statistical significance was set at $P < 0.05$.

Results

The mean rabbit weights were comparable in two groups. The comparisons of mean apoptosis scores of ovarian surfaces, follicular epithelial, and stromal cells in pneumoperitoneum and no pneumoperitoneum groups are given in Figure 1. Statistically significantly higher apoptosis scores in ovarian surface epithelial cells (2.57 ± 0.53, vs. 1.14 ± 0.38, $P = 0.002$, Mann–Whitney *U*-test, respectively), follicular epithelial cells (2.85 ± 0.38, vs. 1.85 ± 0.38, $P = 0.002$, Mann–Whitney *U*-test, respectively), and stromal cells (2.71 ± 0.49, vs. 1.29 ± 0.49, $P = 0.002$, Mann–Whitney *U*-test, respectively) were observed in pneumoperitoneum group, compared with no-pneumoperitoneum group.

Discussion

The experimental model suggested that alteration in IAP had a detrimental effect on ovaries. This study confirmed the damage of ovarian surface, follicular epithelial, and stromal cells during CO_2 pneumoperitoneum by increase in ovarian apoptosis even under the 12 mmHg IAP level.

Gynecologic endoscopy is an important and diagnostic and treatment procedure in gynecological practice all over the world.

Less bleeding, reduced postoperative pain, smaller surgical incision, lower risk of postoperative delirium, and shorter hospital stay have been reported as accepted benefits of gynecologic endoscopy over conventional surgery.[13-15] The reported rate of complications derived from gynecologic endoscopy was ranged from 0.1% to 10%.[16] Some reported complications are gas embolism, blood acid-base disturbances, and increased or altered IAP-related tissue damage.[10,16] CO_2 pneumoperitoneum may increase tissue gas absorption; this process may cause acidosis, especially in patients subjected to high operation time. CO_2 pneumoperitoneum associated such complications may be observed in 5.5% of patients.[17] There has been no study investigating ovarian apoptosis induced by pneumoperitoneum.

CO_2 during pneumoperitoneum may cause some physiologic changes in solid organs. The main mechanism underlined this complication was ischemia–reperfusion. Since CO_2 insufflation may case ischemia, the desufflation of CO_2 may cause reperfusion injury. Routine CO_2 insufflation and desufflation attacks may increase the release of free radicals and may alter the oxidative stress signal pathway. This oxidative stress and lipid peroxidation process may have a major role in reperfusion injury of intra-abdominal organs. The main pathophysiologic mechanism of some adverse clinical conditions following laparoscopic procedures may be the disturbed functions of intra-abdominal organs related by the increase in oxidative stress.[6,9,10,18] The pneumoperitoneum associated oxidative stress may alter ovarian apoptosis. To the best of our knowledge, this issue has not been investigated and reported previously. Recent studies also focused on the preventive molecules of CO_2 pneumoperitoneum-induced ischemia–reperfusion ovarian damage.[19,20]

The process of mammalian survival may require mature oocyte development and release from the ovary. The human ovary secretes sex hormones, produces and releases mature oocyte, and supports pregnancy through complex and well-designed mechanisms. This mechanism has not been well known. There have been many unknown mysteries regarding these mechanisms. These reported functions of the ovary may require sufficient oxygen and normal microvascular supply.[21] Oxidative stress induced by CO_2 pneumoperitoneum-associated ischemia–reperfusion injury may affect such ovarian functions and increase ovarian apoptosis.

Recently, most studies about pneumoperitoneum focus on the effect of CO_2 pneumoperitoneum on cancer cells and focus on apoptosis, invasion, and proliferation of cells. In these studies, it was observed that pneumoperitoneum increased apoptosis in normal CO_2 pressure and decreased cancer cell proliferation.[22,23] Based on preliminary study results on the effects of pneumoperitoneum on abdominal organs, apoptosis was not changed in normal CO_2 pressure. However, apoptosis increased in high CO_2 pressure.[24] In experimental studies, it was shown that high IAP directly increased renal[25] and hepatic[26] cell apoptosis. In our study, the high ovarian apoptotic scores during pneumoperitoneum were also demonstrated by histopathological study, as clearly shown in Figure 2. High IAP level may have a direct effect on the ovarian surface epithelium. Since CD95 shows DNA damage in these cells, CO_2 pneumoperitoneum may alter apoptosis in ovarian cells at safe pressures. One reason for the ovary to be affected at safe pressures may be the absence of a protective membrane on the ovary. The increased CD95 antigens may also cause further an additional reduction in ovarian reserve by means of

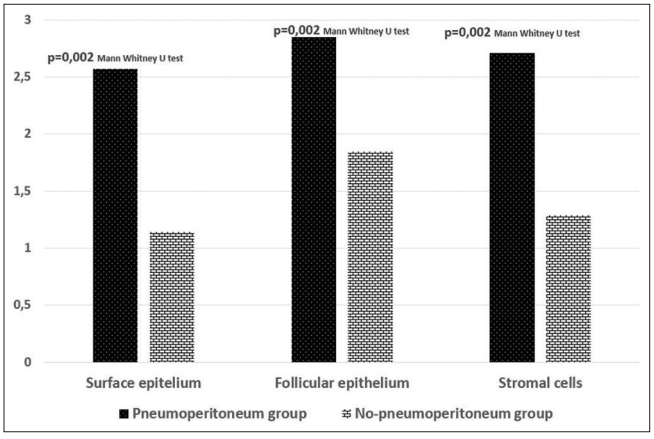

Figure 1: The comparisons of mean apoptosis scores of ovarian surfaces, follicular epithelial, and stromal cells in pneumoperitoneum and no pneumoperitoneum groups. Mann–Whitney *U*-test was used for comparison

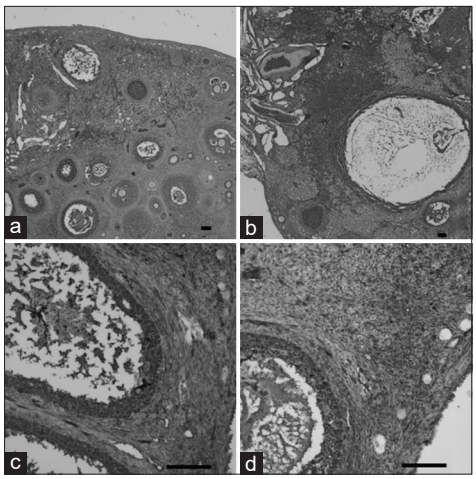

Figure 2: (a) No CD95 staining in ovary cells (surface, follicular, and stromal) in the no-pneumoperitoneum group (Cd95, ×40), (b) High CD95 staining in the pneumoperitoneum group (CD95, ×40). (c) No CD95 staining in ovary cells (surface, follicular, and stromal) in the no-peritoneum group (CD95, ×200), (d) High CD95 staining in the pneumoperitoneum group (CD95, ×200). Scale bars represent 200 μm

apoptosis in the ovarian surface epithelium. This issue also needs further research.

Decreased blood flow caused by laparoscopy leads to ischemia in the abdominal organs.[3] Based on experimental study results, it has been shown that increased abdominal pressure during pneumoperitoneum may cause histopathological and apoptotic significant damage in the testes in a long-term period and may cause infertility depending on the level of the abdominal pressure increase.[3,27] Similarly, the ovaries are also highly sensitive to apoptosis. As reported in our study [Figure 1], significant findings of increasing apoptosis scores were observed in the pneumoperitoneum group.

CD95 antigen has been reported as a cell death mediator.[28] CD95 antigen stimulated granulosa and luteal cell apoptosis.[29,30] At the early stages of human follicular development, Fas (CD95) expression has a major role in granulosa cell apoptosis.[29] Fas antigen activated p53-mediated granulosa cell apoptosis in follicular atresia procedure.[31] These findings may suggest the idea of high apoptosis, may increase follicular atresia, and alter fertility. In a healthy body, Fas receptor activation is a complex process. The activation results in the recruitment of Fas-associated death domain (FADD)-containing protein.[32] FADD then activates procaspase 8,[33] that activates other caspases such as caspases 3, 6, and 7.[34] One study reported that cumulus cells surrounding aging oocytes secrete apoptosis-related Fas ligand and accelerate oocyte aging by binding to Fas receptors.[35] We believed that the direct effect of increased IAP on ovary may increase apoptosis and reduce ovarian reserve and decrease human fertility potential. The apoptosis changes observed in histological specimens [Figure 2] may also support this idea.

The limitations of this study were the small sample size and lack of biochemical oxidative stress marker evaluation and the lack of human subjects.

Conclusion

This experimental study reported that even at safe IAP for an acceptable operation time period, there was a significant increase in apoptosis of ovarian cells. The safety of CO_2 pneumoperitoneum during laparoscopy may be questioned even under the nowadays condition of large amount of usage of gynecologic laparoscopy in all over the world. This study results need clarification with human subjects, so further human studies related with ovarian apoptosis are needed. Such studies may answer the question of the underlined pathophysiologic mechanism and factors of unexplained infertility.

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Conflicts of interest

There are no conflicts of interest.

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