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Effects of carbon dioxide pneumoperitoneum on exocrine and endocrine functions, and oxidative state of rat pancreas

Sefa Sag [a], Mustafa Imamoglu[b], Haluk Sarihan [b], Esin Yulug [c], Ahmet Alver [d], Sukran Geze Saatci [e], and Ali Cay [f]

[a]Department of Pediatric Surgery, University of Health Sciences, Kanuni Education and Research Hospital, Trabzon, Turkey; [b]Department of Pediatric Surgery, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey; [c]Department of Histology and Embryology, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey; [d]Department of Medical Biochemistry, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey; [e]Department of Anesthesia and Critical Care, Faculty of Medicine, Karadeniz Technical University, Trabzon, Turkey; [f]Department of Pediatric Surgery, Faculty of Medicine, Bezmialem Vakif University, Istanbul, Turkey

ABSTRACT

We investigated the effects of increased intra-abdominal pressure during laparoscopy on the endocrine and exocrine functions, oxidative stress and histopathology of the pancreas in rats. We established three experimental groups of eight animals. Group 1 was the untreated control. Forth other two groups, pneumoperitoneum with CO_2 was established for 60 min at 6 mm Hg for group 2 and 12 mm Hg for group 3; groups 2 and 3 animals were allowed to re-perfuse for 30 min. Amylase, glucagon and insulin levels were analyzed in blood samples and insulin:glucagon ratio was calculated. Histopathology and malondialdehyde assay were performed on pancreatic tissue samples. Histological damage scores for vasocongestion were increased significantly in groups 2 and 3 compared to group 1. Histological damage scores for inflammatory cell infiltration were increased significantly in group 3 compared to group 1. Malondialdehyde levels were increased significantly in group 3 compared to group 1. We found no significant differences among groups for serum amylase levels or histological damage scores for hemorrhage. Insulin and glucagon levels, and the insulin:glucagon ratio was increased significantly in group 3 compared to groups 1 and 2. We found that in rats routine laparoscopy caused increased serum insulin and glucagon levels, and histopathological changes that indicated ischemia-reperfusion injury to the pancreas.

KEYWORDS

Ischemia-reperfusion; laparoscopy; oxidative stress; pancreas; pneumoperitoneum; rat

Laparoscopy is widely used for both diagnosis and treatment. Pneumoperitoneum induced during laparoscopy may cause problems in organs and tissues following surgery. It has been suggested that increased intra-abdominal pressure (IAP) due to pneumoperitoneum may cause hypoperfusion by increasing organ and tissue resistance to venous return (Schafer and Krahenbuhl 2001; Polat et al. 2003). After deflation of pneumoperitoneum, ischemia-reperfusion injury can occur due to resumption of blood flow. Increased IAP can affect extra-abdominal organs such as testes and lungs in addition to intra-abdominal organs including liver, spleen and pancreas (Schafer and Krahenbuhl 2001; Polat et al. 2003; Cay et al. 2006; Imamoglu et al. 2006; Unsal et al. 2006; Guven et al. 2010).

Intra-abdominal pressure between 6 and 12 mm Hg are used routinely during laparoscopy. In patients undergoing laparoscopic surgery, splanchnic blood flow was decreased significantly and blood flow to the stomach, jejunum, colon and liver also were affected when IAP exceeded 10 mm Hg (Schilling et al. 1997). The splanchnic circulation consists of blood flow originating from the celiac, superior mesenteric and inferior mesenteric arteries. Blood flow to the pancreas is supplied by the splanchnic circulation (Harper and Chandler 2016). The pancreas is susceptible to ischemia-reperfusion injury and disturbance of the microcirculation may affect the functions of islet and acinar cells adversely (D'Hoker et al. 2013). It has been reported that edema, inflammation and necrosis occur in pancreatic tissue exposed to IAP (Otto et al. 2010; Boyuk et al. 2011). Because the pancreas is affected by IAP that produces intra-abdominal hypertension, it is possible that pathological changes may develop in the pancreas even under the routine pressures used for laparoscopy. We have found no study of the effects of pneumoperitoneum on endocrine and exocrine functions of the pancreas exposed to routine laparoscopic pressures. We investigated the effects of

CONTACT Sefa Sag drsefa51@gmail.com; sefa.sag@sbu.edu.tr Department of Pediatric Surgery, University of Health Sciences, Kanuni Education and Research Hospital, Trabzon, Turkey

IAP during laparoscopy on the endocrine and exocrine functions, oxidative stress status and the histopathological structure of the pancreas.

Material and methods

Animals and experimental groups

Our study was conducted in the Pediatric Surgery Department of the Karadeniz Technical University and was approved by the Faculty of Medicine Committee on Animal Research and Ethics (protocol no. 2012/23). We used 24 250 – 300 g female rats. The animals were housed at 20 – 24 °C, 50 – 60% relative humidity with a 12 h light:12 h dark cycle. Standard feed and water were provided *ad libitum*. The animals were housed at the Research Center of Karadeniz Technical University. The animals were divided randomly into three groups of eight.

Pneumoperitoneum was achieved by peritoneal cavity puncture using an 18 gauge (G) angiocatheter (Nova Cath IV cannula; Medipro, Istanbul, Turkey) inserted caudal to the sternum. Carbon dioxide (CO_2) was insufflated through this catheter and an electronic gas insufflator (Karl Storz, Tuttlingen, Germany) was used for controlling the IAP during the pneumoperitoneum period. All animals were anesthetized for 90 min. The animals in group 1 were not exposed to IAP, but were connected to a laparoflator by an 18 G angiocatheter placed in the abdomen. IAP insufflation was performed continuously to maintain the IAP at 6 mm Hg for 60 min in group 2 and at 12 mm Hg for 60 min in group 3. Reperfusion for 30 min followed pneumoperitoneum.

Experimental protocol

Following anesthesia with 50 mg/kg ketamine (Ketalar; Parke-Davis, Berlin, Germany) and 10 mg/kg xylazine (Rompun; Bayer, Leverkusen, Germany) injected intramuscularly, the abdomen and neck regions of the rats were shaved and cleaned. Tracheostomy was performed and a 16 G (Nova Cath IV cannula; Medipro, Istanbul, Turkey) angiocatheter was inserted; ventilation was provided by a ventilator (Versamed Tvent 201; Kadima, Israel). Pneumoperitoneum was induced by CO_2 intra-abdominal insufflation at a rate of 0.1 – 0.2 l/min using 18 G angiocatheter (Nova Cath) placed caudal to the sternum. IAP was kept constant using an automatic laparoflator (Karl Storz, Tuttlingen, Germany) and reperfusion for 30 min followed pneumoperitoneum for 60 min.

Blood samples were obtained by intracardiac puncture for biochemical assays 30 min after desufflation of the abdomen. The abdomen was opened with a median incision and the pancreas was removed completely. Each pancreas was gently cleaned of fat and blood using 0.9% saline solution, then divided sagittally into two parts. One part was used for histopathological examination and the other for biochemical analysis. The latter specimen was stored at −80 °C until analysis.

Histopathology

Pancreatic tissue was fixed in 10% formaldehyde for 48 h. Fixed tissues were dehydrated through a graded alcohol series, cleared with xylene and embedded in paraffin. Sections were cut at 5 µm using a RM 2255 automated microtome (Leica RM 2255, Tokyo, Japan). Sections were deparaffinized, rehydrated, then stained with hematoxylin and eosin (H&E) (Stevens and Wilson 1996).

Pancreatic tissue was evaluated for vascular congestion, hemorrhage, inflammatory cell infiltration, edema and other degenerative changes. Sections were examined using a BX51 light microscope (Olympus, Tokyo, Japan). Sections were inspected by an experienced histologist blinded to the group identity of the specimens. The mean histopathologic score was calculated for each group (Gunduz et al. 2009). For scoring, five different areas of sections from each group were evaluated semiquantitatively at 200 x. Semiquantitative scores were assigned as 0, normal tissue; 1, mild changes; 2, moderate changes; 3, severe changes.

Malondialdehyde (MDA) in pancreas

Pancreatic tissue was weighed, then homogenized with an ice-cold 1.15% potassium chloride solution containing 0.5 ml/l 10% (w/v) Triton-X 100. Tissue was homogenized at 9,500 rpm four times for 10 sec at 4 °C using an Ultra-Turrax T25 homogenizer (IKA-Labortechik, Staufen, Germany). MDA levels of pancreas homogenates were measured using the method of Uchiyama and Mihara (1978). The basic principle of this test is the formation of a pink color as a result of the reaction of thiobarbituric acid with lipid peroxides. Tetraethoxypropane was used as a standard and tissue MDA levels were calculated as nmol/g wet tissue.

Serum hormone and enzyme levels

Blood samples were centrifuged at 1,000 x g for 10 min and serum samples were frozen at −80 °C until assay. Insulin (DRG International, Inc., Springfield NJ), glucagon (Cusabio Biotech Co. Ltd., Wuhan, China)

and amylase (Cusabio Biotech Co. Ltd.) levels were measured using commercial ELISA kits according to the manufacturer's instructions. Insulin:glucagon ratio also was calculated.

Statistical analysis

Statistical analyses were performed using SPSS v.16 Med Calc 12.3 statistical software (SPSS Inc., Chicago, IL.). Data are means ± SD. Kruskal Wallis analysis of variance was used to compare differences between group parameters. Dual comparisons between groups exhibiting significant differences were evaluated using the Mann-Whitney U-test with corrected Bonferroni test. Statistical significance was accepted for all tests at $p \leq 0.05$.

Results

Histopathology

No hemorrhage, congestion or inflammatory cell infiltration was observed in group 1. In group 2, mild hemorrhage and inflammatory cell infiltration and moderate vasocongestion were observed. In group 3, mild hemorrhage and moderate congestion and inflammatory cell infiltration were observed (Figure 1). Histological damage scores for hemorrhage indicated no statistically significant differences between groups 1 and 2, groups 2 and 3, or groups 1 and 3 (Figure 1, Table 1). Histological damage scores for vasocongestion were increased significantly in groups 2 and 3 compared to group 1. No significant difference was found between groups 2 and 3 (Figure 1, Table 1). Histological damage scores for inflammatory cell infiltration were significantly greater for group 3 than for group 1. No statistically significant differences were found between groups 1 and 2, and groups 2 and 3 (Figure 1, Table 1).

MDA

MDA levels were increased significantly in group 3 compared to group 1. No significant differences were found between groups 1 and 2, and groups 2 and 3 (Table 2).

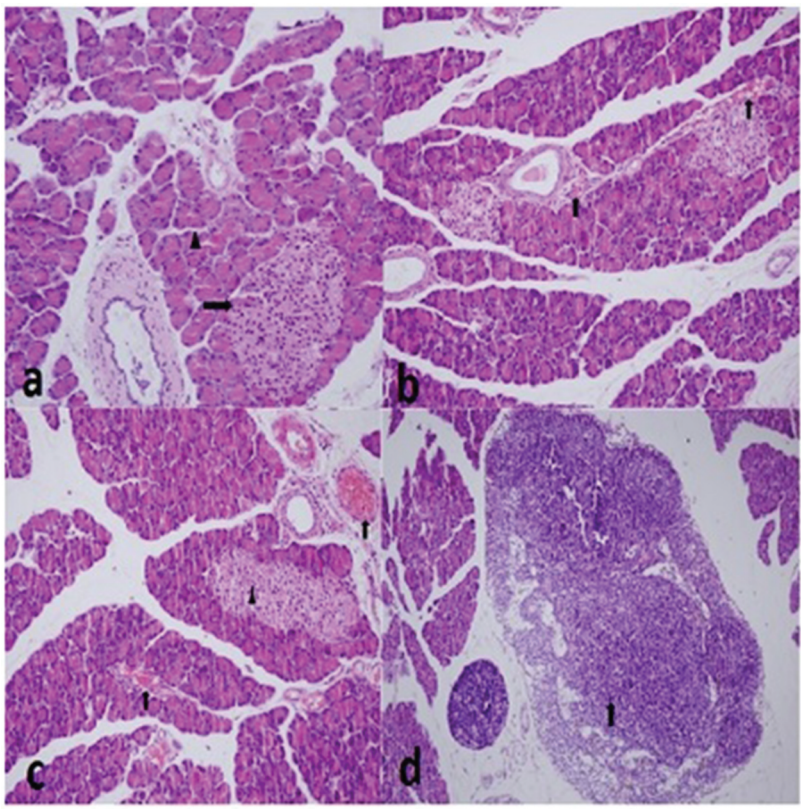

Figure 1. Representative micrographs of pancreatic tissue of all groups. a) Normal seroacinus cells (▲) and islet of Langerhans (↑) in group 1. H & E staining. x 200. b) Vasocongestion (↑) in interlobular area in group 2. H & E staining. x 200). c) Vasocongestion (↑) in interlobular area and between Langerhans cells (▲) in group 3. H & E staining. x 200. d) Inflammatory cell infiltration (↑) in interlobular area in group 3. H & E staining. x 200.

Table 1. Histological damage scores.

Group	Hemorrhage	Vasocongestion	Inflammatory cell infiltration
1	0.12 ± 0.35	0.12 ± 0.35[a]	0.12 ± 0.35[d]
2	0.25 ± 0.46	1.5 ± 0.53[b]	0.25 ± 0.46
3	0.37 ± 0.51	1.62 ± 0.51[c]	1 ± 0.75[e]

No statistically significant differences were found for hemorrhage. Histological damage scores for vasocongestion decreased significantly in group 1 vs. groups 2 and 3 ($^{a-b, a-c}p < 0.05$). Histological damage scores for inflammatory cell infiltration were increased significantly in group 3 vs. group 1 ($^{d-e}p < 0.05$). $^{a-b, a-c, d-e}p < 0.05$

Evaluation of exocrine functions

Serum amylase levels of the groups exhibited no statistically significant differences between groups 1 and 2, groups 2 and 3, or groups 1 and 3 (Table 2).

Evaluation of endocrine functions

Serum insulin levels were increased significantly in group 3 compared to groups 1 and 2. No significant difference in insulin levels was found between groups 1 and 2 (Table 2).

Serum glucagon levels were increased significantly in group 3 compared to groups 1 and 2. No significant difference was found in glucagon levels between groups 1 and 2 (Table 2).

The insulin:glucagon ratio was increased significantly in group 3 compared to groups 1 and 2. No significant difference was found in insulin:glucagon ratio between groups 1 and 2 (Table 2).

Discussion

We investigated the effects of IAP on pancreatic tissue and pancreatic functions under conditions resembling routine laparoscopy. We found that IAP caused vasocongestion and inflammatory cell infiltration in pancreatic tissue. The MDA level, which is an indicator of ischemia-reperfusion injury, increased in the IAP groups. Serum amylase, which is an indicator of exocrine function, did not increase, but serum insulin and glucagon levels, which are indicators of endocrine function, increased as IAP increased.

Previous investigations of the effects of increased IAP on the pancreas employed pressures and durations that were greater than typical clinical conditions (Otto et al. 2010; Boyuk et al. 2011). By contrast, we used pressures and periods comparable to clinical conditions. Our findings suggest that 12 mm Hg pressure for 60 min, which is routine for laparoscopy, may cause histopathological damage to the pancreas.

Ischemia-reperfusion damage may develop in various organs during laparoscopy. MDA is a breakdown product of lipid oxidation and is widely used as an indicator ischemic damage. Yol et al. (2004) induced acute pancreatitis by biliary pancreatic duct ligation and applied IAP for 30 min at 12 mm Hg with CO_2 in rats. These investigators reported no significant change in pancreatic MDA in the group with IAP compared to sham operated and laparotomy without pneumoperitoneum groups. We found that the MDA level was increased in group 2 compared to the control group, but the difference was not statistically significant. The increased MDA levels in group 3 were significant compared to controls. Our findings indicate that IAP with 12 mm Hg pressure for 60 min causes ischemia-reperfusion injury in the pancreas.

The pancreas has both exocrine and endocrine functions; amylase is the usual indicator of its exocrine functions. Mujicić (2006) reported no significant differences in amylase levels at 2, 4, 6, 8, 12, 24 and 48 h in postoperative in patients who were treated by laparoscopic cholecystectomies at 15 mm Hg pressure. Yol et al. (2004) used increased IAP for 30 min at 12 mm Hg in rats and induced pancreatitis by bilio-pancreatic duct ligation; no significant change in amylase was found compared to controls. Consistent with the literature, we found that the exocrine function of the pancreas was not affected significantly by 6 or 12 mm Hg pressure.

Aktan et al. (1994) compared pancreatic hormone levels following laparoscopic (10 – 12 mm Hg IAP) and open cholecystectomies. These investigators reported increased insulin levels at 12 and 24 h postoperatively. Engin et al. (1998) reported that post-operative insulin and glucagon levels were increased compared to pre-operative values 6 h following both open and laparoscopic cholecystectomies; however, the increase in glucagon was greater following laparoscopic surgery than for open surgery. While trauma due to disruption of splanchnic blood supply by increased

Table 2. Amylase, glucagon, insulin and MDA levels, and insulin:glucagon ratios.

Parameters (units)	Group I	Group 2	Group 3
Amylase (U/l)	17 ± 4.4	17.4 ± 4.8	18.9 ± 3.8
Glucagon (pg/ml)	881.8 ± 217.4[a]	889.98 ± 300.9[b]	1906.2 ± 395.2 [c]
Insulin (μIU/ml)	0.351 ± 0.2[d]	0.357 ± 0.2[e]	2.2 ± 0.9 [f]
MDA (nM/mg)	91.4 ± 20.9 [g]	143.9 ± 40.8	171.4 ± 27.2 [h]
Insulin:glucagon ratio	0.0004 ± 0.00023[ı]	0.0004 ± 0.00031 [j]	0.0012 ± 0.00059 [k]

No statistically significant differences were found for serum amylase levels. Serum glucagon, insulin levels and insulin:glucagon ratio were increased significantly in group 3 vs. groups 1 and 2 ($^{a-c, b-c, d-f, e-f, ı-k, j-k}p < 0.05$). MDA for pancreatic tissue was increased significantly in group 3 vs. group 1 ($^{g-h}p < 0.05$). $^{a-c, b-c, d-f, e-f, g-h, ı-k, j-k}p < 0.05$

IAP affects insulin and glucagon levels, systemic neuroendocrine effects also may affect pancreatic endocrine activity. Also, histopathological and biochemical changes are correlated with the severity of surgical trauma (Jan and Lowry 2010). We found no significant difference in the insulin and glucagon levels following 6 mm Hg IAP compared to the control group, but we did observe a significant increase of these hormones at 12 mm Hg compared to 6 mm Hg IAP. We believe that increased insulin and glucagon levels may be caused by structural damage to the pancreas due to ischemia-reperfusion as well as a possible systemic metabolic response due to surgical trauma. We believe that the increased insulin and glucagon secretion are caused primarily by ischemia-reperfusion injury. Also, the insulin:glucagon ratio was higher in group 3 than the other groups. Approximately 70 – 80% of islet cells are β-cells, which secrete insulin; glucagon secreting α-cells constitute approximately 15% of the cells (Longnecker 2014). β-Cells are sensitive to oxidative stress (Lenzen 2008; D'Hoker et al. 2013; Park et al. 2014); there is no comparable information about α-cells. Although we do not know whether β-cells were affected more than α-cells by increased IAP, our findings suggest that ischemia-reperfusion injury due to IAP increased insulin levels more than it increased glucagon levels.

A limitation of our study was the small number of subjects, which was due to ethical constraints. Nevertheless, statistical differences were found among groups.

We found that in a rat model, routine laparoscopy caused increased serum insulin and glucagon levels and caused histopathological changes that indicated ischemia-reperfusion injury to the pancreas. Further studies are required to apply our findings to the clinical setting.

Disclosure statement

The authors report no conflict of interest.

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ORCID

Sefa Sag http://orcid.org/0000-0002-0904-315X
Haluk Sarihan http://orcid.org/0000-0002-0991-8235
Esin Yulug http://orcid.org/0000-0002-8857-9234
Ahmet Alver http://orcid.org/0000-0002-9617-6689
Sukran Geze Saatci http://orcid.org/0000-0001-9115-8257
Ali Cay http://orcid.org/0000-0002-2037-4451

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