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Effects of carbon dioxide pneumoperitoneum on renal function in obstructive jaundice: an experimental study in a rat model

Efectos del neumoperitoneo por dióxido de carbono sobre la función renal en la ictericia obstructiva: un estudio experimental en un modelo de rata

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RESUMEN

Introducción: Se sabe que tanto el neumoperitoneo (PNP) como la ictericia obstructiva (IO) conducen potencialmente a una lesión renal aguda (IRA), pero no se ha investigado el efecto combinado. **Objetivo:** Este estudio tuvo como objetivo investigar los efectos del PNP en las funciones renales en un modelo de rata de IO. **Métodos**: Cuarenta y ocho ratas se dividieron en ocho grupos de seis ratas. El grupo 1 fue el grupo de control (operado de forma simulada); A los grupos 2, 3 y 4 se les indujo 5, 10 y 15 mmHg de CO2 PNP respectivamente (Grupos 1-4: "Grupos no OJ"); el grupo 5 fue el grupo IO; y los Grupos 6, 7 y 8 eran grupos IO que fueron inducidos con 5, 10 y 15 mmHg de CO2 PNP respectivamente (Grupos 5-8: "Grupos OJ"). Se ligó el conducto biliar común y se dividió en grupos IO. A las 48 horas se indujo una PNP de 5-10-15 mmHg mediante minilaparotomía con aguja de Veress en los Grupos 6, 7 y 8, mantenida durante 60 minutos. **Resultados**: No hubo diferencias estadísticamente significativas entre los grupos en cuanto a los niveles de Nitrógeno ureico en sangre y Creatinina (p> 0,05). Los valores de lipocalina asociada a gelatinasa de neutrófilos (NGAL) fueron significativamente más altos en los grupos IO que en los grupos no IO (p < 0,05). Los valores séricos de cistatina-C fueron significativamente más altos en los grupos IO con PNP de 10 y 15 mmHg que en los grupos sin IO (p < 0,05). Conclusión: en la etapa temprana de AKI, los niveles de NGAL y Cystatin-C pueden ser más altos, mientras que las pruebas estándar de función renal fueron normales. Nuestros hallazgos destacan el aparente efecto desfavorable de IO con PNP sobre las funciones renales y el reconocimiento temprano de AKI con la medición de NGAL y Cystatin-C en estas condiciones.

PALABRAS CLAVE: Insuficiencia Renal Aguda (IRA); Cistatina-C; laparoscopia; lipocalina asociada a gelatinasa de neutrófilos (NGAL); ictericia obstructiva (OJ); neumoperitoneo (PNP)

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ABSTRACT

Introduction: Both pneumoperitoneum (PNP) and obstructive jaundice (OJ) are known to lead to acute kidney injury (AKI) potentially, but the combined effect has not been investigated. **Aim**: This study aimed to investigate the effects of PNP on renal functions in a rat model of OJ. **Methods**: We divided forty-eight rats into eight groups of six rats. Group 1 was the control (sham-operated) group; Groups 2, 3, and 4 were induced 5, 10, and 15 mmHg of CO2 PNP, respectively (Groups 1-4: "non-OJ Groups"); Group 5 was the OJ group; and Groups 6, 7 and 8 were OJ groups that were induced with 5, 10, and 15 mmHg CO2 PNP was respectively (Groups 5-8: "OJ Groups"). The common bile duct was ligated and divided into OJ Groups. After 48 hours, a 5-10-15 mmHg PNP was induced by mini-laparotomy with a Veress needle in Groups 6, 7, and 8, maintained for 60 minutes. **Results**: There were no statistically significant differences between groups regarding blood urea Nitrogen and Creatinin levels ($p > 0.05$). Neutrophil gelatinase-associated lipocalin (NGAL) values were significantly higher in OJ Groups than in non-OJ Groups ($p < 0.05$). Serum Cystatin-C values were significantly higher in OJ Groups with 10 and 15 mmHg PNP than in non-OJ Groups ($p < 0.05$). **Conclusion**: In the early stage of AKI, NGAL and Cystatin-C levels might be higher, while standard renal function tests were normal. Our findings highlight the apparent unfavorable effect of OJ with PNP on renal functions and early recognition of AKI with the measurement of NGAL and Cystatin-C in these conditions.

KEYWORDS: Acute kidney injury (AKI); Cystatin-C; laparoscopy; neutrophil gelatinase associated lipocalin (NGAL); obstructive jaundice (OJ); pneumoperitoneum (PNP)

INTRODUCTION

The laparoscopic approach has been the first choice of technique in many areas of surgery. Understanding the increment of intra-abdominal pressure during carbon dioxide (CO2) pneumoperitoneum (PNP) as a part of laparoscopic surgery is essential as systemic changes due to CO2 have become important. Despite advantages, laparoscopic surgical procedures and CO2 PNP affect many systems and organs, such as the brain, lungs, and liver (1,2). The kidney is one of the critical organs where such effects may be observed (3-5).

When considering physiological changes associated with PNP, it has been reported that the decrease in blood flow might cause ischemic changes in intra-abdominal organs (6). Experimental studies have demonstrated significant and reversible decreases in the glomerular filtration rate (GFR), urinary outflow, and renal blood flow (3,5-7). Those effects may depend on the pressure applied during PNP, with several other mechanisms such as decrement of cardiac output, renal vein, parenchyma imprinting, and hormonal effects (4,6,8-12).

Another condition having an effect on renal functions is obstructive jaundice (OJ) (13). The obstruction of the biliary tree and obstruction of bile flow causes the accumulation of many substances with a systemic toxic effect, primarily bile salt and bilirubin (14). Acute tubular necrosis (ATN) is one of the complications encountered in OJ, with a present rate of 8% (15). Hence, deterioration in renal function may be due to both OJ along with PNP (16).

Although there are various human and animal studies in the literature on the effects of PNP and OJ on renal functions, to the best of our knowledge, no study has documented the relation between acute kidney injury (AKI) and PNP along with OJ, especially analyzing with NGAL and Cystatin-C assessment (3-6,14-16).

Cystatin-C is a basic protein with a low molecular weight (13 kDa) and is a non-glycolysis polypeptide cysteine proteinase inhibitor (17,18). It is synthesized at a steady rate in all nuclear cells in the body. Cystatin-C is present in all tissues and biological fluids in measurable amounts. Although the primary structure, physical, chemical, and immunological characteristics of Cystatin-C have been determined, its biological role still needs to be well defined (17). During the structural analysis of the Cystatin-C gene, it has been demonstrated that there is a steady production rate, even in inflammatory conditions, unaffected by any condition (18). Due to its small molecular weight and basic iso-electric pH, it drains more freely in the glomerulus than other proteins. Almost all are reabsorbed by proximal tubules and catabolized in tubular cells resulting in no return to the blood

flow (19). Hence, Cystatin-C has advantages over serum Creatinine in measuring renal functions (4,20,21). Serum Creatinine levels depend on muscle mass, age, gender, muscle metabolism, and hydration condition. When there are acute changes in glomerular filtration function, serum Creatinine values reflect the kidney functions once the stable condition balance is ensured. A meta-analysis of 46 studies showed that Cystatin-C was superior to serum Creatinine in determining AKI (21).

Neutrophil gelatinase-associated lipocalin (NGAL), also known as lipocalin-2 or siderocalin, is a protein, which creates a covalent bond with neutrophil gelatinase, weighs 25 kDa and consists of 178 amino acids (22,23). Various biological functions of NGAL have been identified, such as triggering apoptosis, suppressing bacterial reproduction, and regulating inflammatory response (22). Neutrophil gelatinase-associated lipocalin is expressed in bone marrow, colon, kidneys, lungs, stomach, uterus, prostate, and trachea (22,23). The increase in NGAL expression occurs together with inflammatory and epithelial damage (23,24). It has been documented that NGAL levels increase in blood and urine analyses after toxic or ischemic damage to the kidney (4,23,25-26).

Based on laparoscopic surgery practices for either benign (choledocholithiasis, benign biliary strictures) or malign (pancreas carcinoma, cholangiocarcinoma, ampulla tumor, choledochal lower-end tumor) conditions that might cause OJ, we aimed in this study to determine the effects of PNP applied at different pressures to rats along with the OJ model on renal functions by using new identifiers, NGAL and Cystatin-C.

MATERIALS AND METHODS

A total of 48 male Wistar albino rats weighing between 350-400 grams and having an average age of six months were used in the study. The rats were divided into eight groups of six rats each. Throughout the experiment, the lighting arrangement was 12 hours of the day and 12 hours of the night. The rats were placed in cages in groups of six, maintaining room temperature at 21±2.5 °C. The rats were fed with standard pellets and given tap water throughout the study. Surgical anesthesia was applied through intramuscular injection of Xylazine hydrochloride (Rompun, Bayer HealthCare) 5 mg/kg dose in addition to 50 mg/kg of ketamine hydrochloride (Ketalar, Parke Davis and Eczacibasi, Istanbul).

Surgical Procedure and Operative Details

After ensuring the required sterile conditions following anesthesia, the front abdominal walls of the rats were shaved and cleaned with a 10% povidone-iodine solution. We fixed a Veress needle (Ethicon Endo-Surgery, UV120, USA) to the abdominal cavity with a 3/0 silk suture through a 5-millimeter incision to the abdominal wall. After fixing the Veress, an electronic insufflator (Karl Storz GmbH, Tutlingen, Germany) was connected, and five mmHg, ten mmHg, and 15 mmHg of CO2 constant pressure were insufflated. The insufflator was adjusted so that in case there was an absorption of CO2 gas on the peritoneal surface or leakage, it would automatically insufflate gas in order to ensure that intra-abdominal pressure would be maintained at the desired level.

Minilaparotomy was performed on the rats in Group 1, and after placing a Veress needle into the abdomen without applying PNP and waiting for 60 minutes, intracardiac blood samples were taken, and the rats were sacrificed. After 60 minutes of CO2, PNP was applied at pressures of 5, 10, and 15 mmHg, respectively, as mentioned above, to the rats in Groups 2, 3, and 4; intracardiac blood samples were taken, and they were sacrificed. After performing laparotomy on the rats in Group 5, the main bile duct extending to the duodenum from the liver hilum was found and dissected from the surrounding adipose tissues. It was cut after ligating from its proximal and distal with 4/0 silk sutures. Then, 1 mL of normal saline was physiologically injected into the abdomen, the folds were closed as primary, and the procedure was finalized. Following the operation, the rats were fed for 48 hours with standard rat feed and providing tap water for fluid resuscitation purposes. At the end of this period (48 hours), after placing a Veress needle into the abdomen without applying PNP and waiting for 60 minutes, intracardiac blood samples were taken, and the rats were sacrificed. For the rats in Groups 6, 7, and 8, OJ was created in the same manner mentioned above. After the operation, fluid resuscitation was applied to the rats in the same manner, and they were fed with standard rat feed. In the 48th postoperative hour, after placing a Veress needle in sterile conditions with mini-laparotomy

on rats in Groups 6, 7, and 8 and after PNP at pressures of 5, 10, and 15 mmHg were applied for 60 minutes with an electronic insufflator (Karl-Storz GmbH, Tutlingen, Germany) controlling the intra-abdominal pressure, intracardiac blood samples were taken, and the rats were sacrificed. Therefore, experimental animals were divided as follows: Group 1, Sham rats; Group 2, PNP 5 mmHg; Group 3, PNP 10 mmHg; Group 4, PNP 15 mmHg; Group 5, OJ; Group 6, PNP 5mmHg + OJ; Group 7, PNP 10 mmHg + OJ and Group 8, PNP 15 mmHg + OJ.

Blood Samples

The analyses consisted of Cystatin-C, NGAL, BUN (blood urine Nitrogen), Creatinine, AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), GGT (Gamma-glutamyltransferase), ALP (Alkaline phosphatase), total and direct bilirubin levels. Blood samples taken in tubes with ethylenediaminetetraacetic acid (EDTA) were centrifuged for 10 minutes at 3000 revolutions, and serum samples were prepared. Cystatin-C levels were expressed in mg/dL, NGAL levels as ng/mL; BUN, Creatinine, AST, ALT, GGT, ALP, total, and direct bilirubin levels were expressed in mg/dL.

Statistical Analysis

The data were analyzed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The normality of the variables was assessed with Kolmogorov-Smirnov Test. All data were compared with the non-parametric test due to the abnormal distribution. Groups without OJ (Groups 1, 2, 3, 4: "non-OJ Groups") and groups with OJ (Groups 5, 6, 7, 8: "OJ Groups") were compared separately with the Kruskal-Wallis Variance Analysis. When there were significant differences, paired comparisons were conducted with the Mann-Whitney U Test, comparing the pressure between OJ Groups and non-OJ Groups to determine the source of differences between groups. Means were provided as mean ± standard deviation or median, interquartile range where needed. The significance level for all analyses was taken considered 0.05.

Ethics Committee Approval

This experimental study was conducted at the Gazi University Faculty of Medicine Experiment Animal Research Laboratory with the consent no B.30.2.GUN.0.05.06/78-6487 of the Gazi University Local Ethics Board on Animal Experiments.

RESULTS

No animal loss occurred in any group in the study, and all the animals tolerated the surgical procedure. Non-OJ Groups (Groups 1, 2, 3, and 4) were compared regarding serum AST, ALT, ALP, GGT, total and direct bilirubin levels, and no significant difference was found between the groups (p= 0.57, p= 0.1, p=0.1, p= 0.1, p= 0.2 and p= 0.25, respectively). OJ Groups (Groups 5, 6, 7, and 8) were compared in terms of serum AST, ALT, ALP, GGT, total and direct bilirubin levels, and no significant difference was found (p= 0.38, p= 0.7, p=0.42, p= 0.79, p= 0.22 and p= 0.41, respectively) (**Table 1**).

Table 1. Comparisons of hepatic enzyme levels, BUN and Creatinine levels in non-OJ Groups and OJ Groups

Parameters (median, min-max)	Group 1 (control)	Group 2 (PNP 5 mmHg)	Group 3 (PNP 10 mmHg)	Group 4 (PNP 15 mmHg)	p
AST (U/L)	195 (104-311)	209 (134-400)	165 (115-204)	173 (121-250)	0.57
ALT (U/L)	100 (89-119)	77 (57-94)	75 (48-89)	82 (69-96)	0.10
GGT(U/L)	1 (1-1)	1 (1-1)	1 (0-1)	1 (1-2)	0.10
T. Bil. (mg/dL)	0.14 (0.09-0.17)	0.13 (0.08-0.17)	0.15 (0.10-0.19)	0.17 (0.17-0.41)	0.20
D. Bil. (mg/dL)	0.02 (0.01-0.03)	0.03 (0.02-0.04)	0.03 (0.01-0.05)	0.02 (0.01-0.06)	0.25
ALP (U/L)	249 (228-418)	245 (192-395)	227 (187-258)	210 (155-228)	0.10
BUN (mg/dL)	65 (56-75)	63 (46-85)	58 (44-63)	58 (52-65)	0.09
Creatinine (mg/dL)	0.50 (0.40-0.54)	0.50 (0.40-0.59)	0.47 (0.45-0.52)	0.51 (0.47-0.77)	0.39

Parameters (median, min-max)	Group 5 (OJ)	Group 6 (PNP 5 mmHg+OJ)	Group 7 (PNP 10 mmHg+OJ)	Group 8 (PNP 15 mmHg+OJ)	p
AST (U/L)	1046 (903-1269)	772 (459-1260)	869 (246-1146)	813 (628-1232)	0.38
ALT (U/L)	887 (854-991)	934 (842-1063)	848 (556-991)	1014 (952-1032)	0.07
GGT(U/L)	18 (15-24)	19 (12-45)	24 (11-43)	21 (12-48)	0.79
T. Bil. (mg/dL)	10.11 (8.20-10.71)	8.63 (7.29-9.93)	10.04 (2.09-17.35)	9.29 (6.06-11.25)	0.22
D. Bil. (mg/dL)	6.0 (5.19-6.23)	5.11 (4.65-5.93)	5.78 (1.21-8.79)	5.50 (3.75-7.09)	0.41
ALP (U/L)	963 (817-1279)	955 (792-1807)	1047 (729-1473)	933 (705-1217)	0.42
BUN (mg/dL)	47 (32-61)	57 (49-62)	59 (51-84)	60 (45-90)	0.11
Creatinine (mg/dL)	0.54 (0.45-0.73)	0.54 (0.45-0.62)	0.66 (0.52-0.74)	0.60 (0.51-1.05)	0.12

PNP= pneumoperitoneum, OJ= obstructive jaundice

Non-OJ Groups (Groups 1, 2, 3, and 4) were compared regarding serum BUN and Creatinine levels, and no significant difference was found between the groups (p= 0.09 and p= 0.39, respectively). OJ Groups (Groups 5, 6, 7, and 8) were compared in terms of serum BUN and Creatinine levels, and no significant difference was found between the groups (p= 0.11 and p= 0.12, respectively) (**Table 1**).

Hepatic enzyme (total bilirubin, direct bilirubin, AST, ALT, GGT, and ALP) levels were higher in OJ Group 5 compared to non-OJ Group 1 (control) (p= 0.002, for all parameters). There was no significant difference between BUN and Creatinine levels between these two groups (p= 0.08 and p= 0.18, respectively). Hepatic enzyme levels were higher in OJ Group 6, which was administered PNP at five mmHg pressure, compared to non-OJ Group 2, which was administered PNP at five mmHg pressure (p= 0.002 for all parameters). The difference between BUN and Creatinine levels was not significant (p= 0.24 and p= 0.485, respectively). Hepatic enzyme levels were higher in OJ Group 7, administered PNP at ten mmHg pressure, compared to non-OJ Group 3, which was administered PNP at ten mmHg pressure (p= 0.002, for all parameters). The difference between BUN and Creatinine levels was not significant (p= 0.423 and p= 0.061, respectively). Similarly, hepatic enzyme levels were higher in OJ Group 8, which was administered PNP at 15 mmHg pressure, compared to non-OJ Group 4, which was administered PNP at 15 mmHg pressure (p= 0.002 for all parameters). The difference between urea and Creatinine levels was not significant (p= 0.394 and p= 0.24, respectively) (**Table 2**).

Table 2. Comparison of hepatic enzyme levels, BUN and Creatinine levels between pneumoperitoneum groups with and without obstructive jaundice

Parameters (median, min-max)	Group 1	Group 5	p	Group 2	Group 6	p
AST (U/L)	195 (104-311)	1046 (903-1269)	0.002	209 (134-400)	772 (459-1260)	0.002
ALT (U/L)	100 (89-119)	887 (854-991)	0.002	77 (57-94)	934 (842-1063)	0.002
GGT (U/L)	1 (1-1)	18 (15-24)	0.002	1 (1-1)	19 (12-45)	0.002
T. Bil. (mg/dL)	0.14 (0.09-0.17)	10.11 (8.20-10.71)	0.002	0.13 (0.08-0.17)	8.63 (7.29-9.93)	0.002
D. Bil. (mg/dL)	0.02 (0.01-0.03)	6.0 (5.19-6.23)	0.002	0.03 (0.02-0.04)	5.11 (4.65-5.93)	0.002
ALP (U/L)	249 (228-418)	963 (817-1279)	0.002	245 (192-395)	955 (792-1807)	0.002
BUN (mg/dL)	65 (56-75)	47 (32-61)	0.08	63 (46-85)	57 (49-62)	0.24
Creatinine (mg/dL)	0.50 (0.40-0.54)	0.54 (0.45-0.73)	0.18	0.50 (0.40-0.59)	0.54 (0.45-0.62)	0.485

Parameters (median, min-max)	Group 3	Group 7	p	Group 4	Group 8	p
AST (U/L)	165 (115-204)	869 (246-1146)	0.002	173 (121-250)	813 (628-1232)	0.002
ALT (U/L)	75 (48-89)	848 (556-991)	0.002	82 (69-96)	1014 (952-1032)	0.002
GGT (U/L)	1 (0-1)	24 (11-43)	0.002	1 (1-2)	21 (12-48)	0.002
T. Bil. (mg/dL)	0.15 (0.10-0.19)	10.04 (2.09-17.35)	0.002	0.17 (0.17-0.41)	9.29 (6.06-11.25)	0.002
D. Bil. (mg/dL)	0.03 (0.01-0.05)	5.78 (1.21-8.79)	0.002	0.02 (0.01-0.06)	5.50 (3.75-7.09)	0.002
ALP (U/L)	227 (187-258)	1047 (729-1473)	0.002	210 (155-228)	933 (705-1217)	0.002
BUN (mg/dL)	58 (44-63)	59 (51-84)	0.423	58 (52-65)	60 (45-90)	0.394
Creatinine (mg/dL)	0.47 (0.45-0.52)	0.66 (0.52-0.74)	0.061	0.51 (0.47-0.77)	0.60 (0.51-1.05)	0.240

Group 1: Control, **Groups 2, 3, 4:** Pneumoperitoneum without obstructive jaundice, **Group 5:** Obstructive jaundice, **Groups 6, 7, 8:** Pneumoperitoneum with obstructive jaundice

Non-OJ Groups (Groups 1, 2, 3, and 4) were compared regarding serum NGAL and Cystatin-C levels, and no significant difference was found (p= 0.29 and p= 0.15, respectively). OJ Groups (Groups 5, 6, 7, and 8) were compared in terms of serum NGAL and Cystatin-C levels, and no significant difference was found for Cystatin-C levels (p= 0.42) (Table 3). However, within comparison analyses for OJ Groups, the highest serum NGAL level was found in OJ Group 8 (p= 0.001) (**Table 3, Figure 1**).

Table 3. Comparisons of serum NGAL and Cystatin-C levels in non-OJ Groups and OJ Groups

Parameters (median, min-max)	Group 1 (control)	Group 2 (PNP 5 mmHg)	Group 3 (PNP 10 mmHg)	Group 4 (PNP 15 mmHg)	p
NGAL (ng/mL)	0.31 (0.22-0.57)	0.37 (0.30-0.62)	0.34 (0.25-0.50)	0.39 (0.34-0.75)	0.29
Cystatin-C (mg/dL)	0.14 (0.12-0.17)	0.17 (0.14-0.22)	0.14 (0.11-0.17)	0.14 (0.13-0.27)	0.15
	Group 5 (OJ)	**Group 6 (PNP 5 mmHg+OJ)**	**Group 7 (PNP 10 mmHg+OJ)**	**Group 8 (PNP 15 mmHg+OJ)**	**p**
NGAL (ng/mL)	1.0 (0.61-1.21)	1.15 (1.09-1.27)	1.42 (1.14-1.53)	1.56 (1.44-1.70)	0.001
Cystatin-C (mg/dL)	0.16 (0.14-0.25)	0.20 (0.18-0.24)	0.21 (0.14-0.27)	0.19 (0.17-0.33)	0.42

PNP= pneumoperitoneum, OJ= obstructive jaundice

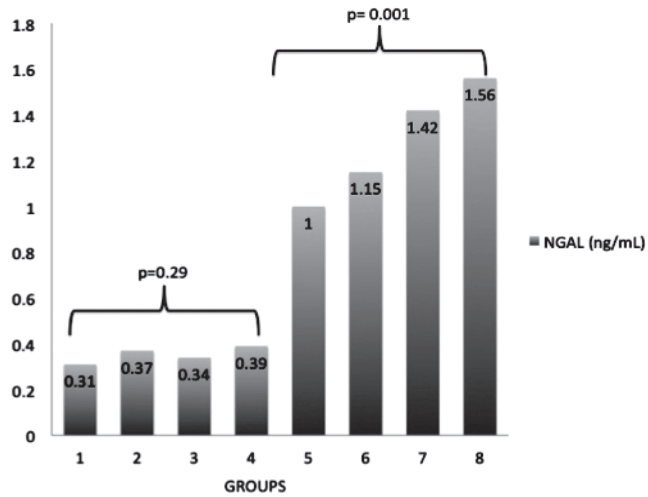

Figure 1. The comparison between all groups for serum NGAL levels

There were no statistically significant differences in serum Cystatin-C level comparison between non-OJ Group 1 and OJ Group 5 (p= 0.13), non-OJ Group 2, and OJ Group 6 (p= 0.09). However, the levels were significantly higher in OJ Groups 7 and 8 compared to non-OJ Groups 3 and 4 (p= 0.009 and p= 0.04, respectively) (Table 4, Figure 2). All OJ Groups showed significantly higher serum NGAL levels compared to non-OJ Groups with PNP (between non-OJ Group 1 and OJ Group 5, non-OJ Group 2 and OJ Group 6, non-OJ Group 3 and OJ Group 7, non-OJ Group 4 and OJ Group 8) (p= 0.002, for all comparisons) (**Table 4, Figure 2**).

Table 4. Comparisons of serum NGAL and Cystatin-C levels between non-OJ Groups and OJ Groups for pneumoperitoneum

Parameters (median, min-max)	**Group 1**	**Group 5**	**p**	**Group 2**	**Group 6**	**p**
NGAL (ng/mL)	0.31 (0.22-0.57)	1.0 (0.61-1.21)	0.002	0.37 (0.30-0.62)	1.15 (1.09-1.27)	0.002
Cystatin-C (mg/dL)	0.14 (0.12-0.17)	0.16 (0.14-0.25)	0.13	0.17 (0.14-0.22)	0.20 (0.18-0.24)	0.09
	Group 3	**Group 7**	**p**	**Group 4**	**Group 8**	**p**
NGAL (ng/mL)	0.34 (0.25-0.50)	1.42 (1.14-1.53)	0.002	0.39 (0.34-0.75)	1.56 (1.44-1.70)	0.002
Cystatin-C (mg/dL)	0.14 (0.11-0.17)	0.21 (0.14-0.27)	0.009	0.14 (0.13-0.27)	0.19 (0.17-0.33)	0.04

Group 1: Control, **Groups 2, 3, 4:** Pneumoperitoneum without obstructive jaundice, **Group 5:** Obstructive jaundice, **Groups 6, 7, 8:** Pneumoperitoneum with obstructive jaundice

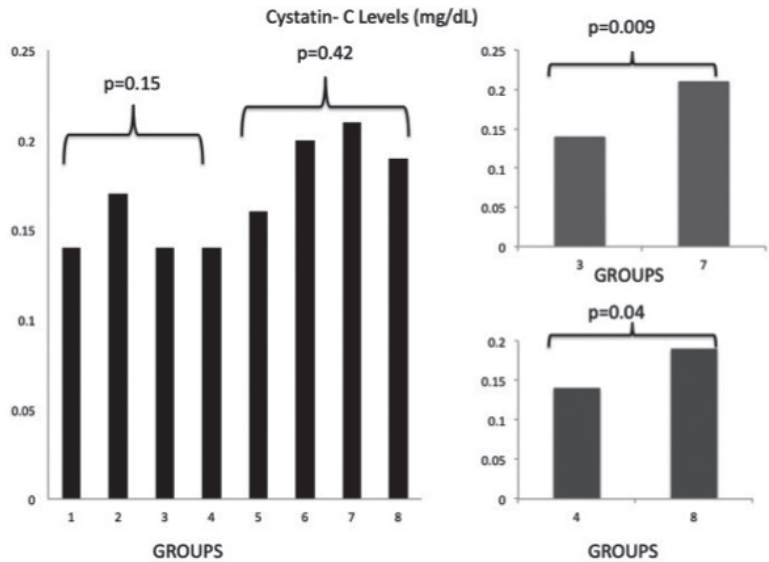

Figure 2. The comparison of all groups for serum Cystatin-C levels

DISCUSSION

Laparoscopic interventions are widely used in emergent and elective fields of surgery. In recent years, hepatobiliary and pancreatic surgeries in the advanced laparoscopic surgery field have been used increasingly for malign or benign obstructive diseases. (27-28).

The CO2 gas used in laparoscopic surgery reveals the operation area by providing the PNP, and CO2 is preferable due to its superiority over helium, Nitrogen protoxide, and argon (29,30). Adverse effects of CO2 PNP on renal functions have been reported in several studies (3-5,20,31,32). The underlying mechanisms were discussed, being an increase in intra-abdominal pressure, hypercarbia, the preoperative position of the patient, and hemodynamic characteristics (4,8,10,33). The other factors that might lead to AKI are reduced

cardiac output, decrement in renal blood flow and vein compression, increased renal vascular resistance, vasopressin, endothelin, angiotensin-II, and secretion of vasoconstrictor mediators such as catecholamine (3,5,6,7,9-12,14,34-36). To the best of our knowledge, no study analyzed the effects of PNP with OJ on renal functions.

In this experimental study, we first examined the effects of PNP with OJ on liver enzymes. We found no significant effect on liver function in PNP with the OJ model. Our results were similar to the literature (37). In our study, besides the conventional biochemical parameters used for renal functions, we also used new-generation indicators such as Cystatin-C and NGAL. Regarding the traditional renal function tests such as Creatinine and BUN, we found no difference between group analyses. To demonstrate the effect of PNP on kidney functions, the comparative analyses between non-OJ Groups 2, 3, and 4 with controls showed no significant difference in serum NGAL levels. This result suggests that PNP may not be a cause of AKI by itself if kidneys have no prior damage. However, OJ Group 5 (control) showed higher NGAL levels compared to non-OJ Groups with PNP (Groups 2, 3, 4).

Moreover, OJ Groups with PNP (Groups 6, 7, 8) revealed significantly higher levels of NGAL compared to PNP Groups and even compared with the non-PNP OJ Group (Group 5). No significant differences were observed between OJ Groups 6, 7, and 8 in comparison analyses. According to these results, we can conclude that the main bile duct obstructions may cause renal damage, and this effect may be increased with PNP from the pressure applied during the procedure. Some authors also emphasize that renal functions deteriorate in patients with OJ with possible mechanisms including reduced cardiac outflow, reduced peripheral vasoconstriction, hypovolemia, increased renal vasoconstriction, and decrease in glomerular filtration rate (38-40). Furthermore, it is also concluded that bile acids and conjugate bilirubin may cause both ischemia and direct tubular necrosis by itself, in addition to possible damaging effects of PNP, such as increased kidney structure (13,41).

Several studies indicate the superiority of Cystatin-C over Creatinine for evaluating glomerular filtration rate (4,20,21). Here, we also found that serum Cystatin-C levels significantly increased in OJ Groups 7 and 8, suggesting the rising levels of Cystatin-C are more evident in pressures of 10 mmHg and above. Hence, keeping PNP pressure under ten mmHg or gasless laparoscopic techniques is suitable for patients with prior kidney function disorders.

These results lead us to conclude that NGAL and Cystatin-C levels are more sensitive in early recognition of renal damage in laparoscopic surgery for patients with OJ, according to the literature in various settings.

In conclusion, AKI is a significant complication in the context of laparoscopic surgeries for OJ. PNP is not an indicator for AKI in these settings unless OJ is present. The early phase of renal damage might not always be determined with conventional biochemical methods. Using novel renal biomarkers, such as Cystatin-C and serum NGAL, is an alternative way to reach an early diagnosis of AKI and improve the patient's postoperative prognosis. This experimental study might shed light on developing prospective clinical studies, including the correlation of novel biomarkers with histopathological changes in this area.

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