

# Effects of pneumoperitoneum with carbon dioxide on renal and hepatic functions in rats

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## Abstract

**Introduction:** Laparoscopic surgery is a preferred method based on its many benefits. However, increasing abdominal pressure by CO<sub>2</sub> insufflation during the implementation of this technique poses challenges.

**Aim:** To determine the degree of renal and liver injury that occurs in a pneumoperitoneum (PP) model of prolonged CO<sub>2</sub> insufflation.

**Material and methods:** Twenty-one female Sprague Dawley rats were separated randomly into three groups. Group 1 was the control group and given anesthesia for 3 h. In group 2, PP was administered under anesthesia for 1 h. In the last group, PP was administered under anesthesia to animals for 3 h. We measured renal and liver injury biomarkers and made a histopathological evaluation to estimate the degree of injury and assessed the correlation of biomarkers including kidney injury molecule-1 (KIM-1) with histopathological findings.

**Results:** Histopathological analysis according to the kidney ischemia tubular damage score showed a statistically significant difference between the 3 groups ( $p < 0.001$ ). There was an increase in KIM-1 levels in the groups, although it was not statistically significant ( $p = 0.062$ ,  $p = 0.156$ ,  $p = 0.350$  respectively). According to the correlation test in this research, KIM-1 results had a statistically significant association with creatinine, urea, aspartate aminotransferase and alanine aminotransferase levels in all control and study groups.

**Conclusions:** According to our results, the increase in KIM-1 was correlated with Cr levels and compatible with histopathological analysis. Moreover, intra-abdominal pressure statistically significantly increased the degree of kidney injury and there was not a significant increase in the levels of KIM-1. There was no difference in liver damage between groups.

**Key words:** liver, pneumoperitoneum, kidney, injury, kidney injury molecule-1.

## Introduction

Laparoscopic surgery is a preferable technique all around the world for the treatment of many diseases. In this technique, pneumoperitoneum due to CO<sub>2</sub> insufflation has become widely used. Shorter operative time, less morbidity and better postoperative recovery period are major advantages of this technique. On the other hand, decreased venous return, subcu-

taneous emphysema, impaired ventilation, a risk of gas emboli, and abdominal compartment syndrome due to increased intra-abdominal pressure (IAP) are some disadvantages of this technique [1, 2]. Moreover, pneumoperitoneum (PP) may cause ischemia in kidneys and other intra-abdominal organs (such as the liver) because of decreased splanchnic blood flow [3]. The IAP-related decrease in renal blood flow and glomerular filtration rate (GFR) may cause oliguria due

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to ureteral obstruction, systemic hormonal effects, and vascular or direct renal compression [1, 2]. Normally, acute kidney injury (AKI) is diagnosed based on elevated levels of serum creatinine (SCr) and depends on the duration and severity of oliguria [4]. However, the level of serum creatinine is not a sufficiently specific or sensitive biomarker to indicate nephron injury especially for patients with decreased muscle mass, but rather an indicator of the balance between creatinine production and excretion. AKI is described as a reversible decrease in GFR due to intrarenal events such as prolonged ischemia and sepsis causing tubular or glomerular cell necrosis, resulting in increased levels of creatinine in the plasma and a temporary decrease in renal blood flow.

There is a need for a sensitive and specific biomarker better than SCr for AKI patients. Kidney injury molecule 1 (KIM-1), a 104 kDa protein, is expressed at very low levels in the normal kidney. KIM-1 is a type I membrane protein, discovered by Ichimura *et al.* in 1998 [5]. The extracellular portion of KIM1 can cleave and rapidly enter tubule lumens after kidney injury, and can then be detected in the urine. It has been confirmed that the urine KIM-1 level is closely related to tissue KIM-1 level and correlated with kidney tissue damage. Not only is KIM-1 proven to be an early biomarker of acute kidney injury but it also has a potential role in predicting the long-term renal outcome. Many studies show that KIM-1 is a sensitive and specific marker of kidney injury as well as a predictor of prognosis, especially in acute kidney injury [6, 7]. However, there are also many studies on the role of KIM-1 in chronic kidney disease.

We aimed to detect the degree of renal and liver injury caused by pneumoperitoneum (PP) and prolonged PP. We measured renal and liver injury biomarkers (such as Cr, AST, ALT, KIM-1) and made a histopathological evaluation to determine the degree of injury and compared the correlation of the biomarkers with the histopathological findings.

## Aim

We aimed to determine the degree of renal and liver injury that occurs in a PP model of prolonged CO<sub>2</sub> insufflation.

## Material and methods

### Study design

A total of 21 female Sprague-Dawley rats were included, which were 5 to 6 months old and weighed

300 to 350 g. They were randomly separated into three groups. Group 1 was the control group (sham) and was given anesthesia for 3 h. Group 2 was administered PP under anesthesia for 1 h, and group 3 was administered pneumoperitoneum under anesthesia for 3 h. The study was approved by the Turkish Medicines and Medical Devices Agency and the Local Ethical Committee on Animal Experiments (Acibadem Mehmet Ali Aydınlar University, 2018/17). The subjects were kept in standardized laboratory conditions of 20–24°C, 50–60% relative humidity, controlled light (day-night cycle of 12 h: 8/20 h), fed on standardized rodent food, and given filtered and chlorinated water. There was no data loss during the research. The animals were anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and xylazine (5 mg/kg). A 10 mm Hg CO<sub>2</sub> PP was created through a catheter inserted in the left lower abdomen using a Sopro 640 pneumatic 30 l insufflator (Sopro comeg S640-3005, Germany). The PP pressure was set using an intraabdominal catheter with a valve system in groups 2 and 3. This system was composed of a chest drainage bottle, filled with water to form a column length up to the desired PP pressure level, connected to the insufflator and a three-way stopcock that allows gas to escape if the pressure is exceeded. The pressure value was used equally in all groups. Also, the KIM-1 measurements were made in blood samples taken preoperatively as well as at the 1<sup>st</sup> and 24<sup>th</sup> postoperative hours; creatinine, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in serum were measured preoperatively and at the 1<sup>st</sup> and 24<sup>th</sup> postoperative hour. The rats were sacrificed at the 24<sup>th</sup> h and histopathological analysis of the kidneys and liver was performed. The exclusion criteria of this study included whether the mice are male, very young or old.

The limitation of this study was that only AST and ALT biochemical tests were used for evaluation of the liver injury and we could not measure the urine concentration of KIM-1 due to the technical inadequacy of urine collection in rats. On the other hand, we did not have more rats, so we determined three groups that included seven rats per group. This was the minimum level for the statistical methods.

### Tissue sampling and histopathological examination

All samples were fixed in a 10% formaldehyde solution. Liver and kidney tissues were embedded in

paraffin and 5 µm tissue sections were obtained for hematoxylin and eosin (H&E) staining. The cut sections were examined for completeness and one representative section of each kidney was selected for tissue processing. The histological damage examined under a light microscope by a pathologist who was blind to the study design (sham vs. IRI). Histological damage in liver tissues was evaluated in portal areas, biliary and vascular structures, and lobular parenchyma. Kidney biopsies were quantified using the EGTI scoring system devised specifically for animal research on kidney tissue in the context of injury [8]. The scoring system consists of histological damage in 4 individual components – Endothelial, Glomerular, Tubular, Interstitial – and is scored from 0 to 4. The scoring was performed in both the preserved and damaged parts of the renal cortex.

### Biochemical analysis

The creatinine (Cr), urea, AST and ALT concentrations in the serum were determined by an enzymatic assay (Creatinine Plus, Roche Diagnostics GmbH, Mannheim, Germany) and serum ALT/AST levels were determined using a kinetic method (Cobas c311, Roche Diagnostics GmbH, Mannheim, Germany).

The KIM-1 concentration was assessed using a rat KIM-1 ELISA kit (SEA785Ra Cloud-Clone Corp, USA). Serum samples for the KIM-1 measurement were collected and frozen at  $-80^{\circ}\text{C}$  until the analysis was carried out. All laboratory investigators were blind to each rat's clinical information.

### Statistical analysis

SPSS 21.0 (SPSS Inc., Chicago, IL) for MacBook Air was used for statistical analysis. The descriptive statistics for categorical variables were given as the number, percentage means  $\pm$  standard deviation (SD), and for numerical variables as the median. The categorical variables for the three groups were compared using the chi-square ( $\chi^2$ ) test. The Kolmogorov-Smirnov test was used to check the normality of the data distribution. Parametric variables were normally distributed. Mean values were compared among groups with the 1-way analysis of variance (ANOVA), followed by the Tukey multiple-comparison test. Correlations were examined by Pearson standard linear regression analysis (normal distribution). Statistical significance was assumed for  $p < 0.05$ . The Pearson correlation coefficient ( $r$ ) evaluated a range of values from +1 to  $-1$  (95% CI). Details are displayed in the description of the results.

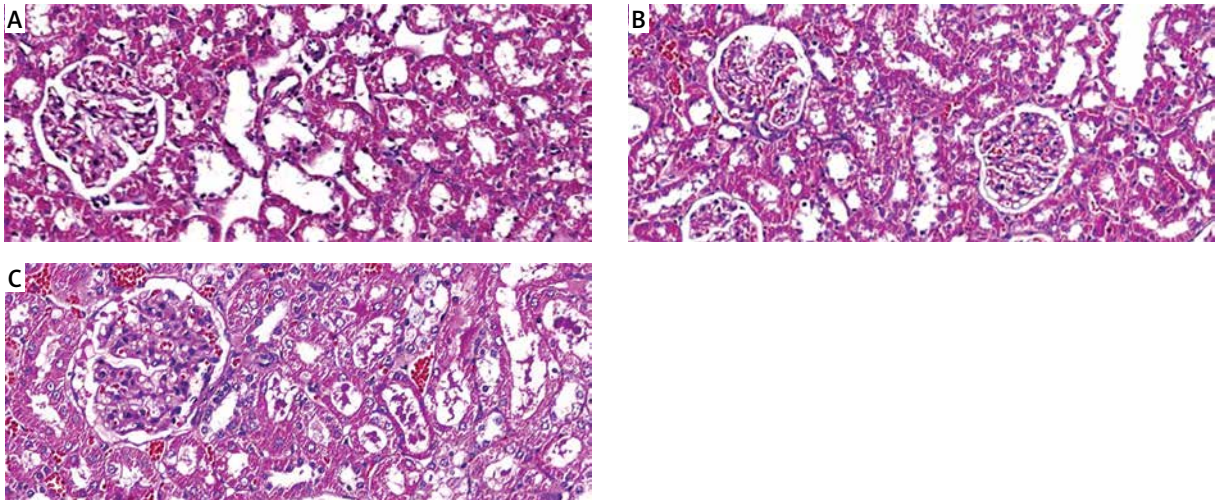
### Results

Results of biochemical analysis (Cr, Urea, AST, ALT, KIM-1) are shown in Table I. Results of histopathological analysis according to the kidney ischemia tubular damage score in sacrificed rats (EGTI histology scoring system) showed statistically significant differences between the groups (Pearson  $\chi^2$  value = 17.365, SD = 2,  $p = 0.001$ ). Only 1 rat showed ischemic tubular damage in group 2 (1/7, 14.3%) and none of the rats in group 1 (Photos 1 A, B). On the other hand, all members of group 3 showed renal

**Table I.** Biochemical serum parameters in all groups

Parameter	KIM-1 Mean $\pm$ SD	Urea Mean $\pm$ SD	Cr Mean $\pm$ SD	AST Mean $\pm$ SD	ALT Mean $\pm$ SD
Group 1 (Sham) before anesthesia	64.0 $\pm$ 1.7	4.0 $\pm$ 0.5	0.1 $\pm$ 0.0	12.1 $\pm$ 4.1	6.1 $\pm$ 1.2
Group 1 (Sham) 1 h after	93.9 $\pm$ 45.7	5.0 $\pm$ 0.8	0.1 $\pm$ 0.0	8.4 $\pm$ 2.5	5.2 $\pm$ 1.7
Group 1 (Sham) 24 h after	61.4 $\pm$ 1.7	4.5 $\pm$ 1.2	0.17 $\pm$ 0.0	19.2 $\pm$ 11.8	5.5 $\pm$ 2.0
Group 2 before anesthesia	75.5 $\pm$ 23.0	3.5 $\pm$ 0.5	0.1 $\pm$ 0.0	12.0 $\pm$ 3.3	6.1 $\pm$ 0.9
Group 2 postop 1 <sup>st</sup> h	83.1 $\pm$ 54.1	4.5 $\pm$ 0.9	0.2 $\pm$ 0.0	14.2 $\pm$ 6.2	6.4 $\pm$ 1.5
Group 2 postop 24 <sup>th</sup> h	44.0 $\pm$ 30.1	2.0 $\pm$ 1.4	0.1 $\pm$ 0.0	8.0 $\pm$ 5.9	3.8 $\pm$ 2.6
Group 3 before anesthesia	77.6 $\pm$ 38.8	3.8 $\pm$ 2.1	0.2 $\pm$ 0.0	15.4 $\pm$ 5.8	6.2 $\pm$ 2.6
Group 3 postop 1 <sup>st</sup> h	62.3 $\pm$ 1.1	5.0 $\pm$ 1.6	0.1 $\pm$ 0.0	10.8 $\pm$ 4.2	5.2 $\pm$ 1.3
Group 3 postop 24 <sup>th</sup> h	61.8 $\pm$ 2.0	3.5 $\pm$ 0.9	0.1 $\pm$ 0.0	45.0 $\pm$ 55.8	6.0 $\pm$ 2.7

KIM-1 – kidney injury molecule.

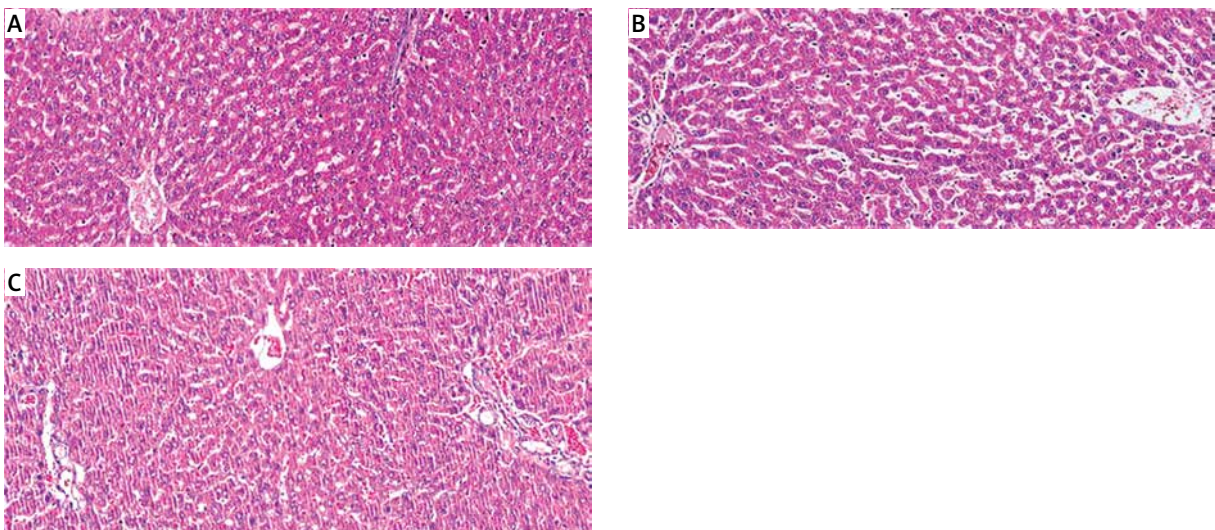


**Photo 1.** **A** – No changes were observed in the renal control group samples (40×, hematoxylin and eosin (H&E) staining). **B** – Only renal tubular injuries of the EGTI system were detected in the samples and no other changes were observed in the group after 1-hour pneumoperitoneum (40×, hematoxylin and eosin (H&E) staining). **C** – Renal tubular damage on histopathological analysis, renal focal tubular necrosis < 25%, score in groups after 1- and 3-hour pneumoperitoneum (EGTI system, 40×, hematoxylin and eosin (H&E) staining)

ischemic tubular damage (Photo 1 C) and the total mean EGTI score of group 3 was the highest.

According to the histopathological analysis, only tubular damage of the EGTI system was detected in the samples and no other changes were observed. Moreover, there were not detected any histopatho-

logical changes in the liver samples in all groups (Photos 2 A–C). One-way ANOVA revealed statistically significant differences among Cr means ( $p < 0.001$ ) and AST means ( $p = 0.038$ ) in group 1 and Tukey’s multiple comparison test revealed a significant difference between pre-anesthesia Cr levels and



**Photo 2.** **A** – No histopathological changes were detected in the liver samples in the control group (40×, hematoxylin and eosin (H&E) staining). **B** – No histopathological changes were detected in the liver samples in the group after 1-hour pneumoperitoneum (40×, hematoxylin and eosin (H&E) staining). **C** – No histopathological changes were detected in the liver samples in the group after 3 h of pneumoperitoneum (40×, hematoxylin and eosin (H&E) staining)

postoperative 24<sup>th</sup> h Cr levels ( $p = 0.015$ ), postoperative 1<sup>st</sup> h Cr levels and postoperative 24<sup>th</sup> h Cr levels ( $p < 0.001$ ), postoperative 1<sup>st</sup> h AST levels and postoperative 24<sup>th</sup> h AST levels ( $p = 0.033$ ). Further, one-way ANOVA revealed statistically significant differences among urea means ( $p = 0.001$ ) and ALT means ( $p = 0.035$ ) in group 2 and Tukey's multiple comparison test revealed significant difference between pre-anesthesia urea levels and postoperative 24<sup>th</sup> h urea levels ( $p = 0.029$ ), postoperative 1<sup>st</sup> h urea levels and postoperative 24<sup>th</sup> h urea levels ( $p = 0.001$ ), postoperative 1<sup>st</sup> h AST levels and postoperative 24<sup>th</sup> h AST levels ( $p = 0.045$ ). There were no statistically significant differences among all biochemical parameters in group 3 according to the one-way ANOVA test.

One-way ANOVA revealed statistically significant differences among Cr means ( $p = 0.019$ ), urea ( $p = 0.001$ ) and AST means ( $p = 0.028$ ) between groups. In addition, Tukey's multiple comparison tests revealed a significant difference between postoperative 24<sup>th</sup> h urea levels in group 1 and postoperative 24<sup>th</sup> h urea levels in group 2 ( $p = 0.009$ ), postoperative 24<sup>th</sup> h AST levels in group 2 and postoperative 24<sup>th</sup> h AST levels in group 3 ( $p = 0.021$ ).

Pearson correlation test (2 tailed) revealed that KIM-1 had a statistically significant association with urea in the postoperative 24<sup>th</sup>-hour group 1 ( $r = -0.790$ ,  $p = 0.035$ ) and Cr in the pre-anesthesia group 2 ( $r = 0.905$ ,  $p = 0.005$ ). Pearson correlation test revealed that Cr had a statistically significant association with KIM-1 in the postoperative 1<sup>st</sup>-hour group 2 ( $r = 0.865$ ,  $p = 0.012$ ). In the postoperative 24<sup>th</sup> h group 2, Pearson correlation test revealed that KIM-1 had statistically significant association with urea ( $r = 0.948$ ,  $p = 0.001$ ), ALT ( $r = 0.975$ ,  $p = 0.001$ ), Cr ( $r = 0.998$ ,  $p = 0.001$ ), and AST ( $r = 0.995$ ,  $p = 0.005$ ). In the same group, urea had statistically significant association with ALT ( $r = 0.970$ ,  $p = 0.001$ ), Cr ( $r = 0.932$ ,  $p = 0.002$ ), and AST ( $r = 0.905$ ,  $p = 0.001$ ). Moreover, in the same group, ALT had a statistically significant association with Cr ( $r = 0.963$ ,  $p = 0.001$ ) and Cr had a statistically significant association with AST ( $r = 0.894$ ,  $p = 0.007$ ).

In the pre-anesthesia group 3, Pearson correlation test revealed that KIM-1 had a statistically significant association with ALT ( $r = -0.860$ ,  $p = 0.013$ ), Cr ( $r = 0.925$ ,  $p = 0.003$ ), and AST ( $r = -0.934$ ,  $p = 0.002$ ). In the same group, ALT had a statistically significant association with Cr ( $r = -0.874$ ,  $p = 0.010$ )

and AST ( $r = 0.841$ ,  $p = 0.018$ ) and Cr had a statistically significant association with AST ( $r = -0.836$ ,  $p = 0.019$ ). In the postoperative 1<sup>st</sup> h group 3, KIM-1 had a statistically significant association with Cr ( $r = 0.839$ ,  $p = 0.018$ ). Lastly, in the postoperative 24<sup>th</sup>-hour group 3, KIM-1 had a statistically significant association with Cr ( $r = 0.872$ ,  $p = 0.011$ ) and ALT had a statistically significant association with AST ( $r = 0.976$ ,  $p = 0.001$ ).

Statistical results revealed differences among KIM-1 mean values in group 1 (control), group 2 and group 3, but the differences were not statistically significant ( $p = 0.062$ ,  $p = 0.156$ ,  $p = 0.350$ , respectively). Moreover, statistical results revealed no significant difference among KIM-1 means between groups ( $p = 0.117$ ).

Spearman's correlation was analyzed in the postoperative 24<sup>th</sup> h group 2 between KIM-1 and kidney ischemia tubular damage score, and Spearman's rho ( $\rho$ ) correlation coefficient was calculated as 0.618 ( $p = 0.139$ ).

## Discussion

Laparoscopy is the most popular minimally invasive surgical technique. It has some advantages compared to the other techniques. However, it can result in high IAP-dependent side effects, such as oliguria. During the laparoscopy, high IAP can result in increased hormonal activity in the renin-angiotensin-aldosterone system and pressure on the renal parenchyma or renal artery. Peters *et al.* reported that complications of the laparoscopy due to the secondary effect of PP were renal failure, respiratory, neuroendocrine, and metabolic problems [9]. The pressure effect of IAP on central veins results in decreased renal perfusion and poor urinary output that is named AKI [10]. The IAP-dependent renal vasoconstriction results in increased sodium excretion in the urine and renal tubular dysfunction. Prolonged IAP and operation time may increase the risk of renal tissue damage [11]. Classical methods of assessing kidney function include measurement of serum urea nitrogen, Cr, and estimated glomerular filtration rate, but they are not sensitive and specific enough. Several biomarkers and laboratory tests can be used to assess GFR and tubular function [12, 13]. Changes in serum Cr are not specific to injury type and not especially sensitive to mild changes in GFR [14, 15].

Kimberley *et al.* reported that PP-induced increases in the serum Cr level occurred within the first 24 h in animal series [16]. The serum Cr level was found to be normal within days and weeks. However, this finding was not valid for some studies using high pressures, other gases and for other parameters of renal function. In this study, serum (Cr levels were found to be higher than associated with high pressures and prolonged PP duration) (> 4 h) [17]. Another study reported that serum Cr increased at the end of the operation and it started to decrease in the 2<sup>nd</sup> h [18]. Another one reported that although urinary output decreased significantly in the laparoscopic group, blood urea nitrogen and Cr levels did not show any significant change [19]. Mc Dougall *et al.* reported a decrease in urinary output at 15 mm Hg PP pressures and in prolonged PP, but no renal injury or histopathological change in animals [20]. They also found a significant decrease in serum Cr. As seen in other studies, serum Cr level is not a good parameter for indicating the early phase of AKI. AKI is defined by the Kidney Disease Improving Global Outcomes criteria as a 50% increase in plasma creatinine concentration over baseline within 7 days or an increase in serum creatinine by 0.3 mg/dl within 2 days [21]. The diagnosis of functional AKI is mainly based on an increase in serum creatinine concentration and urine output; this process may delay the detection of clinically significant kidney damage [22]. However, AKI is a frequent and serious situation observed following laparoscopic operations. For this reason, there is a need for new effective biomarkers for the diagnosis of AKI. One of them, KIM-1, may be a good biomarker that indicates AKI at an early phase. On the other hand, in the literature, there are very rare studies about the KIM-1 biomarker in the high IAP model. This study analyzed the value of the newly introduced biomarker KIM-1 in the prediction of early AKI following laparoscopic operations.

A variant of this molecule, KIM-1a, lacks the tyrosine kinase phosphorylation motif and is mainly expressed in the liver. On the other hand, Sabbisetti *et al.* also found that increased levels of KIM-1 could be detected in the blood and serve as a biomarker of acute kidney injury that was not affected by liver toxicity [23]. KIM-1 measurement can be performed in the plasma or urine [24]. KIM-1 may play a role in the regeneration process of tubule epithelial cells. KIM-1 is a phosphatidylserine receptor that confers a phagocytic phenotype (apoptotic) on the apical aspect

of renal proximal tubule epithelial cells [25]. KIM-1 is expressed at very low levels in the normal kidney. The extracellular portion of KIM-1 can cleave and rapidly enter tubule lumens after kidney damage.

In the literature, there are some studies on the relationship between KIM-1 levels and many diseases, such as acute kidney injury, chronic kidney disease, renal fibrosis and inflammation, IgA nephropathy, diabetic kidney disease, lupus nephritis, polycystic kidney disease, kidney transplant, and renal cell carcinoma. These studies showed that KIM-1 is a sensitive and specific marker of kidney injury as well as a predictor of prognosis especially in acute kidney injury (48–72 h) [26]. In spite of the reported functions of KIM-1 in acute kidney injury, there are some pieces of evidence for its role in chronic kidney disease [27]. It can be used to predict the progress and outcome of kidney disease. Sabbisetti *et al.* also found that plasma concentration of KIM-1 was positively correlated with normalized urinary KIM-1 (correcting for urinary creatinine) ( $r = 0.43$ ,  $p < 0.001$ ) and non-normalized urinary KIM-1 ( $r = 0.24$ ,  $p = 0.02$ ) [23]. Plasma and urinary concentrations of KIM-1 were positively correlated with normalized (correcting for urinary creatinine) and nonnormalized urinary albumin concentration ( $r = 0.33$ ,  $p = 0.001$ ) for plasma KIM-1 ( $r = 0.35$ ,  $p < 0.001$ ) for urinary KIM-1, respectively. Although KIM-1 has been put forward as an alternative to serum Cr for estimating GFR, its clinical use has not been growing because of the small number of studies. In the literature, there are few studies on new biomarkers in renal injury following laparoscopic surgery such as Cys C, but we did not find any study on KIM-1.

We measured KIM-1 levels to evaluate IAP-dependent AKI preoperatively, as well as at the 1<sup>st</sup> and 24<sup>th</sup> postoperative hours. Statistical results revealed differences among KIM-1 mean values in group 1 (control), group 2 and group 3. There was an increase in KIM-1 levels in the groups, although it was not statistically significant ( $p = 0.062$ ,  $p = 0.156$ ,  $p = 0.350$ , respectively). Moreover, statistical results revealed no significant difference among KIM-1 means between groups ( $p = 0.117$ ). These results were not statistically significant between the groups according to the duration of PP.

According to our results, in the sham group, according to results of in-group analyses, data revealed statistically significant differences among Cr means

( $p < 0.001$ ) and AST means ( $p = 0.038$ ); these associations may be dependent on anesthesia.

In the short-term PP (1 h) group, statistical tests revealed statistically significant differences among Cr means ( $p < 0.001$ ) and AST means ( $p = 0.038$ ). Moreover, the multiple comparison test revealed a significant difference between pre-anesthesia Cr levels and postoperative 24<sup>th</sup> h Cr levels ( $p = 0.015$ ), postoperative 1<sup>st</sup> h Cr levels and postoperative 24<sup>th</sup> h Cr levels ( $p < 0.001$ ), postoperative 1<sup>st</sup> h AST levels and postoperative 24<sup>th</sup> h AST levels ( $p = 0.033$ ) in group analyses similar to ALT and urea results. We can infer that PP may be effective in increasing time-dependent Cr. Moreover, in the long-term PP (3 h) group, statistical tests revealed statistically significant differences among Cr means ( $p = 0.019$ ), urea ( $p = 0.001$ ) and AST means ( $p = 0.028$ ) between groups. We can suggest that also the long-term PP

model has similar results due to the same action mechanisms in the liver and kidney.

Silberstein *et al.* induced unilateral ischemia under PP in pigs and observed mild tubular necrosis and tubular regeneration as a result of ischemic injury; they did not observe any such damage in the control group without ischemia [28]. Khoury *et al.* studied the degree of renal injury for different pressures of PP in mice [29]. While finding nothing at 3 mm Hg of pressure, they found glomerular lobulation and microcalcification at 15 mm Hg and moderate acute tubular necrosis at 18 mm Hg. Khoury *et al.* also reported that oxidizing agents, which are formed due to PP-dependent renal ischemia, cause apoptosis and thus renal injury in mice [30].

We found the highest degree of renal tubular damage in the 3 h PP group on histopathological evaluation and the lowest score in the control group (Table II). According to histopathological results, the kidney ischemic tubular damage score (EGTI histology scoring system) showed statistically significant differences between the groups ( $\chi^2 = 17.365$ ,  $SD = 2$ ,  $p = 0.001$ ). The more prolonged the duration of PP was, the greater was the injury to the kidney (Table III). In light of our findings, increases in KIM-1 levels did not directly correlate with the duration of PP and IAP, which are not, therefore, adequate biomarkers for diagnosing acute renal injury in its early phase.

**Table II.** Histopathological results, focal tubular necrosis < 25%, score 1 (EGTI system)

Parameter	Control	1 <sup>st</sup> h	3 <sup>th</sup> h
Tubular	0	1	7
Glomerular	0	0	0
Endothelial	0	0	0
Tubulo/interstitial	0	0	0

**Table III.** Kidney ischemia tubular damage scores of control and study groups in sacrificed rats

Kidney ischemia tubular damage score in sacrificed rats	Sham and experiment order			Total	
	Sham (group1)	Group 2 (1 h of pneumoperitoneum)	Group 3 (3 h of pneumoperitoneum)		
No signs of ischemia	Count	7	6	0	13
	% within kidney ischemia tubular damage score in sacrificed rats	53.8	46.2	0.0	100.0
	% within sham and experiment order	100.0	85.7	0.0	61.9
25% loss of brush border	Count	0	1	7	8
	% within kidney ischemia tubular damage score in sacrificed rats	0.0	12.5	87.5	100.0
	% within sham and experiment order	0.0	14.3	100.0	38.1

According to a correlation test, KIM-1 results had a statistically significant association with Cr levels in all control and study groups; moreover, similar results were obtained from urea, AST and ALT results. This correlation did not change with IAP levels or duration.

Serum Cr level, contrary to what was expected, it is not a good biomarker of early renal injury. On histopathological analysis of the kidneys, we found that the degree of renal injury varied in proportion to the increase of KIM-1 biomarker, but these results were not statistically significantly dependent on the PP duration. IAP resulted in an increase of the degree of kidney injury and an increase, but not statistically significant, in the levels of KIM-1. KIM-1 can be a valuable biomarker for the diagnosis of kidney injury in the early phase, but there is a need for well-designed and more comprehensive studies on this topic.

## Conclusions

As mentioned above, we aimed to determine the degree of renal and liver injury that occurs in a pneumoperitoneum (PP) model. According to our results, the increase in KIM-1 levels was correlated with Cr levels, and compatible with histopathological analysis. Moreover, in our research, IAP increased the degree of kidney injury and there was an increase, but not statistically significant, in the levels of KIM-1. Additionally, we did not find any difference in liver damage between groups in the context of IAP levels or duration. Therefore, there is a need for well-designed and more comprehensive studies on this topic.

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## Conflict of interest

The authors declare no conflict of interest.

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