TÜBİTAK

Turkish Journal of Medical Sciences

http://journals.tubitak.gov.tr/medical/

Research Article

Turk J Med Sci
(2018) 48: 1278-1284
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doi:10.3906/sag-1602-124

Effectiveness of new parameters in the evaluation of pneumoperitoneum-related acute kidney injury in rats

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Received: 22.02.2016 • **Accepted/Published Online:** 13.05.2018 • **Final Version:** 12.12.2018

Background/aim: Pneumoperitoneum with high pressure results in decreased glomerular filtration rates (GFRs). Cystatin-C (Cys-C), neutrophil gelatinase-associated lipocalin (NGAL), and interleukin 18 (IL-18) are new parameters in the evaluation of GFR instead of creatinine. The aim of this study is to show the effects of pneumoperitoneum on renal function with the help of these new acute kidney injury markers.

Materials and methods: Sixty rats were divided into 10 groups according to the length of time and degree of pneumoperitoneum pressure achieved during CO2 insufflation: 0 mmHg (control) for 1 h; 4 mmHg for 1, 2, and 4 h; 8 mmHg for 1, 2, and 4 h; and 12 mmHg for 1, 2, and 4 h. Serum samples were obtained to measure the serum creatinine, blood urea nitrogen (BUN), Cys-C, NGAL, and IL-18.

Results: There were no differences between the serum creatinine levels of the groups. Serum levels of BUN, Cys-C, NGAL, and IL-18 were significantly increased in the 2nd hour of the experiment. This increase was more prominent at high pressures.

Conclusion: Although serum creatinine is a practical way of estimating GFR, it has been shown that Cys-C, NGAL, and IL-18 are superior in the estimation of decreased GFR in pneumoperitoneum.

Key words: Pneumoperitoneum, acute kidney injury, Cys-C, NGAL, IL-18, creatinine

1. Introduction

Increasing laparoscopic applications in all branches of surgery and the usage of laparoscopy in more difficult and complicated operations cause prolonged operative times, especially in elderly patients and patients with underlying renal diseases. It is known that pneumoperitoneum with high pressure and long exposure time deteriorates renal functions by decreasing renal blood flow (1,2). This deterioration might lead to acute kidney injury (AKI), which is catastrophic in laparoscopic living-donor nephrectomy (3). Laparoscopic living-donor nephrectomy has gained importance due to several advantages, but prolonged pneumoperitoneum is a dangerous risk factor for glomerular and tubulointerstitial damage (4).

The AKI rate of sustained and abrupt deterioration of renal function causes a steady accumulation of toxins, with rapid development of fluid, electrolyte, and acid-base disorders (5). The mortality in patients with AKI is five times higher than in normal patients. However, there is a lack of uniform definition for AKI. The consensus classification for AKI-RIFLE (Risk, Injury, Failure, Loss of kidney function, and End-stage kidney disease) is based on serum creatinine levels, whose sensitivity in detecting early stages of kidney failure is poor and unsatisfactory to estimate glomerular filtration rate (GFR) (5,6). New substances are sought for reflecting the GFR.

Cystatin-C (Cys-C) is a cysteine proteinase inhibitor with low molecular weight that is produced by nucleated cells and excreted by the glomerulus, thus reflecting GFR. Cys-C levels were shown to be more sensitive than serum creatinine for detecting impaired GFR and the 'modification of diet in renal disease' equation in the detection of AKI (7). Neutrophil gelatinase-associated lipocalin (NGAL) is a member of a family comprising over 20 proteins called lipocalins, which function in intracellular chemical signaling. The level of NGAL expression appears to be related to the degree of kidney dysfunction and may even be able to predict which

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patients are going to have faster declines in their kidney functions (8).

Interleukin 18 (IL-18) is a proinflammatory cytokine released after organ inflammation and ischemic tissue damage. Isolated proximal tubular damage, hypoxia, and ischemic acute renal injury increase the level of IL-18 (9,10).

This research aimed to show the effects of pneumoperitoneum on renal functions with the help of new AKI markers Cys-C, NGAL, and IL-18 in a rat model.

Materials and methods

2.1. Animals

Sixty male albino Wistar rats weighing 350–400 g were included in this study. All rats were kept under 12-h light and 12-h dark cycles and given rat food and water ad libitum.

2.2. Experimental design

This study was designed as an experimental, randomized, controlled trial with blind assessment of outcome. The local ethics committee approved all procedures. The protocol was designed in accordance with the 1996 revised version of the Guide for the Care and Use of Laboratory Animals. The rats were divided into 10 groups according to the length of time and degree of pneumoperitoneum pressure achieved during CO_2 insufflation: 0 mmHg (control) for 1 h; 4 mmHg for 1, 2, and 4 h; 8 mmHg for 1, 2, and 4 h; and 12 mmHg for 1, 2, and 4 h.

2.3. Operative procedures

All animals were allowed free access to standard rat chow and water prior to surgery. Each animal was anesthetized with intramuscular ketamine (Ketalar, Parke Davis and Eczacıbaşı, İstanbul, Turkey) (30 mg/kg) and xylazine hydrochloride (Rompun, Bayer Health Care, İstanbul, Turkey) (3 mg/kg). CO_2 insufflation was performed by inserting a Veress needle (UV120, Ethicon Endo-Surgery, Cincinnati, OH, USA) percutaneously into the peritoneal cavity, connected to an insufflator (Karl Storz GmbH, Tuttlingen, Germany). Pneumoperitoneum was not applied to the control group. The rats were sacrificed after blood sampling at the end of pneumoperitoneum.

2.4. Biochemical analysis

Obtained blood samples were kept in tubes containing ethylenediaminetetraacetic acid (EDTA) at –80 °C until the analysis. The levels of serum blood urea nitrogen (BUN) and serum creatinine were measured by prepared kits with a Beckman Coulter AU 2700 autoanalyzer (Beckman Coulter Inc., Indianapolis, IN, USA). Values are given as mg/dL. Serum NGAL levels were measured with the prepared kits LIPOCALIN-2, NGAL RAT ELISA, and 96 Test BioVendor KIT 8 (BioVendor-Laboratorni Medicana, Brno, Czech Republic). In this setting, a Stat Fax 2600 Microplate Washer was used, and spectrophotometry was done with an enzyme-linked immunosorbent assay (ELISA) reader (EL x 800 UV Universal AM Microplate Reader, Bio-Tek Instruments, Winooski, VT, USA). Absorbance levels were measured with a Chromate Model 4300 Microplate Reader. Values are given as pg/mL. Cys-C levels were measured by immunonephelometric method (BNTMII, Siemens, Munich, Germany) with the prepared kit Siemens LATEX Cystatin C. Values are given as mg/dL. IL-18 levels were measured by ELISA with the Rat Interleukin 19 Elisa Kit (Cusabio, Invitrogen Corporation, Carlsbad, CA, USA). Absorbance levels were measured with a Chromate 4300 Microplate Reader. Values are given as pg/mL.

2.5. Statistical analysis

Statistical analysis was performed with the help of SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Values were analyzed by the Kolmogorov–Smirnov test for distribution control. As the values were not in normal distribution, nonparametric tests were used for evaluation. Statistical analysis was performed by Kruskal–Wallis and Mann–Whitney U tests. $P < 0.05$ was considered to be significant.

3. Results

The results are shown in Figures 1–3. There were no differences between the creatinine levels of the rats at the same intraabdominal pressures with different durations of pneumoperitoneum. There were no differences between the creatinine levels of the rats at the same duration of pneumoperitoneum with different intraabdominal pressures.

There were no differences between the BUN levels of rats of different intraabdominal pressure groups at the 60th and 120th minutes of pneumoperitoneum. The BUN levels of the rats at the 240th minute of pneumoperitoneum at different intraabdominal pressures were significantly higher in than the control group ($P = 0.02$, $P = 0.02$, $P = 0.02$ for 4 mmHg, 8 mmHg, and 12 mmHg, respectively) and rats with different intraabdominal pressures at the 60th minute ($P = 0.04$, $P = 0.04$, $P = 0.04$ for 4 mmHg, 8 mmHg, and 12 mmHg, respectively) (Figure 1).

There were no differences between the NGAL levels of rats of different intraabdominal pressure groups at the 60th and 120th minutes of pneumoperitoneum. The NGAL levels of rats at the 240th minute of pneumoperitoneum with intraabdominal pressures of 8 mmHg and 12 mmHg were significantly higher than in the control group ($P = 0.04$, $P = 0.02$ for 8 mmHg, 12 mmHg, respectively) and the rats with 8 mmHg and 12 mmHg intraabdominal pressures at the 60th minute ($P = 0.04$, $P = 0.02$ for 8 mmHg, 12 mmHg respectively) (Figure 2).

There were no differences between the Cys-C levels of rats of different intraabdominal pressure groups at the

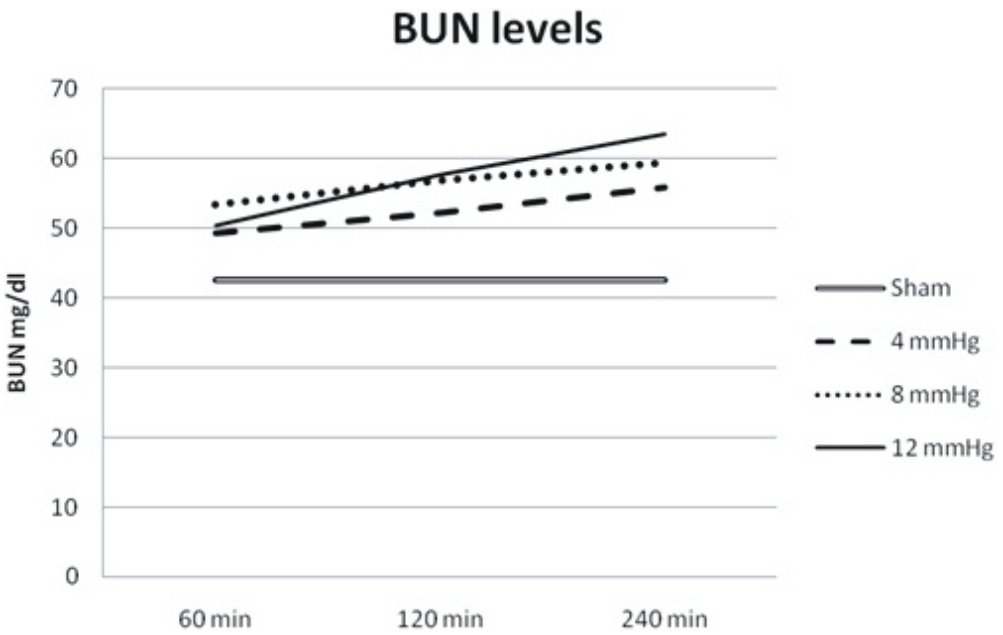

Figure 1. Serum BUN levels of rats with different intraabdominal pressures for different durations of pneumoperitoneum. Comparisons of groups were performed by Kruskal–Wallis and Mann–Whitney U tests.

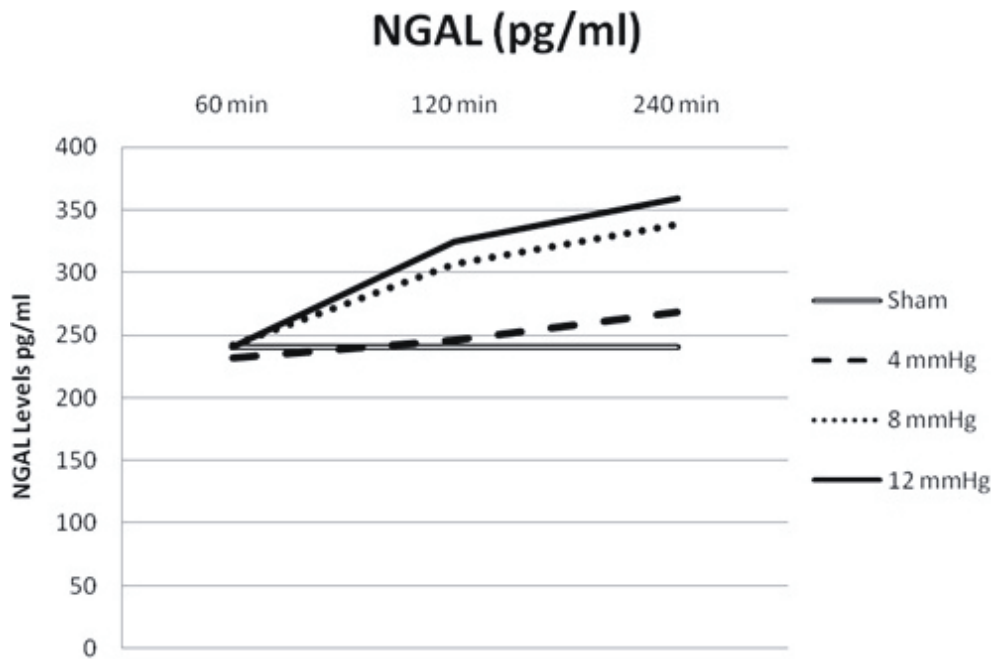

Figure 2. Serum NGAL levels of rats with different intraabdominal pressures for different durations of pneumoperitoneum. Comparisons of groups were performed by Kruskal–Wallis and Mann–Whitney U test.

60th and 120th minutes of pneumoperitoneum. The Cys-C levels of rats at the 240th minute of pneumoperitoneum at 12 mmHg intraabdominal pressure were significantly higher than the Cys-C levels of the control group (P = 0.03) (Figure 3).

There were no differences between the IL-18 levels of rats of different intraabdominal pressure groups at the 60th and 120th minutes of pneumoperitoneum. The IL-18 levels of rats at the 240th minute of pneumoperitoneum with 12 mmHg intraabdominal pressure were significantly

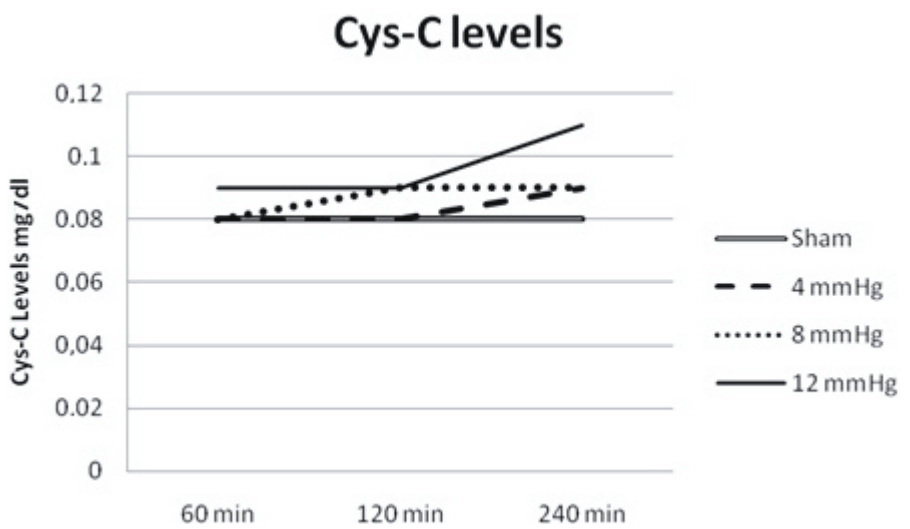

Figure 3. Serum Cys-C levels of rats with different intraabdominal pressures for different durations of pneumoperitoneum. Comparisons of groups were performed by Kruskal–Wallis and Mann–Whitney U tests.

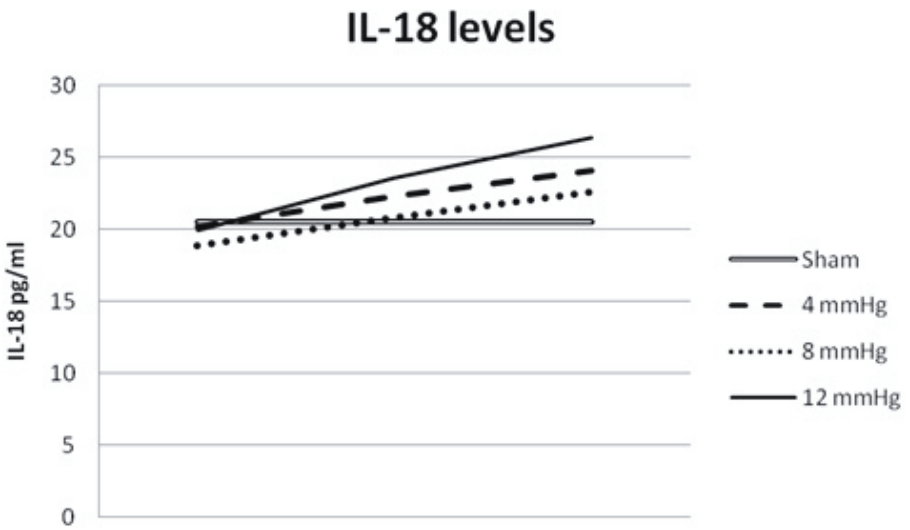

Figure 3. Serum IL-18 levels of rats with different intraabdominal pressures for different durations of pneumoperitoneum. Comparisons of groups were performed by Kruskal–Wallis and Mann–Whitney U tests.

higher than IL-18 levels of the control group and IL-18 levels of the rats with 12 mmHg at the 60th minute of pneumoperitoneum (P = 0.03) (Figure 4).

4. Discussion

Although there are several advantages of laparoscopic surgery over open surgery, laparoscopic operations are not free of complications. Prolonged pneumoperitoneum may also impair perfusion in many organs and systems. Long operation time increases the adverse effects of pneumoperitoneum on cerebral oxygenation (11). Increased intraabdominal pressure due to pneumoperitoneum may lead to several undesirable results like oliguria and AKI (12–14). Pressure on the renal parenchyma and renal vein together with decreased cardiac output and systemic hormonal effects may lead to AKI. Renal vein pressure is 5 mmHg in rats and oliguria is seen during pneumoperitoneum over 5 mmHg. Furthermore, insufflation pressure of 10 mmHg in rats corresponds to an insufflation pressure of 15 mmHg in humans (1). For this reason, we evaluated the effects of insufflation pressures of 4, 8, and 12 mmHg in our study. We identified the highest pressure group as 12 mmHg, which is not used in laparoscopy, but we wanted to understand the sensitivity

limits of these new parameters in the relationship of AKI with high pressure. Insufflation pressure above 12 mmHg in rats is equal to an insufflation pressure of more than 20 mmHg in humans, which is never used. Intraabdominal pressures over 20 mmHg are accepted as intraabdominal hypertension and may lead to abdominal compartment syndrome, which is catastrophic (15,16). Thus, pressure of 20 mmHg or more in humans is not used in laparoscopy for insufflation. In this respect, the results obtained in the high pressure group of our study may be a guide in the evaluation of renal damage progression in intraabdominal hypertension and abdominal compartment syndrome. We also evaluated the effects of pneumoperitoneum at 60, 120, and 240 min. Attempts at introducing 4-h pneumoperitoneum resulted in the death of rats in previous studies (17). Elevated pressures in renal veins may decrease renal plasma flow and GFR, shunting blood away from the cortex and functioning glomeruli and leading to ischemia. Estimating the reliable and safe range for pneumoperitoneum-related AKI is an important subject of investigations, especially for laparoscopic living-donor nephrectomy. For this reason, prevention and early estimation of AKI has gained much more importance.

Several scoring systems and biomarkers are used to show decreased GFR and related AKI (6,18,19). Serum creatinine is the most commonly used serum marker of renal function. It is the basic parameter in RIFLE scores. However, serum creatinine is insensitive regarding the detection of small decreases in GFR, thus suggesting the existence of a so-called creatinine-blind GFR (40–70 mL min^{-1} 1.7 m^{-2}) (20). Serum creatinine levels are also not appropriate for evaluating initial kidney injury because they vary widely with age, sex, lean muscle mass, muscle metabolism, and hydration status (17). In our study, serum creatinine levels increased with time and insufflation pressures, but this was not statistically significant. This might be due to the creatinine-blind GFR levels. In this study, the BUN levels in the 4th hour of pneumoperitoneum at different intraabdominal pressures were significantly higher. Therefore, BUN can be used as a marker of prolonged pneumoperitoneum-related acute kidney injury independently of pressure. Cys-C, NGAL, IL-18, and urinary kidney injury molecule 1 (KIM-1) are new parameters used to predict the AKI. It has been shown that Cys-C is superior to creatinine for the detection of impaired GFR in cross-sectional studies and metaanalysis (14,21,22). Cys-C is also superior to creatinine in determining the early estimation of decreased GFR in the elderly, diabetics, renal transplant recipients, and liver cirrhosis patients (7,23,24). In our study, significantly increased Cys-C levels were seen at 12 mmHg at the 4th hour. Although creatinine did not show any significant change, Cys-C was able to show a difference. As the sensitivity of Cys-C is higher than that of creatinine, GFR is decreased at high pressure levels with time. At low pressures, Cys-C levels did not show any significant difference at the 4th hour. This is probably due to the unchanged GFR at low insufflation levels. High pressure and longer times made for a much more significant GFR decrease.

NGAL is a novel, sensitive, and noninvasive biomarker for renal ischemia but is not specific to AKI (5). Local release of cytokines from neutrophils trapped in the microcirculation after ischemia due to pneumoperitoneum led to upregulation of NGAL in renal tubules (25,26). IL-18 is a well-known mediator of inflammation, activated by caspase-1. Beside the neutrophil-dependent NGAL, IL-18 is another potential marker for AKI in a neutrophil-independent way. NGAL and IL-18 are the most promising biomarkers for AKI (14,25–29). It was concluded that NGAL and IL-18 are not predictors of GFR, but they show inflammation in the kidneys. In our study, NGAL and IL-18 levels were significantly increased at 12 mmHg insufflation pressure at the 4th hour. In the study of Barros et al., there was no significant difference between NGAL levels of the pneumoperitoneum and control groups (17). This is because they applied 10 mmHg insufflation, which is lower than in our study. Pressure of 10 mmHg is not enough to upregulate NGAL gene expression. However, higher pressures are critical for kidney injury. Also, previous studies investigated urinary IL-18 and NGAL levels, and the tissue sample NGAL levels were different from those of our study (17,25–28). In our study, we measured the serum levels of NGAL and IL-18. Our results were in accordance with previous studies performed using urine and tissue samples. Our study showed that serum levels of NGAL and IL-18 in pneumoperitoneum give results similar to the urinary levels of IL-18 and tissue levels of NGAL. Serum levels of NGAL in the 2nd hour in high pressure groups were significantly higher than in the shorter durations. This shows us that the inflammation is progressing with time at high pressures. This situation may be explained by other mechanisms like systemic hormone activity rather than GFR. This may be a subject of future study.

In conclusion, high insufflation levels for more than 1 h lead to decreased GFR and increased inflammation reactions in the rat kidney. Insufflation at 8 mmHg for rats is the counterpart of 10–12 mmHg pressure in humans used for laparoscopic surgery. Significant increase was detected in BUN and NGAL markers in this pressure group. As a result, these two parameters can be considered as effective markers for the influence on renal function of insufflation pressure in laparoscopic surgery. Significant elevations of IL-18 and Cys-C levels in the rats in the 12 mmHg pressure group indicate that these parameters can be used as biomarkers for AKI in intraabdominal hypertension and abdominal compartment syndrome with BUN and NGAL. It must be kept in mind that laparoscopic operations with high pressures and long durations may cause AKI.

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