The Effects of Low versus Standard Pressure Pneumoperitoneum on Renal Tubular Epithelial and Peritubular Endothelial Cells Injury in Living-Donor Nephrectomy: A Randomized Controlled Study

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Abstract

**Background:** Laparoscopic nephrectomy is a preferable technique for living kidney donation. However, positive-pressure pneumoperitoneum may have an unfavorable effect on the remaining kidney and other distant organs due to increased systemic inflammation, endothelial vascular response and renal tubular injury. Early detection of renal injury due to increased intraabdominal pressure is needed. The aim of this study was to evaluate whether using low-pressure pneumoperitoneum reduces syndecan-1 shedding and soluble vascular endothelial growth factor receptor-2 (sVEGFR-2) expression as the early markers of vascular endothelial and renal tubular cell injury, in comparison to the standard pressure.

**Methods:** We conducted a prospective randomized study on 44 patients undergoing laparoscopic donor nephrectomy that were allocated into standard pressure (12 mmHg) group or low-pressure (8 mmHg) group. The serial plasma interleukin-6, syndecan-1, and sVEGFR-2 were collected at baseline, intra- and postoperative, and were quantified by ELISA. We analyzed syndecan-1 and VEGFR-2 expression from renal cortex tissue by immunohistochemistry and examined the ultrastructure of renal tubules and peritubular capillaries using electron microscopy. The perioperative hemodynamic, end-tidal CO2, serum creatinine, blood urea nitrogen and urinary KIM-1 were recorded.

**Results:** Forty-four patients were analyzed, the patients’ baseline and demographic characteristics were compared between groups. The low-pressure group showed significantly lower intra- and postoperative heart rate, intraoperative plasma IL-6 and sVEGFR-2 level compared to the standard pressure group. Plasma syndecan-1 level was lower in the low-pressure group although insignificant. Syndecan-1 expression in proximal tubules was significantly higher in the low-pressure group. The expression of VEGFR-2 in proximal, distal tubules and peritubular capillaries endothelium were significantly lower in the low-pressure group. The low-pressure group showed better morphological ultrastructure of renal tubules and peritubular capillaries.

**Conclusion:** The low-pressure pneumoperitoneum reduced the inflammatory response of plasma IL-6, the shedding of syndecan-1 and VEGFR-2 expression as the marker for renal tubular injury in laparoscopic nephrectomy.
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Keywords: Pneumoperitoneum pressure, renal resistive index, interleukin-6, syndecan-1, VEGFR-2, laparoscopic nephrectomy

Background

Minimally invasive surgery is increasingly performed in many institutions. Laparoscopic nephrectomy technique is a less-invasive technique for living donor allograft kidney procurement and becomes a preference to promote early postoperative recovery. The increased intra-abdominal pressure (IAP) as a result of pneumoperitoneum insufflation may have an unfavorable effect on the kidney and other distant organs due to several mechanisms. As laparoscopic techniques are advancing, living donors are patients undergoing surgery to save others and their postoperative condition becomes a priority. So, it is important to ensure the safety level and risk of the technique to the allograft kidney function, both for the recipient and kidney donor function furthermore.¹

The increased IAP is frequently present in surgical or critically ill patients and becomes an independent predictor for morbidity and mortality. The mean IAP in a healthy patient while supine is 1.8 mmHg with range -1–6 mmHg.² The World Society of the Abdominal Compartment Syndrome (WSACS) define that upper normal limit for IAP is approximately 5–7 mmHg in adults.³ The kidneys are at risk of injury induced by the increased IAP. A direct effect of increased IAP secondary to pneumoperitoneum is correlated with renal venous congestion and decreasing renal blood flow due to compression of the renal vasculature and renal parenchyma.⁴

A prospective clinical study of living kidney donor undergoing transperitoneal laparoscopic nephrectomy with pressure at 12 mmHg showed increasing inflammatory responses and early signs of kidney injury, compared with patients undergoing open retroperitoneal nephrectomy.¹ An animal study showed applying pneumoperitoneum to isolated perfused rat kidney demonstrated early onset of inflammation and renal apoptosis.⁵ The decreased renal blood flow lead to tissue hypoperfusion
that triggers inflammatory responses. After desufflation, reperfusion occurs when renal blood is normalized that further stimulates the synthesis of inflammatory cytokines. Inflammatory cytokine has been postulated to mediate the association between blood flow changes with endothelial and epithelial cells injury. Vascular endothelial dysfunction and tubular cell injury in response to inflammatory cytokine has an important role in acute kidney injury (AKI).

Syndecan-1 is a cell surface proteoglycan that consists of heparan sulfate and chondroitin sulfate that is expressed on various epithelial and vascular endothelial cells. Syndecan-1 is involved in many cellular functions to promote cell proliferation and survival. The shedding of syndecan-1 may be one of the important mechanisms of tubular epithelial injury in ischemia and inflammation conditions. The elevated serum syndecan-1 predicted acute kidney injury (AKI) and mortality in patients with acute heart failure and in pediatric patients undergoing cardiac surgery. Higher expression of tubular epithelial syndecan-1 promoted tubular cell survival and repair that was correlated with prolonged allograft survival in kidney transplantation patients.

Activation of Vascular Endothelial Growth Factor (VEGF) binding to VEGF Receptor-2 (VEGFR-2) has important roles in maintaining angiogenesis and microvasculature permeability. Overstimulation of VEGF-VEGFR-2 induced renal tubulointerstitial injury through altered endothelial proliferation, abnormal angiogenesis, and extracellular matrix deposition, that was enhanced in endothelial nitric oxide deficiency. These findings indicate the inhibition of syndecan-1 shedding and VEGF-VEGFR-2 stimulation is a novel target in preventing or managing AKI, since serum BUN, creatinine and urine output are the delayed signs of deteriorating kidney function.

We aimed to evaluate the effect of lower intraabdominal pressure in preventing kidney injury in transperitoneal laparoscopic living donors nephrectomy as human models of mild increasing intraabdominal pressure. The present study was designed to evaluate the low-pressure
pneumoperitoneum in attenuating systemic inflammatory response and renal tubular cell injury in comparison to standard pressure. We hypothesized that short-term increasing of intraabdominal pressure could alter renal perfusion and induce a systemic inflammatory response that lead to tubular cell injury, and using lower pressure pneumoperitoneum could reduce those effects. The primary outcome was detecting the shedding of syndecan-1 and activated VEGFR-2 as the novel markers of early renal injury due to increased intraabdominal pressure condition. The secondary outcome was detecting plasma interleukin-6 (IL-6) as the systemic pro-inflammatory cytokine and urinary KIM-1.

Methods

Ethical Considerations

A prospective single-blinded clinical study on patients undergoing transperitoneal laparoscopic living donor nephrectomy was conducted at the university teaching hospital after receiving approval from the medical ethics committee (protocol no. 17-06-0619, approval date: June 19th, 2017), and was registered on ClinicalTrial.gov (NCT:03219398).

Patients Enrollment

We enrolled 44 patients between July 2017 until February 2018, after obtaining the written informed consent from the patients. The inclusion criteria were age between 18 – 65 years old, American Society of Anesthesiologist (ASA) physical status classification I-II, body mass index 18 – 25 kg/m². Exclusion criteria were hemodynamic instability > 25% of the baseline despite the intervention treatment and conversion of laparoscopy to open nephrectomy. The patients were allocated using block randomization of 4, using a list of random numbers in sealed envelopes then divided into the 12 mmHg (standard pressure) group or 8 mmHg (low-pressure) pneumoperitoneum group. Patients and principal investigator were blinded to group allocation. The principal investigator received the randomization codes after all measurements and calculations had been entered into results database of all patients.

Anesthesia and Pneumoperitoneum
All patients received monitoring of heart rate, electrocardiography, non-invasive blood pressure, pulse oxygen saturation, end-tidal carbon dioxide (IntelliVue MP70 Philips Healthcare, Netherlands), and cardiac output measurement using bioimpedance cardiometry (ICON™, Osypka Germany) were conducted. After midazolam premedication, a standardized anesthesia was induced with propofol 1 – 2 mg/kg i.v and fentanyl 1 µg/kg i.v, intubation was facilitated with atracurium 0.5 mg/kg i.v. General anesthesia maintenance was performed using sevoflurane with end-tidal sevoflurane (ETS) target at 1.5 – 2% (Aisys C2, GE Healthcare, Illinois USA) to maintain bispectral index value between 40 to 50 (BIST™, Covidien, Minneapolis USA) and maintenance of atracurium 0.005 mg/kg/min i.v to achieve train of four between 0.15 – 0.25 (TOF-Watch, Organon, Ireland).

Under general anesthesia, the patient was positioned on the lateral decubitus position. The research assistant opened the sealed envelope and allocated the patient into the standard group or lower pressure group based on inclusion number. After introducing the Hasson trocar, pneumoperitoneum was established by carbon dioxide (CO₂) insufflation. The patients received 12 mmHg or 8 mmHg pneumoperitoneum pressure (Olympus, Tokyo Japan) depend on the randomization. The surgeon inserted an endoscopic 30° video then introduced two 5-mm and 10 or 12 mm laparoscopic trocars under direct vision. In this study, all patients underwent left kidney procurement. The kidney was extracted through the Pfannenstiel incision using endobag, immediately was flushed with a cold preservative solution (Custodiol® HTK). At the end of surgery, the pneumoperitoneum was desufflated and the incision was closed. All patients received bilateral Quadratus Lumborum (QL) block using bupivacaine 0.25% before extubation. The patients received a reversal of muscle relaxant if necessary and they were extubated. In this study, all anesthesia and surgeries were performed by the same consultant team with comparable distributions.

Sample Collection and Analysis

Intrarenal Doppler using ultrasound transducer 3.5 – 5 MHz (Logic 7-GE, USA) was performed to
measure interlobar arterial peak systolic and end diastolic velocities, and the resistive index (RI) was calculated by peak systolic velocity minus end diastolic velocity and divided by peak systolic velocity. The RI measurement was performed on the left kidney at the time: before the anesthesia induction as the baseline; 2 hours of gas insufflation intraoperatively; and 2 hours after gas desufflation on the remaining right kidney.

Venous blood samples from brachial veins and urine sample were collected at the same time of RI measurement. All samples were stored in -80° C until analysis and every sample was run in duplicates. Plasma IL-6, syndecan-1, and sVEGFR-2 were analyzed by ELISA method (Human IL-6, Quantikine®, R&D system, USA, Human CD138/Syndecan-1, Diaclone, France, and Human VEGF R2/KDR Quantikine® R&D, Minneapolis, MN)), following manufactures instruction. KIM-1 was determined from a 10 µL urine specimen and were measured by ELISA method (Human Urinary KIM-1, Quantikine®, R&D system, USA). Perioperative hemodynamic profiles were represented by heart rate, systolic pressure, diastolic pressure, mean arterial pressure, and cardiac output were recorded immediately at the same time as blood sample was collected. Pre-postoperative serum creatinine and BUN were recorded.

Immunohistochemistry and Renal Ultrastructure Examination

Cold ischemic time was defined as the interval between the kidney immersed in ice slushed until intravascular perfusion with cold preservative solution. The renal biopsy was performed one time at the end of cold ischemic time. Tissues were immersed in Dubosq solution for 30 minutes and fixed in 10% neutral-buffered formalin, embedded in paraffin, and sectioned. For syndecan-1 immunostaining, the 4 mm thick sections were stained using the periodic acid Schiff (PAS). The sections were incubated with the primary antibody Anti-Syndecan-1 antibody (B-A38, ab714, Abcam, USA) overnight at 4°C. For VEGFR-2 immunostaining, the sections were incubated with the primary antibody Anti-VEGFR-2/KDR antibody (SP123, ab115805, Abcam, USA) overnight at 4°C. After washing, the sections
were incubated with horseradish peroxidase conjugated secondary antibody for 30 minutes at room temperature. The slides were then washed and incubated with 3,3-Diaminobenzidine (DAB)-peroxidase substrate solution for 20 seconds.

The protein expressions of syndecan-1 and VEGFR-2 were determined by immunohistochemistry method and were observed under the light microscope (Leica DM500) and photographed with digital camera (Leica ICC50 HD, Germany). In each slide, 20 different fields at x400 magnification were selected. The Syndecan-1 expressions in the proximal and distal tubular epithelial cells were assessed. A semiquantitative analysis using HER-2 score and H-Score was performed. The total number of proximal and distal tubular cells assessed was 500 cells each from each slide. Tagging and evaluation of intensity (0 – 3+) of 500 cells from each section were based on HER-2 criteria (score 0: no staining; 1+: weak and incomplete membrane staining in less than 10% of the cells; 2+: weak complete staining of the membrane in more than 10% of the cells; 3+: strong complete homogenous membrane staining in more than 30% of the cells) with the help of the ImageJ program then was converted into percentages, and entered into the histological score (H-score) formula, the resulting value is between 0–300:

The VEGFR-2 expressions in arterial endothelial cells, peritubular and glomerular capillaries, podocyte cells, proximal and distal tubular epithelial cells were assessed. The semiquantitative analysis was performed by the percentage of positive VEGFR-2 expressions in 25 peritubular arteries and 50 peritubular capillaries of each sample. The VEGFR-2 expressions in the proximal and distal tubular epithelial cells were assessed using the HER-2 score and H-Score as described above. The scoring was performed by three observers who were blinded to the randomization of sample allocations.

Electron microscopy (EM) was performed to examine the ultrastructure of proximal tubules, distal tubules, peritubular capillaries, and arteries. After perfusion fixation with 4% paraformaldehyde, kidney tissues were fixed in 2.5 % glutaraldehyde and postfixied with 2% osmium tetroxide in 2,5%
K$_3$Fe(CN)$_6$ and 3% sucrose. The samples were dehydrated in graded ethanol, embedded in Spurr resin and vacuumed. Ultrathin sections were stained with 2% uranyl acetate with triple lead citrate and examined by EM (JEOL 1010, Tokyo, Japan) at 80 kV.

Statistical Analysis

Power analysis (a = 0.05, b = 0.20) was used to determine the sample size for detecting significance difference in VEGFR-2 and syndecan-1 between groups. Chi-squared test was used for the categorical variables. Parametric data were presented as the mean ± standard deviation or median (interquartile range), and were compared using unpaired t-test or Mann-Whitney test. Repeated analysis of variance followed by post hoc analysis was performed. Transformed data were analyzed using presented as geometric mean and confidence interval of 95% (minimum–maximum) using a general linear model. All of the analysis was performed using SPSS 20.0 software. P-values < 0.05 was considered statistically significant.

Results

Patients were recruited between August 2017 until February 2018. The CONSORT flow diagram is presented in Figure 1. After exclusion of 2 patients, 44 patients were enrolled and analyzed.

Baseline and perioperative characteristics are presented in Table 1. The basic characteristics of the overall subjects were more men (56%) than women (44%), the age range was 30–35 years, had a BMI between 22–25, BUN level 19.82–26.18 mg/dL and creatinine level 0.8–1.18 mg/dL. Perioperative data of the subjects showed comparable pre- and postoperative urine output, total warm ischemic time, total cold ischemic time, duration of surgery and anesthesia between the two pressure groups.

Table 2. shows the parameters of hemodynamic cardiac index (CI), stroke volume (SV), mean arterial pressure (MAP), and end-tidal CO$_2$ between the 12 mmHg and 8 mmHg group are not significantly different. However, there is an increase in heart rate (HR) in the 12 mmHg group was significantly higher than the 8 mmHg group ($p < 0.001$).
Figure 2 shows a comparison of changes in renal RI values, plasma IL-6, syndecan-1, sVEGFR-2, and urinary KIM-1 starting from baseline, 2 hours of pneumoperitoneum and 2 hours after surgery in both pressure groups. In each group, the RI was significantly increased during pneumoperitoneum and after surgery compared to baseline ($p <0.001$). The comparison of RI between the 8 mmHg and 12 mmHg group were not significantly different perioperatively ($p = 0.746$). The increasing plasma IL-6 levels were significant during 2 hours of pneumoperitoneum insufflation almost 4-8 times and still increases 5-8 times further after surgery compared to baseline ($p < 0.001$). Plasma IL-6 level in the 8 mmHg group were significantly lower during pneumoperitoneum (4.58 vs 8.50 pg/mL) and after surgery (36.18 vs 44.89 pg/mL) compared to the 12 mmHg group ($p = 0.003$). The increasing plasma syndecan-1 levels were significant during 2 hours of pneumoperitoneum and still increases 2 times further after surgery compared to baseline ($p < 0.001$). Plasma syndecan-1 levels during pneumoperitoneum (11.99 vs. 13.09 ng/mL) and postoperatively (30.07 vs 32.03 ng/mL) were consistently lower in the 8 mmHg group compared to the 12 mmHg group. Plasma sVEGFR-2 in the 8 mmHg group were significantly lower during pneumoperitoneum (8105.99 vs. 6841.05 pg/mL; $p = 0.032$) and after surgery (8452.25 vs. 7263.92 pg/mL; $p = 0.044$) compared to the 12 mmHg group. Urinary KIM-1 level was significantly higher during pneumoperitoneum compared to baseline and markedly decreased after surgery in both groups ($p < 0.001$).

The pressure group of 8 mmHg shows the expression of syndecan-1 in the proximal tubules with stronger intensity than the pressure group of 12 mmHg. There is no difference in the intensity of syndecan-1 expression in the distal tubules between the two groups. Syndecan-1 expression is negative in both glomerular and peritubular capillaries in both groups. The H-score of syndecan-1 expression in proximal tubules were significantly higher in the 8 mmHg group (223.48 vs. 209.36; $p = 0.030$) compared to the 12 mmHg group, while the expression in distal tubules was comparable (111.32 vs .108.40; $p = 0.757$) between the two groups (Figure 3).
The expression of VEGFR-2 in the proximal and distal tubules were lower in the 8 mmHg group than the 12 mmHg group. The H-score of VEGFR-2 expression in proximal tubules ($p = 0.030$) and distal tubules ($p = 0.024$) were significantly lower in the 8 mmHg compared to 12 mmHg group. The VEGFR-2 expression in peritubular capillaries in the 8 mmHg group was lower than the 12 mmHg group. The comparisons of VEGFR-2 histological score in peritubular capillaries showed the lower percentage of strong expression cells and a lower histological score in the in 8 mmHg group ($p < 0.001$) compared to 12 mmHg group. The VEGFR-2 expression in peritubular arterial endothelial cells was similar between the two groups (Figure 4).

Electron microscopy studies were performed to determine the early changes in tubular epithelial cells, peritubular capillaries, and glomerulus ultrastructure. The ultrastructure morphology of the proximal tubule, distal tubule, and peritubular capillary endothelial cells from each pressure group are shown in Figure 5. The pressure groups of 8 mmHg had better proximal and distal tubule ultrastructure morphology, intact cell membranes with clear cell boundaries, and intact brush borders compared to the 12 mmHg group. The 12 mmHg group showed the swollen nucleus, the cell membrane was more tenuous that the boundary between cells become more distant, many vacuolizations and the brush border was detached from the cell body, that indicated a greater injury compared to the 8 mmHg group. Vacuolizations was not seen in the distal tubule of 8 mmHg group as much as in the 12 mmHg group. The peritubular capillary in the 8 mmHg group showed intact endothelial cell nucleus, endothelial layer, and basement membrane, compared to the 12 mmHg group that showed swollen endothelial cell nucleus, edematous endothelial layer and basement membrane disruption in the peritubular capillary

Discussion

Many institutions still continue to use standard pressure pneumoperitoneum at 12 – 14 mmHg because of its surgical space convenience, although several studies have demonstrated the negative effects of positive-pressure pneumoperitoneum on cardiovascular and organ perfusion. The unfavorable consequences are not expected during most elective laparoscopic operations in healthy
or low-risk individuals. However, the increased intraabdominal pressure have a significant clinical impact for high-risk patients such as elderly, cardiac dysfunction or critically ill patients.\textsuperscript{4,10}

The kidneys are very susceptible to the increased intraabdominal pressure, even a slight increase in pressure of 10 mmHg has begun to affect kidney function and at a pressure of as high as 20 mmHg kidney function begins to be disrupted.\textsuperscript{5,11} Animal study showed that CO\textsubscript{2} pneumoperitoneum at 12 to 18 mmHg induced renal cell apoptosis in outer medulla and cortex.\textsuperscript{12} In humans, increased intra-abdominal pressure cause hypoperfusion in the abdominal or splanchnic region regardless with or without hypotension. Research on animals showed pneumoperitoneum with a pressure at 12 mmHg resulted in hypoperfusion that induced the release of inflammatory cytokines and neutrophil migration.\textsuperscript{5,12} The advanced venous congestion and decreased renal blood flow lead to tissue hypoperfusion or ischemia that trigger inflammatory responses. After desufflation, reperfusion occurs when renal blood is normalized then leads to oxidative stress that stimulates synthesis of inflammatory cytokines. Inflammatory cytokine has been postulated to mediate the association between blood flow changes and endothelial-epithelial injury.\textsuperscript{4,13}

The increased intraabdominal pressure causes mechanical compression of the inferior vena cava and of the renal vasculature and parenchyma. It increases sympathetic activity, which is regulated through the mechanism of mediated baroreceptors together with the effects of CO\textsubscript{2} that can lead to renal cortical vasoconstriction and its sequelae.\textsuperscript{12,13} The kidneys have autoregulation which is influenced by vascular (myogenic) and tubuloglomerular feedback (TGF) factors. Vascular factors affect autoregulation of renal perfusion through blood flow (pressure) and pressure on blood vessels (pressure), which depends on cardiac output and blood pressure as long as it is on the threshold of autoregulation.\textsuperscript{14} Under normal condition, blood flow is a laminar flow which gives constant pressure to the blood vessel walls. Changes in blood flow will cause shear stress due to turbulent or oscillatory
flow. Shear stress will stimulate the synthesis of endothelial nitric oxide, which will activate the NF-κB signaling pathway through VEGFR-2 which is present on the surface of endothelial cells. Furthermore, stimulation of pro- or anti-inflammatory responses will play a role in the activation, injury, repair, apoptosis of endothelial and epithelial cells. Shear stress in the form of a constant or uniform laminar flow has a protective effect on the endothelium. Low or turbulent shear stress caused by impaired blood flow, not constant and uniform, will stimulate the inflammatory response, increase the expression of endothelial adhesion molecules and their interactions with neutrophils and monocytes in the endothelium.15

When pneumoperitoneum insufflation is given, an increase in RI values represents an increased intra-abdominal pressure will cause a decrease in interlobar arterial blood flow. It will stimulate a systemic inflammatory response that trigger the release of IL-6.15 Our study showed a higher release of IL-6 during the increasing intra-abdominal pressure in the standard pressure group compared to the low-pressure group. Pneumoperitoneum insufflation using CO₂ gas has a disadvantage since it was high solubility gas that can be absorbed by the tissue, resulting in sympathetic stimulation such as tachycardia. Although CO₂ and surgical techniques can contribute to the release of pro-inflammatory cytokines,16 our study showed a short-time and slight increasing intraabdominal pressure result in significantly increasing IL-6 levels, and using the low-pressure pneumoperitoneum can attenuate that response. The low-pressure group showed the trend of heart rate that significantly lower than the standard pressure group.

Studies on the impact of low-pressure versus standard pressure pneumoperitoneum have shown the various results. A laparoscopic cholecystectomy study performed with low-pressure or standard pressure showed no difference in the increase of IL-6, IL-8, and IL-10. Another laparoscopy study found significantly higher IL-1, IL-6, and CRP levels in the standard pneumoperitoneum pressure group compared with low-pressure pneumoperitoneum.13 Yap et al. found that laparoscopic donor
nephrectomy resulted in nearly 50% of their subjects showed an increased tumor necrosis factor alpha (TNF-) excretion at both 5 and 24 hours and increased urine neutrophil gelatinase-associated lipocalin (NGAL) after donor nephrectomy, that was suggesting that elevated cytokine levels may be due in part to increased endogenous production. The results of this study validate their previously published study which demonstrated that animal models of nephrectomy AKI resulted in increased TNF-?, IL-6, and monocyte chemoattractant protein-1 (MCP-1) expression. IL-6 has been shown to induce neutrophil infiltration with increased macrophage infiltration. An animal study showed the extrarenal IL-6 production from liver after unilateral nephrectomy. Our results suggested that the increased of plasma IL-6 was due to the increased endogenous production, not because of decreased renal excretion since the urine output and serum creatinine were within the normal limit before and after the procedure.

As hypothesized, we found the increasing plasma syndecan-1 corresponded to the elevated plasma IL-6. Interleukin-6 is a proinflammatory cytokine that will cause Syndecan-1 activation and shedding from the endothelial surface of blood vessels into the bloodstream. In accordance with the degree of inflammation that occurs, the shedding of syndecan-1 increased in both level of pneumoperitoneum pressure compared to the baseline conditions. However, the increasing plasma Syndecan-1 level was less and Syndecan-1 expression in proximal tubular cells was higher in the low-pressure group compared to the standard pressure group. There was a significant increase in the degradation of syndecan-1 glycocalyx products after major surgery in human and animal studies. The duration of laparoscopic nephrectomy is relatively longer than open nephrectomy with the addition of using high-pressure pneumoperitoneum lead to exposing the kidney with longer time and more profound of warm ischemia that contributes to the syndecan-1 shedding.

The renal tubular epithelium not only passively injured, but also produced an active response to inflammation. The release of proinflammatory and chemotactic cytokines which activates T cells and
its co-stimulating molecules. Proximal tubular cells respond to T-cell ligands through cell surface receptor activation.\textsuperscript{13} The increasing syndecan-1 expression and its shedding into the blood are considered as an adaptive response to repair an early cell injury.\textsuperscript{21} Syndecan-1 plays a role in the process of re-epithelialization during the inflammation including to be involved in promoting the survival of renal tubular epithelial cell in animal models of ischemia/reperfusion and in kidney transplantation patients. In early renal injury, tubular epithelial cells increase the regulation of syndecan-1 to proliferate and repair the injured cells. In respond to a mild inflammatory condition, the increasing tubular syndecan-1 expression showed a better re-epithelialization process in allografts, that correlates with less proteinuria, lower serum creatinine, less tubular atrophy, and lower risk of delayed graft function. Syndecan-1 becomes a tubular marker that correlates with kidney graft function and survival.\textsuperscript{22} In further injury, the epithelial cells will increasingly lose syndecan-1, because of the decreasing of their ability to proliferate and regenerate it. The sustained elevating plasma syndecan-1 and low of syndecan-1 expression correlated with the degree of loss of tubular function in the kidney.\textsuperscript{23} Syndecan-1 expression in the proximal renal tubules is related to the degree of proteinuria in various kidney diseases so that the level of syndecan-1 in plasma could become a sign of renal tubular injury.\textsuperscript{24}

Vascular endothelial growth factor-A (VEGF-A) is a strong angiogenic cytokine that has a role in maintaining the microvascular system and increasing vascular permeability. One of the VEGF-A regulators is VEGFR-2 which is expressed during ischemic or inflammatory conditions.\textsuperscript{25,26} When the inflammation occurs, IL-6 and activated Syndecan-1 in the endothelial cells stimulate the synthesis of VEGF-A molecules and its binding to VEGFR-2 on the endothelial surface, and then increase the VEGFR-2 phosphorylation process in order to repair the endothelial injury.\textsuperscript{23,27} Plasma syndecan-1 level was hypothesized to be correlated with plasma soluble VEGF-A as a marker of endothelial damage, and plasma creatinine and urea levels as a marker of kidney function.\textsuperscript{23} In a normal human
kidney, VEGFR-2 is expressed on glomerular endothelial cells, peritubular capillaries, as well as in tubular epithelial cells in a low degree. Regulation of protein expression through the VEGFR-2 receptor is important for the survival of endothelial cell tissue in the kidneys after ischemic injury.\textsuperscript{25,28}

The synthesis and activation of VEGFR-2 in baseline condition occurs but is very mild. Our study found there was an increase in the synthesis and higher activation of VEGFR-2 in the standard pressure than the low-pressure group. The level of plasma sVEGFR-2 was significantly higher when the standard pressure was used, in comparison to the low-pressure pneumoperitoneum that attenuated inflammation response and produced lower sVEGFR-2 level. Activation of VEGFR-2 as the marker of vascular endothelial permeability factor depended on the extent of inflammation results in an increase in endothelial permeability and increased levels of VEGFR-2 in soluble form (sVEGFR-2) in plasma. Plasma soluble VEGFR-2 is the result of an increase in alternative splicing of mRNA or as a proteolytic product of membrane-bound VEGFR-2 released into the bloodstream. During ischemic / reperfusion injury, VEGFR-2 mRNA expression and sVEGFR-2 increase as the response of VEGFR-2 receptors. The increased VEGFR-2 expression is a direct effect of VEGF released by ischemic tubular epithelial cells to the adjacent endothelial cells to maintain capillary blood supply and to promote tubular cell survival and recovery. As a comparison, previous studies have shown that at laparoscopy there is an increase in protective VGEF-mRNA expression as a response to repair of injured tissue.\textsuperscript{25,29,30}

Increased VEGFR-2 expression in tubular epithelial cells in our result may describe that inflammatory responses that occur in the circulation reach the extracellular matrix and renal tubules. The VEGFR-2 expression in tubular epithelial cells was higher in the standard pressure compared to the low-pressure group. The low-pressure group produced less injury to the kidney due to less inflammation and less stimulation of VEGFR-2 in the renal endothelial and tubular epithelial cell. A previous study
showed the overstimulation VEGFR-2 occurring before unilateral nephrectomy in experimental animals induced endothelial proliferation, abnormal angiogenesis, and extracellular matrix deposition causing acute tubulointerstitial injury.\(^{28}\) There is a hypothesis that syndecan-1 act as a VEGFR-2 co-receptor and has a role in modulating VEGF-VEGFR-2 signal for endothelial cell proliferation and survival. It has been proposed that syndecan-1 and VEGFR-2 can be a new marker for AKI and its treatment.\(^{23,31}\)

From electron microscopy examination, the low-pressure group showed proximal and distal tubule ultrastructure with intact tubular cell membranes with clear cell boundaries and intact brush borders. Those morphologies were better compared to the standard pressure group that had a greater injury seen from the tenuous tubular cell membrane, detached brush border from the cell body and showed more vacuolizations. The extracellular matrix peritubular endothelial cell was also more edematous in standard pressure pneumoperitoneum group. The results supported the use of low-pressure pneumoperitoneum results in a lower degree of ischemia and tissue inflammation, thus reducing injury to the endothelial and tubular epithelial cells. Perioperative ischemia and reperfusion process cause injury to the donor kidney epithelial cells that can continue to induce the response from the vascular endothelium.\(^{20,22}\) Experimental animal studies treated with various pressure gradients of CO\(_2\) pneumoperitoneum indicate that the increased intraabdominal pressure caused reperfusion ischemic injury leading to cell apoptosis.\(^{12}\) Damage or loss of tubular epithelial cells is the main histological finding of tissue damage that occurs in renal ischemic reperfusion injury. In humans, acute tubular necrosis was observed in 44% of patients in the open nephrectomy group and 45% in the laparoscopic group. In patients undergoing laparoscopy nephrectomy, 54% of renal biopsy specimens taken showed subcapsular cortical injury. These injuries indicate that pneumoperitoneum and mechanical injury during laparoscopic surgical manipulation causes acute tubular necrosis accompanied by peritubular capillaries congestion.\(^{30}\)
The increasing urinary KIM-1 during pneumoperitoneum insufflation expressed the stress injury on the proximal tubules cell injury accompanied by the formation of debris cells and apoptotic cells. The process causes an increase of the KIM-1 molecule synthesis that will be released into the lumen of the tubule and can be detected in the urine.\textsuperscript{32} In our study urinary KIM-1 level was lower during low-pressure pneumoperitoneum compared to the standard pressure. The reversible tubular injury that was represented by decreasing KIM-1 level into the baseline level after surgery could be due to the short length of laparoscopy procedure.

We found syndecan-1 expression in proximal and distal tubular epithelial cells, with the negative expression of syndecan-1 within glomerular or peritubular vasculature. Our study result was similar to Adepu et al. who conducted the study and found syndecan-1 in the basolateral layer in proximal tubular epithelial cells in human kidney biopsy samples.\textsuperscript{22} She made a hypothesis that the increase in plasma syndecan-1 levels was partly derived from the extravascular source such as renal tubular epithelial cells. Our study on living donor patients showed the contradictory results to a previous animals study that showed the presence of syndecan-1 protein in the glomerulus and peritubular capillaries.\textsuperscript{23} Syndecan-1 expression was not detected in glomerular endothelium might be because of the dominant type of proteoglycan expression in glomerular endothelial cells was syndecan-4, perlecan, and glypican based on research on human glomerular endothelial culture cells in vitro. Other studies also showed the dominant proteoglycans layer in IgA nephropathy are perlecan and biglycan.\textsuperscript{24,26} Urinary syndecan-1 level can be used as another alternative to detect extravascular shedding of glycocalyx layer.

Our present study found that laparoscopic donor nephrectomy resulted in increased plasma IL-6, syndecan-1, and sVEGFR-2 during pneumoperitoneum insufflation and after gas desufflation. The IL-6 as a mediator in the extrarenal effects of AKI is clinically important, since it may lead to the use of cytokine-binding proteins and other anti-inflammatory agents to improve outcome beyond what
current supportive renal measures can offer. Despite the absence of syndecan-1 from the glomerular and peritubular endothelial glycocalyx, it was found in the membrane of the proximal and distal tubules and was important for the survival of renal tubular cells during inflammation. The VEGFR-2 can be a sensitive marker to detect endothelial injury due to perfusion disturbance and inflammation. Both increasing plasma syndecan-1 and sVEGFR-2 level can be interpreted as an early warning of underlying renal injury, rather than plasma creatine, BUN or urine output.

Endothelial injury and renal tubular injury are the earliest signs of hypoperfusion and inflammation due to increased intra-abdominal pressure.\(^1,5,12\) From our study results, although the pneumoperitoneum time is relatively short, the increased inflammation reaction, endothelial and renal tubular injury markers were higher especially when the standard pressure was used in comparison to the lower pressure pneumoperitoneum. The low-pressure pneumoperitoneum could attenuate systemic inflammatory response and vascular response compared using the standard pressure. Inhibiting syndecan-1 shedding and the release of VEGFR-2 are believed has renal-protective roles.\(^22,23,28,31\) Further experimental and clinical studies on inhibiting syndecan-1 shedding and sVEGFR-2 response to endothelial injury in preventing and reducing kidney injury are warranted.

**Conclusions**
Our findings demonstrated that using low-pressure pneumoperitoneum attenuated the inflammatory response plasma IL-6, that may reduce the shedding of syndecan-1 and VEGFR-2 expression as the marker for endothelial and renal tubular cell injury. We should consider using lower pneumoperitoneum pressure during laparoscopic nephrectomy. Lowering the pneumoperitoneum pressure can be expected to be an effort to reduce the injury to the endothelium and renal tubular epithelium.

**Abbreviations**
IAP, intra-abdominal pressure; AKI, acute kidney injury; HSPG, heparan sulfate proteoglycan; VEGF, vascular endothelial growth factor; sVEGFR-2, soluble vascular endothelial growth factor receptor-2; KIM-2, kidney injury molecule-1; IL-6, interleukin-6; QL, quadratus lumborum; RI, resistive index; BM,
glomerular basement membrane.

Declarations

**Ethics approval and consent to participate**

The study protocol was approved by medical ethics committee of Faculty of Medicine, Universitas Indonesia committee (protocol no. 17-06-0619, approval date: June 19th, 2017), and was registered on ClinicalTrial.gov (NCT:03219398). Written consent was obtained from the participants in this study.

**Consent for publication**

Not applicable.

**Availability of data and material**

The datasets used and/or analyzed of the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declared that they have no competing interest.

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**Authors’ contributions**

Design of study: DA and CAM. Conduct of study: DA and CAM. Data analysis: DA, AL, NCS, NIM, ASM. Manuscript preparation: DA, AL, SS. All authors contributed to the development of interim, final drafts, and read and approved the final manuscript.

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Tables
Table 1. Patients characteristics and perioperative data.
| Characteristics                  | 12 mmHg group (n = 22) | 8 mmHg group (n = 22) |
|----------------------------------|------------------------|-----------------------|
| Sex                              |                        |                       |
| Male (%)                         | 45.5                   | 68.2                  |
| Female (%)                       | 54.5                   | 31.8                  |
| Age                              | 35.14 ± 19.41          | 30.50 ± 20.25         |
| Weight (kg)                      | 63.67 ± 9.02           | 60.19 ± 12.31         |
| Height (cm)                      | 159.80 ± 6.97          | 164.05 ± 8.27         |
| Body Mass Index (BMI)            | 25.75 ± 5.37           | 22.23 ± 3.26          |
| Pre-operative                    |                        |                       |
| BUN (mg/dL)                      | 19.82 ± 5.55           | 23.32 ± 5.64          |
| Creatinine (mg/dL)               | 0.80 ± 0.5             | 0.87 ± 0.20           |
| Post-operative                   |                        |                       |
| BUN (mg/dL)                      | 26.18 ± 4.76           | 28.05 ± 6.67          |
| Creatinine (mg/dL)               | 1.10 ± 0.45            | 1.18 ± 0.31           |
| Post-operative urine output (ml/kg/hour) | 1.04 (0.70-3.30) | 1.26 (0.70-1.98) |
| Duration of surgery (minute)     | 273 (258-288)          | 270 (210-360)         |
| Duration of anaesthesia (minute) | 295 (245-385)          | 300 (230-390)         |
| First warm ischemic time (minute)| 3.50 (2.50-3.40)       | 3.51 (2.51-3.42)      |
| Cold ischemic time (minute)      | 25.40 (23.10-30.40)    | 25.40 (23.12-30.41)   |

Categorical variable presented in n (%).

Numeric variable presented with mean (± standard deviation) or median (minimum–maximum).

Table 2. Intraoperative hemodynamic parameters and end-tidal CO₂.
| Parameters | Mean (CI 95%) |  | Mean Difference |  |
|------------|--------------|---|-----------------|---|
|            | 12 mmHg      | 8 mmHg | CI 95%          | p  |
| 1. Cardiac index (L/minute/m²) |             | | | |
| a. baseline | 2.84 (2.49-3.23) | 2.76 (2.48-3.07) | 0.740 | 1.028 |
| b. at 2 hours intra-insufflation | 3.20 (2.78-3.70) | 3.35 (2.98-3.77) | 0.628 | 0.957 | 0.593 |
| c. after surgery | 3.24 (2.85-3.69) | 3.39 (3.08-3.73) | 0.589 | 0.957 |
| 2. Stroke volume (mL/minute) |             | | | |
| a. baseline | 36.84 (32.77-41.67) | 34.70 (31.05-38.47) | 0.480 | 0.690 |
| b. at 2 hours intra-insufflation | 33.70 (28.98-38.550) | 37.20 (32.34-42.06) | 0.057 | 0.33 | 0.499 |
| c. after surgery | 32.12 (28.57-35.77) | 37.64 (32.6-42.77) | 0.07 | 0.35 |
| 3. End-tidal CO₂ (mmHg) |             | | | |
| a. baseline | 35.59 (33.89-37.29) | 34.86 (33.47-36.25) | 0.494 | 0.727 |
| b. at 2 hours intra-insufflation | 37.77 (36.36-39.18) | 38.00 (36.79-39.21) | 0.800 | 0.23 | 0.339 |
| c. after surgery | 37.14 (35.46-38.81) | 38.32 (37.06-39.58) | 0.248 | 1.182 |
| 4. Mean arterial pressure (mmHg) |             | | | |
| a. baseline | 77.92 (72.75-83.10) | 78.30 (72.85-83.76) | 0.917 | -0.38 |
| b. at 2 hours intra-insufflation | 80.05 (75.72-84.37) | 82.31 (77.59-87.15) | 0.467 | 2.26 | 0.499 |
| c. after surgery | 85.77 (80.48-91.07) | 83.06 (78.79-87.31) | 0.409 | 2.73 |
| 5. Heart rate (beats/minute) |             | | | |
| a. baseline | 76.82 (72.34-81.56) | 65.69 (61.56-70.10) | 0.05 | 1.17 |
| b. at 2 hours intra-insufflation | 86.98 (82.62-91.56) | 74.10 (69.41-79.10) | < 0.001 | 1.18 | 0.033 |
| c. after surgery | 95.39 (89.62-101.53) | 77.66 (72.23-83.52) | < 0.001 | 1.23 |


Data are presented as geometric mean and confidence interval 95% (minimum-maximum), \( p<0.05 \) is significant.

The two groups were compared with unpaired t-test and general linear model.

Table 2. shows the parameters of hemodynamic cardiac index (CI), stroke volume (SV), mean arterial pressure (MAP), and end-tidal CO\(_2\) between the 12 mmHg and 8 mmHg group are not significantly different. However, there is an increase in heart rate (HR) in the 12 mmHg group was significantly higher than the 8 mmHg group (\( p < 0.001 \)).

Figures

![CONSORT flow diagram](image-url)
Comparison of renal RI, plasma IL-6, syndecan-1, sVEGFR-2, and urinary KIM-1 between 12 mmHg and 8 mmHg group. (A) Renal resistive index (RI). (B) Interleukin-6 (IL-6). (C) Syndecan-1. (D) Soluble VEGFR-2. (E) KIM-1. All data are presented as means ± standard deviation. Continuous data were analyzed using repeated ANOVA, the comparison between groups was analyzed using unpaired t-test and general linear model; * p < 0.001, ** p < 0.05.
Figure 3
Syndecan-1 expression of tubular epithelial cells in 12 mmHg and 8 mmHg group. (A)(D)(G) Negative control. B) Reduced intensity of syndecan-1 expression in the proximal tubule of the 12 mmHg group. (C) The Syndecan-1 expression in the proximal tubule of the 8 mmHg group is stronger than the 12 mmHg group. (E) Syndecan-1 expression between the distal tubule of the 12 mmHg group and (F) in the distal tubule of the 8 mmHg group was not different. (H)(I) Syndecan-1 expression is negative in the glomerular and peritubular capillaries of the 12 mmHg and 8 mmHg group. Original magnifications were ×400 and red dashed box for higher magnifications. Red arrows indicate positive syndecan-1 expression, yellow arrows indicate negative syndecan-1 expression. (J). The H-score of syndecan-1 expression in proximal tubules is higher in 8 mmHg group than 12 mmHg (p = 0.030), and not significantly different in distal tubule between both groups (p = 0.757). Data are presented as mean ± standard deviation. The two group was compared using unpaired t-test; * p < 0.05.
Figure 4
VEGFR-2 expressions of tubular epithelial in 12 mmHg and 8 mmHg group. (A)(D) Negative control. (B) Increased of VEGFR-2 expression in the proximal tubule of the 12 mmHg group. (C) VEGFR-2 expression in the proximal tubule of the 8 mmHg group is lower than the 12 mmHg group. (E) Increased VEGFR-2 expression in the distal tubule of the 12 mmHg group. (F) The VEGFR-2 expression in the distal tubule of the 8 mmHg group is lower than the 12 mmHg group. Original magnifications were x400 and red dashed box for higher magnifications. Red arrows indicate positive VEGFR-2 expression. (G) The H-score of VEGFR-2 expression in proximal tubules (p = 0.005) and distal tubules (p = 0.024) are higher in the 12 mmHg group compared to the 8 mmHg group. Data are presented as mean ± standard deviation. The two groups were compared using unpaired t-test; * p < 0.05.
Figure 5

VEGFR-2 expressions of peritubular vascular endothelial cells in 12 mmHg and 8 mmHg pneumoperitoneum pressure group. (A) Negative control (B) Strong VEGFR-2 expression in the peritubular capillaries and artery of the 12 mmHg group. (C) VEGFR-2 expression in the peritubular capillaries and artery of the 8 mmHg group is lower than the 12 mmHg group.
Original magnifications were x400 and red dashed box for higher magnifications. Red arrows indicate positive syndecan-1 expression in peritubular capillaries endothelium, yellow arrows indicate positive syndecan-1 expression in peritubular arteries endothelium.

(D) VEGFR-2 expression score of peritubular capillaries in 12 mmHg is higher than the 8 mmHg group (E) VEGFR-2 expression score of artery between 12 and 8 mmHg group are not significantly different. Score value represents the percentage of positive VEGFR-2 expression in 25 peritubular arteries and 50 peritubular capillaries each sample. Data were analyzed using Chi-square for trend or Mann-Whitney; * p < 0.001, ** p < 0.05.
Figure 6

The ultrastructure of renal tubules and peritubular capillaries in the 12 mmHg and 8 mmHg pneumoperitoneum pressure group. (A) Proximal tubular epithelial cells in the 12 mmHg group, arrows show tenuous epithelial membranes and detached brush borders. (B) Proximal tubular epithelial cells in the 8 mmHg group, arrows indicate the tight epithelial membrane and intact brush border. (C) Distal tubular epithelial cells in the 12 mmHg group show vacuolizations and diffused nuclear border. (D) Distal tubular epithelial cells in the 8 mmHg group show intact nucleus and no vacuolizations. (E) Peritubular capillary in the 12 mmHg group show the swollen nucleus, edematous endothelial layer, arrow shows disrupted basement membrane. (F) Peritubular capillary endothelium in the 8 mmHg with intact nucleus and endothelial layer, the arrow shows intact basement membrane. The red
The proposed mechanism of injury to endothelial cells and kidney tubules that occur in standard pressure and low-pressure pneumoperitoneum. 1. The standard pressure (12 mmHg group) decreases the interlobar artery blood flow and result in more changes from laminar flow into turbulent flow [B] compare to the low-pressure (8 mmHg group) [C]; 2. The inflammation response in the 12 mmHg group produce higher IL-6 level than the 8 mmHg group; 3. Interleukin-6 causes the Syndecan-1 activation and shedding from the endothelial surface into the bloodstream in the 12 mmHg group is higher than the 8 mmHg; 4. (a) Interleukin-6 and Syndecan-1 stimulate the synthesis of VEGF-A molecules that (b) bind to VEGFR-2 on the endothelial surface; 5. Activation of VEGFR-2 increases sVEGFR-2 level higher in the 12 mmHg group than the 8 mmHg; 6. The expression of VEGFR-2 in tubular
epithelial cells is higher in the 12 mmHg group and the expression of Syndecan-1 is lower in the 12 mmHg group than the 8 mmHg group; 7. Due to the inflammation, the tubular epithelial cell injury stimulates the synthesis of KIM-1 molecules that will be released into the tubular lumen (urine).