Author’s response to reviews

Title: Effects of Low Versus Standard Pressure Pneumoperitoneum on Renal Syndecan-1 Shedding and VEGF Receptor-2 Expression in Living-donor Nephrectomy: A Randomized Controlled Study

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Version: 4 Date: 19 Nov 2019

Author’s response to reviews:

November 19th, 2019

Editorial Department of BMC Anesthesiology

Dear Editors,

Please consider our revised manuscript, entitled “Effects of Low Versus Standard Pressure Pneumoperitoneum on Renal Syndecan-1 Shedding and VEGF Receptor-2 Expression in Living-donor Nephrectomy: A Randomized Controlled Study” for publication in BMC Anesthesiology. We appreciate the interest that the editors and reviewers (Anthony Bonavia, MD; Ahmed Abdalla Mohamed, MD; and Hassan Mohamed Hassan Sayed Ahmed, MD, EDIAC, FCAI, PhD) have taken in our manuscript and the constructive reviews they have given to improve our manuscript. We have addressed the major and minor concerns of the reviewers.

We have also included a point-by-point response to the reviewers in addition to the changes described above in the manuscript. We have revised and made the discussion section more concise.

Thank you again for consideration of our revised manuscript.
Yours Sincerely,
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POINTS OF REVISION:

Editor’s comment:

As pointed by Reviewer 3 in the previous round of revisions and now endorsed by Reviewer 2, the discussion session needs attention. We kindly ask you to carefully revise the discussion session taking into account Reviewer 2’s comments.

Clarification:

Thank you for your kind reminder. We would take in the reviewer’s comments especially regarding the discussion session

Reviewer #1
Anthony Bonavia, MD

The authors are to be commended on significant improvement in the manuscript compared to last submission, and for their receptiveness to reviewer feedback in their modifications. However, at this time the biggest weakness of the manuscript which precludes publication, in this reviewer's opinion is the Discussion section.

See comments below Major comments:

The DISCUSSION section is too long and wordy, which significantly detracts from the main message of the paper. It should not be a comprehensive literature review, but discussion of the clinically-relevant results backed up by data. I think it could easily be cut in half the length. Things like the molecular details of potential signalling pathways are not of major interest to readers of a clinically-oriented paper.

Clarification:
Thank you for your comment, we have revised and made the discussion section more concise. Hopefully it’ll meet your expectation:

- We have reduced the discussion:
  - from 2569 words (47.74%) to 1953 (39.84%) words, from total of 4902 words in the manuscript
  - from 10 pages (16 paragraphs) to 7 pages (10 paragraphs)

In Background section, we revised:

- (Page 5 line 105–106) “Laparoscopic nephrectomy is a less-invasive technique…”
- (Page 5 line 112–113) “… increased IAP is frequently present in Surgical or critically ill patients and becomes an independent predictor of morbidity and mortality ” was deleted
- (3rd paragraph was incorporated to 2nd paragraph, page 6 line 135–142) “A prospective clinical study of living transperitoneal laparoscopic donor nephrectomy with 12 mmHg IAP showed … play an important role in acute kidney injury (AKI).”
- (Page 6 line 133) “…patients with acute heart failure and in cardiac surgery.”
- (5th paragraph was incorporated to 3rd paragraph, page 6 line 138–142) “Overstimulation of VEGF -VEGFR-2 induces renal tubulointerstitial injury through altered endothelial proliferation…since serum blood urea nitrogen (BUN), creatinine, and urine output are delayed signs of deteriorating kidney function.”

In Methods section, we revised:

- (Page 8, line 193) “anterior QLB or QLB3” was deleted and revised it to “quadratus lumborum block”
- (Page 8, line 194) We moved QLB procedure to Additional File 1
- (Page 9, line 202–203) Additional files order from additional file 1 to additional file 2 and additional file 2 to additional file 3
- (Page 10, line 247–248) “The semiquantitative analysis of syndecan-1 expressions in the proximal and distal tubular epithelial cells was performed using HER-2 score and H-Score.”
This scoring was converted into percentages and entered into the histological score (H-score) formula; 

\[ H\text{-score} = [3 \times \text{strong intensity cell percentage (3+)}] + [2 \times \text{medium intensity cell percentage} \ldots \] 

The resulting value equates to between 0-300.11

In Discussion section, to be more clinically-relevant and concise, we revised:

- “The increased intraabdominal pressure causes … and can lead to renal cortical vasoconstriction and its sequelae.15,16,17” (Page 21, 4th paragraph, line 487–489 to page 17, line 379–382, 1st paragraph)

- (Page 20, 3rd paragraph, line 475–477, line 478–480 were combined to page 17, 1st paragraph, line 383–386) “Research on animals showed … the release of inflammatory cytokines, neutrophil migration, and renal cell apoptosis in the outer medulla and cortex.7,15”

- (Page 20 line 473–480 to page 17, 1st paragraph, line 386–389) “In humans, increased intra-abdominal pressure caused hypoperfusion … and even a slight increased intraabdominal pressure of 10 mmHg has shown to affect the kidney.3,17”

- (Page 19 line 449–455 to page 17 line 389–395) “While several studies have demonstrated the negative effects of positive-pressure pneumoperitoneum … has a significant clinical impact on high-risk patients including the elderly population, cardiac dysfunction patients or critically ill patients.6,19”

- (Page 21, 4th paragraph, line 490–497 incorporated to page 18, 3rd paragraph, line 416–421) “The renal perfusion is affected by the blood flow and pressure on blood vessels … and increase the expression of endothelial adhesion molecules and their interactions with neutrophils and monocytes.23–27”

- (Page 18, line 422–423) “Pneumoperitoneum insufflation using the high solubility of CO2 gas which is easily absorbable by tissue, resulting in sympathetic stimulation such as tachycardia.”

- (Page 22 line 523–526 was corrected to page 19 line 436–437) “… excretion and urine neutrophil gelatinase-associated lipocalin (NGAL) after donor nephrectomy.”

- (Page 22 line 530–533 was corrected to page 19 line 441–444) “As urine output and serum creatinine were within the normal limit before and after the procedure, our results suggest … due to increased endogenous production and not because of decreased renal excretion.”

- (Page 23, 8th paragraph, line 554–564 was incorporated to page 20, 5th paragraph, line 459–463) “In response to a mild inflammatory condition, … therefore it could become an early sign of renal tubular injury and repair.36”
This result was similar to the study of Adepu and colleagues, ..., was partly derived from an extravascular source such as the renal tubular epithelial cells.34

Our findings showed contradictory results to a previous animal study that showed the presence of syndecan-1 protein in the glomerulus and peritubular capillaries.37

From our observation, tubular epithelial cell VEGFR-2 expression was higher in the standard than in the low pressure group ... the extracellular matrix and renal tubules.

In a normal human kidney, VEGFR-2 is expressed on glomerular and peritubular capillaries endothelial cells, as well as tubular epithelial cells at a low degree.39,40

When inflammation occurs, IL-6 and activated syndecan-1 in the endothelial cells stimulate the synthesis of VEGF-A molecules and its binding to its regulator VEGFR-2 on the endothelial surface.

Increasing VEGFR-2 expression on ischemic tubular epithelial cells to the adjacent endothelial cells ... the extent of inflammation and results in an increased endothelial permeability.37,43

...resulting in a lower degree of ischemia, less tissue inflammation, reduced endothelial and tubular epithelial cell injury.

Plasma syndecan-1 level is hypothesized to correlate ... increasing plasma syndecan-1 and sVEGFR-2 levels, rather than plasma creatinine, BUN or urine output, can be interpreted as an early warning of the underlying injury.

...was increased during pneumoperitoneum but not significantly ...that may be due to the short length of the pneumoperitoneum duration during laparoscopy procedure.

The use of low pressure pneumoperitoneum may have attenuated these systemic and vascular inflammatory responses.

We have deleted several parts of the discussion for conciseness purpose such as:
The DISCUSSION lacks supporting references in several places.

Examples include (but are not limited to) the following:

- "In humans, increased intra-abdominal pressure caused hypoperfusion in the abdominal or splanchnic regions with or without hypotension." - need reference

Clarification:

We add the references:


- "Advanced venous congestion and decreased renal blood flow leads to tissue hypoperfusion or ischemia that triggers an inflammatory response" - need reference

Clarification:

We add the references:

"A laparoscopic cholecystectomy study performed with low and standard pressures showed no differences in the increase of IL-6, IL-8, and IL-10." - Need reference

Clarification:

We add the references:


- "In early renal injury, tubular epithelial cells increase syndecan-1 regulation to repair injured cells." - needs reference

Clarification:

We put the references:


Minor comments:

In METHODS:

-Patient Enrollment is misspelled in the manuscript

Clarification:
We have revised it as suggested (Page 7, line 165 & 166)

- Remove URL on line 20 of page 8.

Clarification:

We have removed it as suggested (Page 7, line 173)

- Either include full protocol in methods or in supplementary material section, depending on journal requirements

Clarification:

We moved the QLB procedure description to supplementary material as additional file 1, the methods section became 1365 words of 4902 total words in manuscript (from 1520 words previously)

- "all patients underwent electrocardiography and monitoring of heart rate" should be changed to "all patients were continuously monitored by bedside telemetry"

Clarification:

We have revised it as suggested (Page 8, line 181)

- spell out abbreviations such as i.v. and QLB

Clarification:

We have revised it as suggested (Page 8, line 185, 186, 190, 193 and page 18 line 405)

In DISCUSSION:

- "Unfavorable consequences are not expected" - unfavorable should be changed to "adverse"

Clarification:

We have revised it as suggested (Page 17, line 392)

- "We did not expect any ….CO2 outcomes" should be deleted. It is irrelevant here.
Clarification:

We have deleted it as suggested (Page 17, line 400)

- "the heart rate in the low pressure group trended significantly lower" - "trended" should be deleted - it was significantly lower

Clarification:

We have revised it as suggested (Page 17, line 401)

-"One effect of low pressure pneumoperitoneum was reduced postoperative pain due to lower visceral pain" - you cannot determine causation here. "Due to" should be changed to "may have been due to"

Clarification:

We have revised it as suggested (Page 18, line 403)

-"We assumed the higher heart rate in the standard pressure group was a response to the higher inflammatory response due to higher pneumoperitoneum" - you cannot assume this. You can only hypothesize

Clarification:

We have revised it as suggested (Page 18, line 408–409)