Peer Review File

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Reviewer A

Comment 1: First I want to congratulate you for your interesting job. I would like to know which kind of ureteral anastomosis do you use.

Reply 1: We routinely performed anti-refluxing extravesical ureteroneocystostomy using Lich-Gregoir technique. The ureter was anastomosed to the bladder mucosa using absorbable sutures, maxon 4-0. The seromuscular layer was closed over the ureter creating the tunnel with chromic 3-0. Ureteral stent was placed for 7 days and Foley catheter was placed for 14 days.

Changes in the text: We added the detail about operative technique used in our study in the Methods part "All patients were performed antirefluxing extravesical ureteroneocystostomy using Lich-Gregoir technique. The ureter was anastomosed to the bladder mucosa using maxon 4-0. The seromuscular layer was closed over the ureter creating the tunnel with chromic 3-0. Foley catheter was placed for 7 days and ureteral stent was placed for 14 days.". (see Page 7, line 145)

Comment 2: How long does it takes the ICG analysis?

Reply 2: Ten minutes after vascular anastomosis was completed, a single dose of ICG was injected intravenously. Fluorescent videography was then used to evaluate ureter perfusion. Digital photographs and video recordings of the area were captured immediately after ICG injection until 5 min after ICG injection. The picture with maximal perfusion, which was defined as the picture when the perfusion colour persisted, was used for the analysis. Quantification of perfusion was then performed on the saved images after completing the operation

Changes in the text: We clarified the steps of ICG analysis in the Methods part (see Page 6, line 135).

Comment 3: It Will be nice to see long term results of those patients

Reply 3: Thank you very much for the suggestion. We totally agree with you. Long term clinical outcome of these patients such as rate of urinary leakage is very important. Further studies evaluating clinical outcomes should be warranted. Unfortunately, this was not within

the scope of this study. We hope further studies evaluating clinical outcomes will be conducted in the near future.

Changes in the text: We have discussed about the limitation of our study in the Discussion part "Further studies evaluating clinical outcomes are therefore warranted." (see Page 12, line 268).

Reviewer B

Comment 1: Do you think this technique could applied to a cold stored or cold perfused kidney prior to implantation?

Reply 1: This is a very interesting point. However, no previous data regarding ICG evaluation during cold stored kidney was available. In our opinion, this technique may not be feasible for cold perfused kidney. According to the ICG information, the storage in the ICG solution at low temperature (4°C) inhibits decomposition which may preclude the activity of ICG.

(Reference:Alander JT, Kaartinen I, Laakso A, Pätilä T, Spillmann T, Tuchin VV, et al. A review of indocyanine green fluorescent imaging in surgery. International journal of biomedical imaging. 2012;2012:940585.)

Changes in the text: We did not discuss about this point, because the information is still lacking and perfusion evaluation for cold perfused kidney is out of the scope of our study.

Comment 2: Very nice application of fluorescence imaging and findings.

Reply 2: Thank you very much.

Changes in the text: None

Reviewer C

Comment 1: The authors investigate a very interesting topic in the context of kidney transplantation. It is supposed that ureter perfusion has a high impact on later complications such as leakage or stenosis, however intraoperative assessment methods are lacking. Therefore, this topic is highly clinical relevant.

However, I have some questions:

- The patients number is very low, so I would recommend to name the study as pilot-assessment unless the authors did not provide a power calculation

Reply 1: Thank you for your suggestion. We have added the discussion about "pilot study" in the Discussion part.

Changes in the text: We added "the number of participants was small and our study can be considered a pilot study." in the Discussion part. (see Page 12, line 263).

Comment 2: Introduction:
- The Introduction part can be shorter in my opinion. Especially lines 71-80 are not directly relevant for the study

Reply 2: We have modified the introduction to be more concise and related to the study. However, according to another reviewer's comment, we have added about the incidence of CKD.

Changes in the text: The first paragraph of introduction was summarized. "Chronic kidney disease (CKD) ranks among the most common diseases and negatively affects quality of life and life expectancy. Based on the registry from Asian-Pacific, Australian and New Zealand, Canada, Europe, Japan and United States, the prevalence of CKD has increased by 29.3% since 1990 (1). KT is the most cost-effective procedure for renal replacement therapy whichwas found to be performed around 69,400 KTs annually worldwide. Among them, 64% were from deceased donors (2). Compared with dialysis, KT reduces the risk of death by over 60%, doubles expected survival time, and greatly improves quality of life (3)." (see Page 4, line 71).

Comment 3: - Line 98: There is a new study 12/2020 demonstrating the predictive value of intraoperative perfusion on kidney function. This should be included:
o Quantitative Assessment of Intraoperative Laser Fluorescence Angiography with Indocyanine Green Predicts Early Graft Function after Kidney Transplantation. Gerken ALH, Nowak K, Meyer A, Weiss C, Krüger B, Nawroth N, Karampinis I, Heller K, Apel H, Reissfelder C, Schwenke K, Keese M, Lang W, Rother U. Ann Surg. 2020 Dec 30;Publish Ahead of Print. doi: 10.1097/SLA.0000000000004529.

Reply 3: Thank you very much.

Changes in the text: We added the detail about this study in Background part. "Furthermore, fluorescence angiography with ICG allows intraoperative quantitative assessment of delayed graft function during kidney transplantation" and cited as in references number14. (see Page 5, line 97).

Comment 4: - From my point of view, the aim of investigating the safety of this method is not beyond the scope of this study. There are only 10 patients included; I would not expect relevant complication rates in this small study cohort. Therefore, this aim should be excluded.
Reply 4: You are right. The safety of ICG cannot conclude from the small number of patients. However, we aimed to report the safety of ICG in patients with ESRD, so we added more discussion about this point in Discussion part.
Changes in the text: We added more discussion about the safety in Discussion part … "No adverse events from ICG were observed in our study. However, the safety of ICG cannot be concluded because our study has small number of patients. Larger study with adverse events assessment will be required." (see Page 12, line 258).

Comment 5: Methods:
- How did you determine your dosage of ICG for ureter perfusion? Line135: this is not correct. Your dosage is higher than previously used dosages. The cited study used a dosage of 0.02 mg/kg body weight.
Reply 5: We chose the dosage based on the initial report of ICG and the leaflet. According to the initial use of ICG and the published leaflet, the ICG dosage of 0.25 mg mg/kg was recommended. However, later study reported the use of a dosage reduction to 0.02 mg ICG per kg body weight.
Changes in the text: We have revised the citation to make it correlate better. (see Page 7, line 138).

Comment 6: - Did you also think about quantifying your ureter perfusion on base on a grey scale like previously published studies did for organ parenchyma investigation? On base of this it would be possible to gain more precise insights on perfusion values and inflow characteristics.
Reply 6: Thank you very much for your comment. The use of perioperative doppler ultrasonography for ureteral perfusion evaluation is a very interesting topic. It may provide a useful and non-invasive tool which will be beneficial for intraoperative decision-making. Unfortunately, the reports about this are limited. In our opinion, I think it may be possible to use perioperative doppler ultrasonography for this indication, but further study and information will be needed such as peak flow velocity of normal ureter for the reference of poor perfusion.

Changes in the text: We have not added the discussion about this because it is considered out of our scope.

Comment 7: Discussion:
- Line 236: Again there are more studies on this topic than you cited, for example:
o Quantitative Assessment of Intraoperative Laser Fluorescence Angiography with Indocyanine Green Predicts Early Graft Function after Kidney Transplantation. Gerken ALH, Nowak K, Meyer A, Weiss C, Krüger B, Nawroth N, Karampinis I, Heller K, Apel H, Reissfelder C, Schwenke K, Keese M, Lang W, Rother U. Ann Surg. 2020 Dec 30;Publish Ahead of Print. doi: 10.1097/SLA.0000000000004529.
o Quantitative assessment of microperfusion by indocyanine green angiography in kidney transplantation resembles chronic morphological changes in kidney specimens.Rother U, Amann K, Adler W, Nawroth N, Karampinis I, Keese M, Manap S, Regus S, Meyer A, Porubsky S, Hilgers K, Krämer BK, Lang W, Nowak K, Gerken ALH. Microcirculation. 2019 Apr;26(3):e12529. doi: 10.1111/micc.12529. Epub 2019 Feb 8.
Reply 7: We added the additional studies about this topic in background and discussion part and cited the articles you have suggested.
Changes in the text: We have added the discussion according to the suggested references "Rother et al., found that fluorescence angiography reflects preexisting morphological changes of the renal cortex which may serve as a method for the assessment of microperfusion of the kidney allograft(7)." and cited as the reference number 14 and 32. (see Page 5, line 97and Page11, line 235).

Comment 8: - Figure 2: This flow chart is not very clear… What was the index test? Final diagnosis…are the n= missing?
Reply 8: We modified the flow of participants to make it clearer.
Changes in the text: Change the text in Figure2 as attached file "Figure2".

Reviewer D
Comment 1: The authors investigated the accuracy of intravenous indocyanine green fluorescence imaging in the assessment of ureter perfusion. For this, the authors conducted a prospective study in kidney transplant recipients from deceased donors, comparing the results with histologic features. This is a very interesting subject – however, some issues need to be pointed out:

Introduction:

- In the first paragraph, explain the period and the local where occurs the CKD prevalence increased.

Reply 1: We added the detail regarding the period and where the prevalence was reported.

Changes in the text: We added more explanation as, “Based on the registry from Asian-Pacific, Australian and New Zealand, Canada, Europe, Japan and United States, the prevalence of CKD has increased by 29.3% since 1990 (1, 2).”. (see Page 4, line 72).

Comment 2: Methods:

- It is essential to explain the graft implantation steps, such as pre-implant graft preparation, preservation solution, vascular and ureteral anastomoses procedures.

Reply 2: Kidney graft was prepared, preserving in Euro-Collins solution and renal vessels and ureter were identified. After that, kidney graft was perfused and vascular anastomosis was subsequently performed using external iliac artery and vein. All patients were performed antirefluxing extravesical ureteroneocystostomy using Lich-Gregoir technique. The ureter was anastomosed to the bladder mucosa using maxon 4-0. The seromuscular layer was closed over the ureter creating the tunnel with chromic 3-0. Foley catheter was placed for 7 days and ureteral stent was placed for 14 days.

Changes in the text: We have added the step of the kidney transplantation operation to the methods part. “Kidney graft was prepared, preserving in Euro-Collins solution and renal vessels and ureter were identified. After that, kidney graft was perfused and vascular anastomosis was subsequently performed using external iliac artery and vein.” and “All patients were performed antirefluxing extravesical ureteroneocystostomy using Lich-Gregoir technique. The ureter was anastomosed to the bladder mucosa using maxon 4-0. The seromuscular layer was closed over the ureter creating the tunnel with chromic 3-0. Foley catheter was placed for 7 days and ureteral stent was placed for 14 days.” (see Page 6, line 133 and Page 7, line 145).

Comment 3: Results:

- What was the average age of the donors? Since old donors have more frequent vascular alterations that favor ureteral ischemia, this information must be related in the results section.

Reply 3: Among 10 donors, 8 were male and 2 were female. Mean age of the donor was 29.2 years old, with SD of 14.46 years old.

Changes in the text: We have added “Regarding the donors, 8 were male and 2 were female. Mean age of the donor was 29.2 years old, with SD of 14.46 years old.” in results part. (see Page 8, line 184).

Comment 4: Overall, English editing throughout the whole manuscript is needed.
Reply 4: Thank you very much. We have asked Mr.Stephen Pinder who is a native British speaker and has experience in manuscript editing for ages. We also have read through the whole manuscript again and hope this should be fine with you.
Changes in the text: We did English revision for the whole manuscript. We have attached the addendum concerning Mr. Stephen Pinder in separate file.