Author’s response to reviews

Title: High-throughput sequencing analysis of intestinal flora changes in ESRD and CKD patients

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Olga C. Balafa (Reviewer 1):

1. In general - from a clinical point of view - this study found that PD patients had a "microbiota profile" much more ureamic and pro-inflammed comparing to HD patients, while HD population was nevertheless like CKD population. Two possible clinical reasons - dialysis vintage and lower albumin levels in PD patients - could explain these findings. Now, in CKD populations albumin levels are correlated with systemic inflammation and PD patients in this cohort seem "more inflamed "than HD population (ILs levels, CRP levels etc). Possible reasons for this in this specific cohort could be a) different primary renal diseases. In 24.5 % of PD patients, hypertensive nephropathy was the cause of CKD, while in HD was 0%. b) different co-morbidities eg Cardiovascular disease (there is no reference to comorbidities between groups). I suggest that the authors should compare PD -HD populations with same comorbidities and dialysis vintage in order to conclude if the type of dialysis defines the microbiota.

a) It was not correctly written in the previous manuscript, thank you for bringing it up. We have corrected “Heymann nephritis” to “Hypertensive nephropathy”. The number of Hypertensive nephropathy in CKD, HD, and PD was 10(58.8%), 27(55.1%) and 13(24.5%) was corrected in table 1 and the descriptions in the manuscript.
b) The comparison of comorbidities and dialysis vintage was carried out between PD and HD populations: Comorbidities including coronary disease and hypertension showed no difference between PD and HD populations (p=0.305 and p=0.327). No significant difference was found on distribution of NYHA classifications (P=0.117). CKD patients with PD showed a trend of longer dialysis vintage compared to those with HD (39.85±39.44 month vs. 35.35±32.33 month, p=0.532) (Table 1).

2. The discussion section seems deficient. There is no reference to relevant studies in HD and PD populations (eg Wang IK, Appl Environ Microbiol, 2012), while the authors refer to one Chinese study only (there is a more recently one published Feng Xia Li et al, Front Cell Infect Microbiol, 2019). The authors should compare and mention their findings in relation to published ones and comment on differences or similarities. Finally, they should propose specific aims in future studies.

1) The comparison of published findings was added in discussion as followed:
Microbiota dysbiosis, which was differed between modes of dialysis, was considered a main risk factor in promoting chronic systemic inflammation in CKD patients [22]. Besides dialysis modes, age and dialysis vintage also contributed to the microbiome diversity [20]. Another Chinese study found that probiotic bacteria was less frequently detected in PD patients, which may impair host intestinal barrier and increase the risk of enteric organism invasion [23]. Diabetes was thought to be the main cause of kidney disease, whereas kidney disease is an important risk factor of cardiovascular [24][25]. Studies have shown that diabetic cardiomyopathy influenced the bacterial metabolism and presented as a risk factor of cardiovascular events [26]. Considered that diabetic patients were exclusive and no significantly different was found on cardiac dysfunction, suggested that different dialysis modes were critical contributors to microbiota alterations found in our study.

2) “The influence of different primary renal diseases on microbiota alteration needs further investigation.” was added in discussion as specific aims in future studies.

3. Heymann nephritis (in table 1 and in text) is an experimental type of glomerulonephritis. Which primary renal disease do you mean?

It was not correctly written in the previous manuscript. Thank you for bringing it up. We have corrected “Heymann nephritis” to “Hypertensive nephropathy”.

4. In dialysis vintage, you write "h". What is the time unit?hours? or months?

It should be “month”. We have corrected in table 1 and the relevant description.

5. In the abstract, you should mention the number of patients included in each group.

From 2017 to 2019, a total of 166 patients from Guangzhou Red Cross Hospital were recruited and divided into four groups with 17 cases in healthy control group, 47 cases in CKD non-dialysis group, 49 cases in HD group, and 53 cases in PD group.
6. Why the authors state "in elderly" hemodialysis patients? The mean age in each group is <60 years old.

Thank you for bringing it out, the patients should be defined as “middle-aged adults” according to the criteria of WHO and we have changed to “ESRD and CKD patients”.

7. The title should change "in ESRD and CKD patients” instead of "elderly hemodialysis".

The title was changed.

Mostafa Abdelsalam (Reviewer 2):

1- Could you explain what was the cause for exclusion of diabetic patients from your study population? As we all know, the main cause of CKD is diabetes, and do you think the exclusion of such group of patients will not affect the conclusion of your study?

Studies focused on diabetic kidney disease started early and have clearly confirmed that diabetic kidney disease patients presented with a significant change in the intestinal flora intestinal. The reason we excluded diabetic patients is to try to avoid the effects of blood glucose factors on the intestinal flora, reducing the interference of different dialysis modes HD or PD on the altered intestinal flora.

2- In table 1, you did not mention if there was any difference in-between the different groups as regard inflammatory markers

Whether CRP and IL-6 can regarded as inflammatory markers was added in discussion as followed:

In our study, similar CKD and HD intestinal flora was correlated with similar expression of CRP and IL-6. However, significantly decreased diversity and altered communities of intestinal flora between PD and HD was found correlated with excessive expression of CRP and IL-6. Butyrate-producing taxa as Faecalibacterium in genera level and Bifidobacteria and Prevotella in family level were dominant genus in CT, CKD, and HD groups, while urease containing, indole- and p-cresol-forming taxa as Escherichia in genera and Enterobacteria in family level was dominated genus in PD group. Our result indicated that beneficial and harmful bacteria was imbalanced in PD patients, which was more likely to induce inflammatory response of ESRD patients. CRP and IL-6 as inflammatory markers need further analysis.

3- Heymann nephritis??

It was not correctly written in the previous manuscript. Thank you for bringing it up. We have corrected “Heymann nephritis” to “Hypertensive nephropathy”.