

Response to Reviewer #1:

1) This is a retrospective analysis of 144 patients who received WMT for various indications on a 4-year period for different indications. The authors assessed the variations of the levels of serum uric acid before and after WMT. They found a role of WMT in lowering SUA in patients with hyperuricaemia. The article is within the journal's scope and the topic is quite important. The study design is done very well.

Reply: Thank you for your positive comments.

2) However, I think that baseline disease and WMT indication could constitute a bias since they determine a profound heterogeneity of microbiota status. Authors should have studied patients' outcome in relation to their baseline disease and WMT indication, at least for those cohorts of patients with the same indication that are numerically significant (i.e. IBS, FC, UC, GERD, NAFLD).

Reply: This study focused on the effect and safety of washed microbiota transplantation (WMT) on serum uric acid (SUA) levels in patients with hyperuricaemia and people with normal uric acid levels. As you mentioned, we admitted that baseline disease and WMT indication may constitute a bias since they determine a profound heterogeneity of microbiota status. Because of the small sample size, it was difficult to study patient outcomes in relation to their baseline disease and WMT indication. Our future study will expand our sample size to improve this bias.

3) References are up to date and relevant.

Reply: We have revised the references as required.

4) I would like to recommend language revision. I would recommend typos revision as well.

We sent our revised manuscript to a professional English language editing company, American Journal Experts, to polish the manuscript further.

Response to Reviewer #2:

1) The ~10% reduction of serum uric acid levels is noted as statistically significant.

Reply: Student's t test was used for comparisons between normally distributed groups. Otherwise, the Wilcoxon signed rank test was used. The difference was defined as statistically significant when  $P < 0.05$ .

2) Further, the side effects in the reviewed studies need to be delineated. Reduction in gout attacks or improvement in renal function? What is missing is documentation that such minimal reduction in levels is clinically significant.

Reply: As shown in Table 1, most patients' baseline diseases were not gout. There were only 5 gout patients included in this study, and these patients had reduced gout attacks. Due to the small sample size, it was difficult to investigate whether WMT truly reduced gout attacks. Our future study will expand our sample size to study the effect of WMT on the reduction in gout attacks. We studied serum creatinine levels before and after WMT treatment to assess renal function. Our results suggest that WMT had no impact on serum creatinine levels (Table 5). This difference encourages us to explore the mechanism of WMT reduction of SUA.

Response to Reviewer #3:

1) How authors categorized the high and normal range of uric acid levels

Reply: Patients who received WMT treatment were divided into an HUA group and a normal UA (NUA) group based on their SUA level before treatment. The HUA group comprised patients with an SUA  $>416 \mu\text{M}$ . The NUA group comprised patients with an SUA  $\geq 202 \mu\text{M}$  and  $\leq 416 \mu\text{M}$ . These criteria are based on instructions of the uric acid assay kit used in this study.

2) “...., it is urgent for us to develop a new therapeutic approach ...” this sentence could be novel therapeutic approaches are needed in the treatment of hyperuricemia.

Reply: We have revised the sentence in the manuscript.

3) In introduction section, references for current treatment options for HU and side effects of current therapies are missing.

Reply: We have added the relevant references to the manuscript.

4) There are several spelling mistakes and spaces in the manuscript. The manuscript needs to be checked for grammatical errors.

Reply: We sent our revised manuscript to a professional English language editing company, American Journal Experts, to polish the manuscript further.

5) Some sentences “In recent years, faecal microbiota transplantation (FMT), which refers to the transplantation of the functional flora of a healthy individual into the gastrointestinal tract of a patient to build a new intestinal

microbiota to treat intestinal and extraintestinal diseases, has emerged as a treatment strategy” is very long and difficult to understand.

Reply: We have revised this sentence in the manuscript.

6) Why there is higher number of patients in NUA group than HUA in both short term and long term ? the number of patients should be equally randomized in the all the groups.

Reply: This study was a retrospective study, and a total of 144 patients who received WMT treatment from July 2016 to April 2020 in the First Affiliated Hospital of Guangdong Pharmaceutical University and had SUA data before treatment were selected. Patients who received WMT treatment were divided into an HUA group and a normal UA (NUA) group based on their SUA level before treatment. The number of patients in the HUA group was not equal to that in the NUA group. We focused on the comparison of the serum uric acid levels of each patient before and after treatment.

7) Table 3 is not necessary in the manuscript

Reply: We have deleted Table 3 in the manuscript.

8) The abstract should contain the actual number of patients in the HUA and NUA groups of the study.

Reply: We have revised the abstract in the manuscript.

9) Figure3, 4 and 8, should have clear reporting of the values of SUA before and after. As it is difficult to predict the effect response.

Reply: We added the relevant values to the annotation of respective figures.

10) These should a table for pre and post treatment in both short term and long term treatment group.

Reply: Table 3 includes relevant values of pre- and post-treatment in the short-term and long-term treatment groups.

11) Can author include SUA of mid-term effect treatment groups ?

Reply: We included SUA of mid-term effect treatment groups in Table 3 and Figures 3 and 4.

12) The actual sample size that shows the WMT effect is very small.

Reply: Because of the strict inclusion and exclusion criteria, the actual sample size that showed the WMT effect was inevitably small. Our future study will expand the sample size to further confirm this effect.

13) In discussion, authors mentioned the reson for ineffectiveness of the WMT. They should impliment this in inclusion criteria. This as a inclusion criteria will increase the WMT applicatiblity and the effect of WMT.

Reply: Because most patients did not receive intestinal flora assays, it was difficult for us to diagnose these patients because HUA results from intestinal flora imbalance. We considered a high-purine diet as an exclusion criterion, but we could not completely determine whether these patients had a high-purine diet during treatment.

14) Authors did not provided justification for reduced effect of WMT in mid

term. Why effect reduced in mid term group.

Reply: This result is an important finding, but due to the small sample size, we could not further investigate it. This finding may be related to the small number of samples included in the mid-term observation. Alternatively, WMT may have no effect on mid-term SUA levels in HUA. Follow-up studies should further expand the sample size and extend the follow-up time to clarify the mid-term and long-term effects of WMT treatment. We added these issues to the discussion section.