22/11/2018

Dear editorial team,

We appreciate the thorough work and comments you and the reviewers made in order to improve our manuscript entitled "*Five years of fecal microbiota transplantation – an update of the Israeli experience*", manuscript NO: 40145, and we are thankful for the opportunity to resubmit the manuscript for consideration for publication in the *World Journal of Gastroenterology*. In accordance with the reviewers' comments we performed a revision of the manuscript, which we feel increases the quality of the paper. All revisions are highlighted in <mark>yellow</mark> in the manuscript. Please find below our point-by-point answers and corrections according to the reviewers' comments.

Thank you very much in advance,

Dr. Sharon A. Greenberg and Dr. Nitsan Maharshak, on behalf of the authors.

Reviewer's code: 02941324

Reviewer's country: Italy

1. **ABSTRACT - Please define that LGI is represented only by colonoscopy.**
   The definition was added to the abstract and text

2. **Why did you not use enemas? -**
   We found it generally less successful, and it is not in use in Israel.

3. **INTRODUCTION - please refer to guidelines on FMT for CDI when telling that FMT is widely recognized as a treatment for recurrent CDI (e.g. Surawicz et al, AJG 2013; Debast et al - CMI 2014; Cammarota et al - Gut 2016; Mullish et al - Gut 2018; Sokol et al - DLD 2016).**
   The references were added (reference number 8-10)

4. **METHODS - What do you mean for variables? In this paragraph you talk about outcomes, please clarify.**
   This paragraph was revised and now the outcomes and variables are correctly

stated:

"The <u>primary outcome</u> was FMT success (at least 2 months free of CDI-related diarrhea post-FMT). <u>Secondary outcomes</u> included initial response to FMT (see above) and recurrence at 6 months. The <u>key variables</u> were age, Charlson comorbidity score, and the risk factors for CDI in the 3 months preceding the infection (hospitalization, exposure to antibiotics, IBD and chemotherapy)."

5. **RESULTS - please define, if possible, antibiotic classes used before CDI (e.g. b-lactamics, fluoroquinolones, etc) –**

   We do not have the records of antibiotic used before CDI. This fact was added to the limitations: *"some of the data were collected a posterior and information on laboratory findings, class of antibiotic used prior to CDI… were not available".*

6. **You describe several parameters that are correlated with FMT success or failure. I strongly suggest to make this analysis more appropriate with a multivariate analysis.**

   As suggested, multivariate analysis was carried out and it is presented in table 4. The next paragraph was added to the results: *"The multivariance analysis revealed severe disease and inpatient status as being independently inversely related to FMT success, with ORs of 0.14 (p < 0.05) and 0.19 (p < 0.05), respectively (Table 4). The Charlson score did not affect FMT success or failure".*

7. **Moreover, I suggest to compare, in the discussion, your results with those achieved by Fischer et al (AJG 2017) and Ianiro et al (Clin Microbiol Infect 2017) that identified predictors of FMT failure.**

   As suggested, the next paragraph was added to the discussion:

   "Multivariant analysis revealed that severe CDI (OR = 0.14, p < 0.05)s and inpatient FMT (OR = 0.19, p < 0.05) were each independently inversely related to FMT success, while patients' background illnesses as reflected by the Charlson comorbidity score were not associated with either success or failure of FMT. Similar results were reported by Ianiro et al. in their single-center cohort study that showed that severe CDI and inadequate bowel preparation were independent predictors of FMT failure[28], and by Fischer et al. in their multi-center study, in which predictors for FMT failure included

severe or severe-complicated CDI, inpatient status during FMT and previous CDI-related hospitalization[29]. Taken together, these data imply that the severe form of CDI is less likely to be successfully treated with FMT, and that future studies are warranted in order to find the optimal treatment. Other factors, including the patients' comorbidities as determined by the Charlson comorbidity score, did not seem to affect the FMT outcome. "

8. **Please explain which treatment the 5 patients who did not respond FMT and subsequently died were offered before death.**

   The next paragraph was added to the relevant section in the discussion:

   "while the condition of the others continued to deteriorate despite broad-spectrum antibiotic coverage treatment and intensive care unit support, including mechanical ventilation and vasopressors support".

9. **A 10% mortality is quite high for FMT. Please discuss and compare with previous cohorts in the discussion section.**

   The next paragraph was added to the relevant section in the discussion:

   "Although ~10% mortality rate is quite high for FMT, it represents the natural history of debilitated senior patients with multiple comorbidities in a long cohort rather than the FMT itself, as reported earlier[19]. Similar numbers could be found in other long follow up cohorts, such as by Brandt et al. which reported death of 7 of 77 patients in their long-term cohort (mean follow up 17 months)[12]".

10. **How many patients underwent multiple infusions, according to different routes? In two recent metanalyses (Ianiro et al- UEG Journal 2018; Quraishi et al - APT 2018) the efficacy of single and multiple infusions was stratified for different routes of delivery. Please compare your results with them.**

    Only 4 patients underwent multiple infusions: 3 had second FMT (of which 2 were via colonoscopy and 1 via capsules), and 1 patient had three FMTs (all via colonoscopy). Only 2 / 4 experienced successful FMT (1 via capsules and 1 via colonoscopy).

    The next paragraph was added to the relevant section in the discussion:

    "Multiple infusions were seldom and relatively unsuccessful in our cohort (4 patients, 50%

success rate), but the numbers are too low to arrive at any conclusions and the results cannot be compared with those of recent meta analyses which showed increased success rates with multiple FMTs [26, 27]."

Reviewer's code: 03714458

Reviewer's country: United States

1. Text edits:

   Title: Five years of fecal microbial--> Five years of fecal microbiota Abstract;
   in Israel 5 years ago--> in Israel in 2013 - capsules on ambulatory--> capsules to
   ambulatory - CDI between 2013 through 2017--> CDI from 2013 through 2017 -
   upper GI (UGI)--> upper gastrointestinal (UGI) - of the were 35 (32%) patients-
   -> There were 35 (32%) patients - rate also associated--> rate also correlated
   Key words: Do not use abbreviation Background; - Edit as ;
   nasogastric/nasojejunal tube - and it generally occurred--> and generally
   occurred - Edit as ; donors in a significantly larger number of patients with
   different disease - Therefore, our aim was to examine whether despite this
   wide range of patients and FMT dependent variables, the procedure is as
   effective in all groups of patients and whether a certain FMT route is more
   effective than others. - consider re-wording to be easier to read; for example "
   Therefore, our aim was to examine whether FMT continued to demonstrate
   efficacy despite this wider range of donors and patients, as well as FMT-
   dependent variables, and to examine the individual FMT routes for efficacy as
   well. " Results; - Edit as ; excluded due to insufficient follow-up. The median
   age of the 111 participating - 6 months since FMT initiation --> 6 months after
   FMT initiation - Edit as ; and another above 60 years of age (mean 77.1±8.9
   years, mean difference 39.8, 95% CI 35.3 - 44.3, p < 0.001) (Table 4). - Edit as ; in
   the intensive care unit - Edit as ; The other 5 showed no clinical response
   Discussions Edit as ; In this multi-center cohort study, we described the real-
   world experience of FMT procedures for CDI in a heterogeneous national
   Israeli population during the five years since the procedure has been approved.
   We examined the distribution of different techniques, routes and success rates
   in 111 FMT procedures. - Edit as ; and success rates rose to 88% at 2 months. -
   Edit as ; while Kassam et al reported a trend for higher resolution rates

through the LGI route compared with the UGI route - Edit as ; Minnesota, USA, community-acquired CDI accounted for 41% of CDI cases and was characterized by a younger population with less severe disease, which is in line with our findings 21. We found a significantly higher percentage (40%) of IBD patients among this group compared to the older group (8%). Interestingly, the waiting period between first CDI episode to undergoing FMT was longer among the younger patients compared to the older ones, possibly due to a delay in diagnosis or to a lower compliance rate to undergo FMT, as well as a lower index of suspicion among physicians caring for younger - - Edit as ; These are important for creating balanced data regarding the efficacy and safety of FMT in real life. Limitations: - Edit as ; There were several limitations in the present study. Firstly, it is retrospective in design, warranting a prospective double-blind randomized placebo-controlled study. Secondly, some of the data were collected a posterior and information on laboratory findings and Charlson scores of some of the patients (especially in the ambulatory patients) were not available.

All the above text editing suggestions were gladly accepted and corrected within the manuscript text and are marked in <mark>yellow</mark>.

2. **Any other limitations? Recommend putting power as a limitation, study population (just Israeli patients were included), etc.**

The next sentence was added to the limitations:

"Other limitations were the power of the study and the fact that the study population consisted only of Israeli patients."

3. **Make comments on if the study results here are generalizable to the whole world—would this info be applicable to other countries? - - Generalizability: (Make comments on if the study results here are applicable to other countries or generalizable to the whole world?)**

As this study encompasses data from 5 different centers which represent different geographic and demographic areas with heterogenous population of patients and stool donors, we find that this study results are generalizable for the

whole world. The according paragraph was corrected and perfected.

4. **The results of this study correlate with previous works of others as described in the literature. (Does it correlate with all previous works? If so, specifically how? And if not, how does it not correlate? This paragraph is too short and should be more explanatory).**

   This paragraph was corrected and perfected and now it is now refer to previous works and it is now more explanatory:

   "As the results of this study correlate with previous works regarding overall success rates[5, 7, 8, 12, 15-19], different routes of FMT administration [17, 19] and predictors of failure[28, 29] (see above); and reflects a multi-center data of heterogenous population from several districts in Israel and from different stool donors, this study results are generalizable for the whole world. "

5. **In conclusion, FMT is a safe and effective treatment for CDI, which has been occurring in growing numbers in both older and younger populations. While both LGI and capsule administration of FMT seem to be more efficient than the UGI endoscopic route, FMT via capsules has emerged as a successful and well-tolerated alternative. Prospective and well-powered studies are needed to conclusively determine the best route of administration. - would ((also add comments on side effects, costs, ease of administration, safety to patients, potential for insurance to cover the expense, etc**

   The remark was gladly accepted and according sentence was added to the conclusions:

   "Prospective and well-powered studies are needed to conclusively determine the best route of administration, <u>regarding patient safety, ease of administration, side effects and costs.</u>"

1. **There are more recent, systematic and meta-reviews on FMT in CDI then the ones listed as ref 7-9, please update. The same is true for references 12-14, please update, e.g. with this one: https://doi.org/10.1111/apt.13868**
   Thank you very much for your remark. Several more recent systematic and meta-reviews were added to the manuscript references: Surawicz et al, AJG 2013 [9]; Cammarota et al - Gut 2017 [8]; Mullish et al - Gut 2018 [10] ; König et al – APT 2017 [18]; Ianiro et al – UEG journal 2018 [27]; Fischer et al – AJG 2016 [29].

2. **No mention is made in this paper if frozen or fresh stool was used. Please explain if fresh and/or frozen material was used, and if data is available, consider adding it to Table 3 (Success and Failure).**
   Thank you for the remark. We added several text edits regarding the stool that was used:
   a. In the methods\donor stool preparation – a paragraph concerning the preparation of fresh and frozen stool
   b. In the results – this sentence was added: "*Frozen stool was used in 91 of all the patients in the cohort, with slightly higher success rates than those obtained by fresh stool (89% vs. 80%, p=0.272)*".
   c. As suggested, these variables were added to table 4 (formerly table 3) – success and failure.

3. **Please include in the first page of the discussion that administration of FMT by capsules also had the highest recurrence rate (although not significant).**
   The suggested sentence was added to the second paragraph of the discussion:
   "*Nevertheless, capsules-treated patients had the highest recurrence rates (22% at 6 months), again not reaching a level of statistical significance.*"

4. *Please include in the discussion that the highest AE rate in this study was in the LGI group which is different from most other reports*

As suggested, a paragraph discussing the high rate of AE in this study was added to the discussion, with reference to the :

"Although a ~10% mortality rate is quite high for FMT, it represents the natural history of weakened senior patients with multiple comorbidities in a large cohort rather than the FMT itself, as reported earlier[19, 32]. Similar numbers can be found in other long follow-up studies, such as the one by Brandt et al. which reported the demise of 7 of the 77 patients in their long-term study (mean follow-up 17 months) [12]. Most of the adverse events were mild and self-resolving, and they included abdominal discomfort, nausea, flatulence and constipation, which can be attributed to the procedure itself (i.e., most of these complications occurred in the LGI group). In addition, they are generally self-limiting and rather common post-colonoscopy events, occurring after up to 33% of colonoscopies [33]. Severe complications were recorded for 2 patients (<2% of the cohort) who were severely ill in an ICU setting and each suffered post-endoscopy aspirations."

5. **According to your data, 18 out of 20 CDI patients that also had IBD were treated with success. Please shortly discuss your data and this article that discuss the lower efficacy of FMT in clearing CDI with IBD compared to without IBD: Khoruts A, Rank KM, Newman KM, et al. Inflammatory bowel disease affects the outcome of fecal microbiota transplantation for recurrent Clostridium difficile infection. Clin Gastroenterol Hepatol 2016; 14: 1433–8.**
A short paragraph discussing these results was added to the discussion:
"Interestingly, the IBD patients in our study experienced higher success rates than reported in the literature (90% compared to 74.4% reported by Khoruts et al. [31]), although the group of IBD patients group in our study is much smaller (n=20) compared with theirs (n=272), and that might explain the difference in results."

6. **Please clarify in tables 2-5 what the percentage refers to, e.g. using footnotes. Also, what does the sigma stand for?**
The request was fulfilled – clarifications about the referral of the percentages in the tables was added as footnotes.
The term sigma was changed to p-value.

7. **That should be clarified in the methods and the table legends. If no correction for multiple testing was performed, it should be stated both in the methods and the table legends that the reported p-values are descriptive.**

   A multivariant analysis was added to the study.

8. **page 5 line 3 - Israeli instead of Isreali; page 12, line 5 - To maintain continuity with a previous report**

   The corrections were made in the text and are marked in <mark>yellow</mark>.