

Reviewer #1:

Scientific Quality: Grade D (Fair)

Language Quality: Grade C (A great deal of language polishing)

Conclusion: Rejection **Specific Comments to Authors:** The manuscript entitled "Effect of probiotics on hemodynamic changes and complications associated with cirrhosis: A randomized controlled trial" reports the effects of administration of a probiotic yeast, *Saccharomyces boulardii*, on clinical evaluation of patients with Child-Pugh class B and C cirrhosis. 24 patients received probiotics, while 16 patients received a placebo over the same period as the controls. The clinical evaluation results suggested that administration of *S. boulardii* was associated with a significant improvement in liver function of patients with cirrhosis. The study is interesting and may contribute to the field. However, clinical evaluations at gene levels and relevant molecular mechanisms with novelty are lacking. The manuscript is more suitable for publication in a specialized journal.

Authors' response: clinical evaluations at the genetic level was not the aim of this study and is not commonly used in research on this topic.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: Authors present a pilot trial testing the utility of probiotics in cirrhosis patients.

Major comments: Is there a preliminary data that authors have worked upon?

Authors' response: yes, this is preliminary data from our large study.

Definitions: Authors need to define clearly about what do they mean by hyperdynamic circulation?

Authors' response:

In the Introduction section: "hyperdynamic circulation indicated by increased cardiac output and decreased systemic vascular resistance [SVR)".

Unfortunately, there are no generally accepted quantitative criteria for hyperdynamic circulation.

If the patient is a presenting in the outpatient clinic in the assumption is that they are asymptomatic in which case the clinical relevance of "hyperdynamic circulation" comes into question.

Authors' response:

Outpatients with cirrhosis are not always asymptomatic. They may have mild or moderate ascites, minimal hepatic encephalopathy, and other manifestations of cirrhosis that do not require hospitalization.

Follow-up: Of frequently with the patient followed up during the 3-month study time? How many visits did these patients have? Was there any attrition in the group of patients?

Authors' response:

This has been added to the Methods section: "There were no additional visits or examinations between these two time points."

This has been added to the Results section: "All patients included in the study completed it."

Did any of the study patients need hospitalization during the study period? (related or unrelated to the study)

Authors' response:

This has been added to the Results section: "None of the patients were hospitalized between the visits."

What is the clinical equipoise? What is the significance of amelioration of hyperdynamic circulation? How is it clinically relevant? Authors should highlight the clinical relevance, just because something can be done does not mean that it needs to be done.

Authors' response:

It was added in the Result section:

"In the tested arm, an improvement in liver function (a decrease in the value of Child-Pugh score: -2 [-3-(-1)] vs. -0.5[-1-0]; p=0.042) and a decrease in the degree of ascites (-1[-1-(-1)] vs. 0[0-0]; p=0.015) was observed only in those patients (n=18) who had a decrease in cardiac output after the course of the probiotic."

Does this benefit the patients in long term? 12-month mortality difference or need for liver transplant?

Authors' response:

The assessment of the long-term prognosis was not included in the objectives of this study, but we plan to do this at the end of the year after inclusion. However, since the patients are already unblinded, this study will no longer be blinded.

Based on the consort diagram the investigators screen 198 patients and were eventually successful in enrolling 40 patients cumulatively in both the arms. The overall accrual was less than 25% which questions the overall generalizability of the study.

Authors' response:

First, since significant hemodynamic changes are observed in patients with decompensated cirrhosis, we excluded patients with compensated cirrhosis, and they accounted for about a third of screened patients. Secondly, since this is the first study on this topic, we tried to exclude all significant interfering factors, such as the use of antibiotics, probiotics, prebiotics, which can affect the gut microbiota and disrupt the natural course of the disease. We plan to include these patients in future studies to see if our results can be generalized to these patients. Thus, we believe that the results obtained are quite representative of patients with decompensated cirrhosis who have not previously taken drugs that can affect their intestinal microbiota.

Table 3 is very busy and nearly impossible to interpret. The analysis plan and presentation needs to be improved. For example If they want to present the difference (or improvement) in BMI for before and after the study the analysis should be performed using differences in proportion etc. I would strongly recommend re-analysis for the entire table 3 with appropriate approach. In addition, in table 3, the authors are presenting the unadjusted analysis for the outcomes observed in the 2 groups, this needs to be adjusted for other variables that could demonstrate a similar response. Adjust for age, disease severity etc.

Authors' response:

Table 3 was edited.

Title needs to be changes, I would recommend using "Pilot trial" to assess the safety and feasibility, based on such small numbers no inference can be drawn in terms of efficacy.

Authors' response:

The title was changed: "Effect of probiotics on hemodynamic changes and complications associated with cirrhosis: A pilot randomized controlled trial"

A lot of information presented in table 3 is statistically significant but have very limited to no clinical relevance. Authors need to have a better discussion of the results and what do they propose based on this information.

Authors' response:

The Discussion section was revised.

Was the trial registered at the Clinicaltrials.gov ?

Authors' response:

In the Methods section: "The study was approved by the Ethics Committee of Sechenov University and was registered at <https://clinicaltrials.gov> (NCT05231772)".

Re-Reviewer #1:

Major comments: Is there a preliminary data that authors have worked upon? Authors' response: yes, this is preliminary data from our large study. R2: This is not the preliminary data. Preliminary data does not start with a randomized controlled study. Preliminary data should include some in-vitro evidence that probiotics have some role in pathophysiology or evidence from observational study(s) that probiotics have shown some effect towards the beneficial outcome. Are there any data from the animal studies supporting this hypothesis?

Authors' response 2:

A small, uncontrolled study (<https://pubmed.ncbi.nlm.nih.gov/24661740/>) has been published showing that probiotics have a positive effect on hemodynamic changes in cirrhosis. This randomized controlled study has planned to prove/refute this statement.

Definitions: Authors need to define clearly about what do they mean by hyperdynamic circulation? Authors' response: In the Introduction section: "hyperdynamic circulation indicated by increased cardiac output and decreased systemic vascular resistance [SVR]". Unfortunately, there are no generally accepted quantitative criteria for hyperdynamic circulation. R2: Then how do you evaluate whether the "hyperdynamic circulation" had any benefit from the intervention?

Authors' response 2:

According to the model (DOI: 10.1080/17474124.2018.1481747) for the development of complications of cirrhosis (ascites, hepatic encephalopathy, etc.), they are based, among other things, on the development of hyperdynamic circulation, which worsened portal hypertension. In our study in the test group, along with a decrease in the signs of hyperdynamic circulation, we revealed a decrease in the severity of these complications and an improvement in liver function. Further research is needed to clarify whether there is a connection between these changes or not.

If the patient is presenting in the outpatient clinic in the assumption is that they are asymptomatic in which case the clinical relevance of "hyperdynamic circulation" comes into question. Authors' response: Outpatients with cirrhosis are not always

asymptomatic. They may have mild or moderate ascites, minimal hepatic encephalopathy, and other manifestations of cirrhosis that do not require hospitalization. R2: None of the manifestations as mentioned by the authors are included as the outcomes, none of the above-mentioned clinical parameters and findings were considered as significant outcomes by the authors.

Authors' response 2:

In our study, the outcomes included changes in the signs of hyperdynamic circulation (cardiac output and SVR), systemic inflammation (serum C-reactive protein levels), severity of ascites and hepatic encephalopathy (included minimal one), serum levels of liver biomarkers, and Child-Pugh scale scores.