Probiotics Prevent Hepatic Encephalopathy in Patients with Cirrhosis: a Randomized Controlled Trial

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‘Probiotics Prevent Hepatic Encephalopathy in Patients with Cirrhosis: a Randomized Controlled Trial’

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Abbreviations –

HE – hepatic encephalopathy

MHE – minimal hepatic encephalopathy

CFF – critical flicker frequency

GHBT – glucose hydrogen breath test

LHBT – Lactulose hydrogen breath test

SIBO – small intestinal bacterial overgrowth

OCTT – orocecal transit time

CTP – Child Turcotte Pugh

MELD – model for end stage liver disease

NCT – number connection test

FCT – figure connection test

PHES – psychometric hepatic encephalopathy score

Conflict of interest – none (nothing to disclose)

Specific author contribution –

Study concept, data collection and analysis, interpretation and critical review: Barjesh Chander Sharma and Manish Kumar Lunia
Abstract:

**Background & Aims**: Hepatic encephalopathy (HE) is associated with poor prognosis in patients with advanced liver disease. Probiotics alter the intestinal microbiota with non-urease–producing organisms that reduce production of ammonia. We investigated the efficacy of probiotics for primary prophylaxis of HE.

**Methods**: We conducted a prospective trial at a tertiary care referral institute in New Delhi, India, from January 2012 through March 2013, of patients with cirrhosis without overt HE (48.6±11.1 y old; 96 men and 64 women); 25 were Child Turcotte Pugh (CTP) class A, 51 class B, and 84 class C. Subjects were randomly assigned to groups given probiotics (1x10^8 CFU, 3 times daily; n=86, 42 with minimal HE) or no test article (control, n=74; 33 with minimal HE). All subjects underwent psychometric analyses, critical flicker fusion (CFF) threshold assessments, glucose hydrogen breath tests to identify small intestinal bacterial overgrowth (SIBO), and lactulose hydrogen breath tests (LHBT) to measure oro-cecal transit time (OCTT). The primary endpoint was development of overt HE.

**Results**: At baseline, subjects in each group had comparable CTP, model for end-stage liver disease, CFF, and psychometric hepatic encephalopathy scores and OCTT. After a mean
follow-up period of 38.6±8.80 weeks for patients given probiotics and 40.3±9.8 weeks for controls, 6 patients given probiotics and 7 controls died (P=.81). Three months of probiotics administration significantly reduced levels of arterial ammonia, SIBO, and OCTT; increased psychometric hepatic encephalopathy scores (PHES); and increased CFF thresholds, compared with baseline. Seven subjects in the probiotic group and 14 controls developed overt HE (P<.05; hazard ratio for controls vs probiotic group, 2.1; 95% confidence interval, 1.31–6.53). PHES, CTP scores, and SIBO correlated with development of overt HE.

Conclusion: In a prospective, randomized controlled trial, probiotics were found to be effective in preventing HE in patients with cirrhosis.

Trial Registration no: CTRI/2012/07/002807

KEY WORDS: cognitive and motor function, treatment, therapy, clinical trial, MHE

(www.ctri.nic.in, number - CTRI/2012/07/002807)

Introduction

Hepatic encephalopathy (HE) is a serious but potentially reversible disorder with wide spectrum of neuropsychiatric abnormalities and motor disturbances that ranges from mild alteration of cognitive and motor function to coma and death.¹ Overt forms are estimated to occur in 30% to 45% of patients with cirrhosis.²⁻⁴ Bustamante et al reported survival probability of 42% at 1 year and 23% at 3 years of follow-up with first episode of HE.⁵ Increases in frequency and severity of such episodes predict an increased risk of death.⁶ Small intestinal bacterial overgrowth (SIBO) is common in cirrhosis and associated with systemic endotoxemia.⁷⁻¹² Various studies have shown small intestine dysmotility in patients with cirrhosis.⁹⁻¹⁰ Abnormal intestinal motility may play an important role in increasing the
growth of pathogenic bacteria and increased absorption of gut toxins. There is association of SIBO and delayed oroecal transit time (OCTT) with minimal HE (MHE). We hypothesise that probiotics may replace the harmful urease producing organisms and alter these processes, thereby preventing the development of HE.

Primary treatment of HE is identification and treatment of precipitating factors. Majority of the drugs used in treatment are directed at reduction or elimination of increased neurotoxic ammonia. Treating patients to prevent development of first episode is classified as primary prophylaxis of HE and preventing recurrence as secondary prophylaxis.

Lactulose, a non absorbable disaccharide, remains the mainstay treatment for HE. Its efficacy has been proven for both primary and secondary prophylaxis. However, patients who do not have any specific symptoms of HE, to be adherent on a medication that could cause diarrhoea, bloating and flatulence is difficult.

Probiotics alter the gut flora, with non urease producing organism, resulting in decreased ammonia production. On electronic search in MEDLINE and Cochrane Library, we found studies demonstrating beneficial effects of probiotics in MHE. Probitics are effective for reversal of MHE in 35 - 60% patients and prevention of recurrence of HE. Probiotics can be used as long-term therapy with no adverse effects. Probiotics act by decreasing ammonia in portal blood, by decreasing bacterial urease activity in intestinal lumen, decreasing ammonia absorption, decreasing intestinal pH and improving nutritional status of gut epithelium resulting in decreasing intestinal permeability, and decreasing inflammation and oxidative stress in the hepatocyte leading to increased hepatic clearance of ammonia. Probiotics are effective in secondary prophylaxis of HE. However, there are
no data on efficacy of probiotics on primary prophylaxis. We hypothesize that probiotics may be effective in primary prophylaxis of HE.

Patients and Methods

This was an open labelled randomised controlled trial. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by institutional ethical committee. Study was conducted at G B Pant Hospital, New Delhi, India from January 2012 – March 2013. Randomization was performed using tables of computer generated random numbers by an independent person who was unaware of patient characteristics. All randomization numbers were concealed in separate envelopes which were sealed, opaque and serially numbered. Patients with age between 18 – 75 years with cirrhosis with no previous history of HE were included. Following patients were excluded; patients on lactulose therapy, history of recent alcohol intake (in past 4 weeks), recent infection or antibiotic use (in past 6 weeks), secondary prophylaxis for spontaneous bacterial peritonitis, recent G I bleeding, hepatocellular carcinoma, previous TIPS and shunt surgeries, use of psychotropic drugs, neurologic disease such as Alzheimer’s disease, Parkinson’s disease and non hepatic metabolic encephalopathies.

Patients in treatment group (n=86) received probiotics (including *Bifidobacterium brev*, *Bifidobacterium longum*, *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus paracasei*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus*, 110 billion CFU, available as VSL#3) one capsule 3 times a day. Control group included 74 patients. Previous treatment and prophylaxis of variceal bleed, if any (endoscopic variceal ligation or beta blocker) were continued as before. Patients were asked to avoid any commercial probiotics yogurt preparation. Laboratory investigations included complete
hemogram, coagulation profile, liver function tests, renal function tests, serum electrolytes, arterial ammonia levels and work up for etiology of liver disease. Severity of liver disease was determined by Child Turcotte Pugh (CTP) score and MELD score. Baseline investigations were repeated after 3 months of follow up. All patients were assessed by psychometric tests, critical flicker frequency (CFF), glucose hydrogen breath test (GHBT) and lactulose hydrogen breath test (LHBT). Follow up was done every month for treatment compliance and for development of any complications till six months after recruitment of last patient. Primary outcome measure was development of overt HE. Secondary outcome measure was predictors of development of HE and adverse effects of probiotics.

Psychometric testing

Psychometric hepatic encephalopathy score (PHES) was calculated to diagnose MHE. PHES includes a combination of psychometric tests including number connection test (NCT) A & B, figure connection test (FCT) part A & B (if illiterate), digit symbol test, line tracing test and serial dotting test. Test time is the time required to complete test, including time needed to correct errors. MHE was diagnosed when PHES was ≤ 5.  

Measurement of critical flicker frequency threshold

Critical flicker frequency (CFF) was done by HEPAtonorm analyzer. Flicker frequencies were measured 8 times and mean value was calculated. Measurements of CFF threshold was done by intrafoveal stimulation with a luminous diode. Decreasing the frequency of light from 60 Hz downward, CFF threshold was determined as frequency when impression of fused light turned to a flickering one. Critical flicker frequency is considered abnormal when value is <38 Hz.  

25,26
Assessment of hepatic encephalopathy

West Haven criteria were used to diagnose overt HE (grade 1-4).

Glucose hydrogen breath test

SIBO was diagnosed by early appearance of hydrogen following administration of a challenge dose of glucose. Study subjects were asked to brush their teeth and rinse their mouth with antiseptic mouthwash (chlorhexidine) before the test, to eliminate early peak in hydrogen due to action of oral bacteria on the test sugars. Test included administration of 100 grams dose of glucose dissolved in 200 ml of water. Fasting breath sample was taken 3 times to take the average. It should be less than 20 ppm (Fasting breath hydrogen >20ppm can be due to poor oral hygiene and dental carries, in that case test is repeated another day after proper brushing and chlorhexidine mouthwash). Breath sample for hydrogen was measured every 15 minutes for at least 3 hrs period of time (SC microanalyzer, Quintron Instrument, Milwaukee, WI, USA). Results were expressed in parts per million; a persistent rise in breath hydrogen > 12 ppm above the baseline value within 3 hours period was considered positive for SIBO. These values are based on normal volunteers.27 Earlier studies have shown that 84% - 91% patients with liver cirrhosis without MHE have values similar to normal volunteers.7,9

Lactulose hydrogen breath test

LHBT was done on following day to detect OCTT. 15 ml syrup containing 10 gms of lactulose was given. Breath samples were measured every 15 minutes interval (beginning 30 minutes after dose of lactulose) for first 2 hours and at 20 minutes interval after 2 hours (total 4 hours). OCTT was calculated as the time when an increase of at least 15 ppm of hydrogen in
two consecutive reading was documented (SC microanalyzer. Quintron Instrument, Milwaukee. WI, USA).

**Statistical analysis and data management**

There is no study on efficacy of probiotics in primary prophylaxis of HE. On the basis of the results of our previously published study on secondary prophylaxis, probiotics are effective in 60% patients. We presumed the efficacy of 20% in placebo group and taking into account 20% of the patients will be lost to follow up, we calculated that a sample size of 75 patients in each group would be required to detect a difference in prophylaxis of HE with a 5% type 1 error and 90% power for a 2 tailed log rank test. Data were expressed as absolute number and percentage for categorical variables and mean +/- SD for continuous variables. Chi square test and fisher’s exact test were used for comparison of categorical variables. For continuous variables, Mann-Whitney test for unpaired and Wilcoxon rank sum test for paired data were used as appropriate. Multiple Cox regression analysis was performed to model the simultaneous effects of covariates and possible interactions. Kaplan-Meier method was used to probability for development of overt HE. We performed per protocol analysis (PPA) and two different intent-to-treat (ITT) analyses; in one, it was assumed that all of the missing patients developed HE and in another, it was assumed that only missing patients in probiotics group developed HE. Probability level of P<0.05 was set for statistical significance. Statistical analysis was performed with SPSS software, version 19 (SPSS inc., Chicago, IL).

**Results**
290 patients with cirrhosis were screened. 130 (44.8%) were excluded due to following reasons; recent alcohol intake (n=20), history of overt HE (n=40), lactulose therapy (n=25), antibiotic use in past 6 weeks (n=20), hepatocellular carcinoma (n=3), spontaneous shunt (n=3), significant systemic illness (n=19). 160 patients (mean age 48.6 ± 11.1 years, M:F, 96:64) who met the eligibility criteria were included in the study and randomised in two groups, Group 1 (probiotics) – 86 patients, Group 2 (control) – 74 patients. Etiology of cirrhosis was alcohol in 82 (51.3%) patients, hepatitis B in 31 (19.4%) patients, hepatitis C in 11 (6.9%) patients, cryptogenic cirrhosis in 28 (17.5%) patients and other causes in 8 (5%) patients (autoimmune hepatitis in 4; primary biliary cirrhosis in 3, Wilson disease 1 patient). 25 (15.6%), 51 (31.9%) and 84 (52.5%) were in CTP class A, B and C respectively. Mean CTP score was 9.74 ± 2.63 and MELD score was 19.32 ± 5.91. There was no difference in distribution of age, sex and etiology in two groups (Table 1). Baseline laboratory parameters, CTP score, MELD score, CFF and PHES were also comparable (Table 1). 42(48.8%) in Group 1 and 33 (44.6%) in Group 2 had MHE (p=0.76) (Table 1).

11/160(6.9%) patients were lost during follow up at median follow up of 2 months (range 1-5). Mean follow up of Group 1 patients was 38.6 ± 8.80 weeks and Group 2 patients was 40.3 ± 9.8 weeks (p=0.67).

After 3 months of follow up, there was significant decrease in number of patients with SIBO and MHE in Group 1 as compared to Group 2. Also, there was significant improvement in arterial ammonia, OCTT, PHES and CFF in Group 1 as compared to Group 2 (Table2).

Development of hepatic encephalopathy -
21/149 (14.1%) patients developed overt HE. According to per protocol analysis, 7/80 [8.8%, Child’s A:B:C, 1:2:4] patients in Group 1 (grade 2:3:4, n=2,3,2 respectively) and 14/69 [20.3%, Child’s A:B:C, 2:6:6] in Group 2 (grade 2:3:4, n=5,6,3 respectively) developed overt HE (p<0.05). On Intention to treat analysis, significantly lower number of patients developed HE in Group 1 as compared to Group 2 (13/86 (15.1%) vs 19/74 (25.7%), p 0.04). On worst case scenario analysis from treatment perspective (all the dropout in Group 1 considered to have had HE but none of the dropout in Group 2), there was no difference in number of patients developing HE in Group 1 and Group 2 (13/84 (15.1%) vs 14/74 (18.9%), p NS). On Kaplan Meier analysis, probability of developing HE in Group 1 was lower than in Group 2 (Figure 2). Hazard ratio for development of HE in no treatment group as compared to probiotics group was 2.1 (95% CI, 1.31-6.53). Significantly higher patients in Child’s B (n=6, 13%) and C (n=13, 16.5%) developed overt HE compared to child class A (Child’s B vs Child’s A, p<0.05, Child’s C vs Child’s A, p<0.01). However, there was no difference in occurrence of HE in Child’s B vs Child C (p=0.36). Median hospital stay for HE in Group 1 was 6 days (range 4-13) while in Group 2 was 7 days (range 5-13) (p=0.26).

In patients with MHE, the absolute risk reduction (ARR) was 23.8% (95% CI, 5.4 – 42.2%) and number needed to treat (NNT) was 4.2 (95% CI, 2.4-18.4). That means, 4 patients of cirrhosis with MHE need to be treated to prevent one episode of overt HE. However, in patients without MHE, ARR was 7.8% (95% CI, 2.2-11.4%) and NNT was 12.8 (95% CI, 11.2-26.4).

Correlation of development of overt hepatic encephalopathy to baseline parameters –

Of 21 patients who developed HE, 15 (71.4%) (4 in Group 1 and 11 in Group 2) had MHE at baseline. On univariate analysis, development of overt HE was associated with presence of
MHE, CTP score, MELD score, CFF, SIBO and prolonged OCTT at baseline. On multivariate analysis, MHE, CTP score and SIBO were found to be significant (Table 4).

No adverse effect noted with probiotics supplementation.

Mortality -

6 (7.5%) patients in Group 1 and 7 (10.1%) in Group 2 died during follow up. Causes of death were acute variceal bleed (n=3), severe infection with sepsis (n=6), hepatorenal syndrome (n=3) and intracranial bleed (n=1). There was no significant difference in age (43.1 ± 13.2 vs 44.5 ± 16.8, p = 0.82), MELD score (20.6 ±5.4 vs 19.8 ± 5.9, p = 0.76), CFF (38.7 ± 7.9 vs 35.8 ± 9.2, p = 0.87) and PHES at baseline (-3.3 ± 2.7 vs -3.1 ± 2.9, p = 0.63) between patients who died versus others during follow up. However, CTP score was higher in patients who died than others (9.3 ± 3.5 vs 7.8 ± 3.7, p = 0.02).

Discussion –

We found that that incidence of overt HE was lower in patients treated with probiotics as compared to patients not treated with probiotics. 21 (14.1%) patients developed an episode of HE over a median follow up of 39.3 ± 9.3 weeks (7 patients in Group 1 and 14 patients in Group 2). Agrawal et al demonstrated the beneficial effect of probiotics on reversal of MHE.\(^{14}\) On multivariate analysis, abnormal psychometric tests were associated with development of HE.\(^{14}\) Lactulose and probiotics were equally effective in secondary prophylaxis of overt HE.\(^{14}\) Bajaj et al demonstrated significant MHE reversal and excellent adherence after probiotic yogurt supplementation.\(^{16}\) None of the patients in our study were using any commercial probiotics yogurt. Mittal et reported reversal of MHE in 35% patients with probiotics.\(^{17}\) Liu et al also reported decrease in arterial ammonia level, endotoxemia and reversal of MHE with synbiotics.\(^{19}\) Malaguarnera et al also found improvement in
neuropsychological tests after treatment with probiotics.\textsuperscript{20, 21} Two metaanalysis also supported treatment of MHE with probiotics.\textsuperscript{22, 23} In present study, of 21 patients who developed HE, 15 (71.4\%) (Group 1, n=4, Group 2, n=11) had MHE at baseline. On multivariate analysis, MHE, CTP score and SIBO were significantly associated with development of HE. In a study on secondary prophylaxis of HE with lactulose, recurrence of HE was associated with two or more abnormal psychometric tests and arterial ammonia level.\textsuperscript{14} Romero-Gomez et al reported that CFF together with CTP was independently associated with development of HE.\textsuperscript{25} We found that treatment with probiotics were associated with improvement in PHES, CFF and reversal of MHE. There was significant reduction in SIBO, OCTT and arterial ammonia. Modulation of gut flora with probiotics leads to these favourable effects. This is the first study to demonstrate effect of probiotic on SIBO. In subgroup analysis in patients with MHE, ARR was 23.8\% and NNT was 4.2. That means, 4 patients of cirrhosis with MHE need to be treated to prevent one episode of overt HE. We have recently reported that lactulose is effective in primary prophylaxis of HE.\textsuperscript{13} On treatment with lactulose, ARR was 21.7\% with NNT was 4.6 which is comparable with the results of present study with probiotics. However, lactulose was associated with significant side effects [diarrhea (24\%), abdominal bloating 8\% and distaste (20\%)] requiring dose reduction. In contrast, treatment with probiotics was not associated with adverse effects. In our earlier study on secondary prophylaxis of HE with probiotics, episodes of HE occurred in 34\% cases over follow up period of 12 months compared to 13.2\% in present study on primary prophylaxis.\textsuperscript{14} This difference could be due to short follow up in present study and difference in relative risk of development of recurrence and first episode of HE.

In this study, frequency of precipitating factors for overt HE were similar in both the groups. There was no significant difference in incidence of infection.
Present study has some limitations. The investigator evaluating development of HE was not blinded to treatment allocation. Therefore treatment bias could not be completely excluded. However effect of such bias would be very small because of objective nature of other parameters evaluated such as arterial ammonia, SIBO, OCTT, PHES and CFF. Patients with HE have abnormal arterial ammonia, SIBO, PHES and CFF. Abnormalities of arterial ammonia levels, psychometric tests and CFF are associated with development of HE on follow up. Patients demonstrating reversal of MHE, abnormal arterial ammonia levels and abnormal CFF value after 3 months of treatment do not develop overt HE on follow up. We did not assess health related quality of life. However, earlier studies with probiotics have shown to improve quality of life. This study showed difference in development of overt HE on per protocol analysis and intention to treat analysis. However, in worst case scenario analysis, there was no difference. Probiotics supplementation was not associated with any side effects and none of the patients required discontinuation of therapy.

In conclusion, MHE, CTP score, SIBO are associated with development of overt HE. Probiotics are effective in primary prophylaxis of HE.

*All authors had access to the study data and had reviewed and approved the final manuscript.

Figure legends –

Figure 1 - Consort flow chart for the study

Figure 2 - Probability of developing hepatic encephalopathy in patients receiving probiotics (dotted line) and control group (continuous line)
References


Table 1

Demographic and laboratory parameters of patients in two groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (n=86)</th>
<th>Group 2 (n=74)</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td>Age (years)</td>
<td>48.5 ± 10.5</td>
<td>49.4 ± 11.5</td>
<td>0.89</td>
</tr>
<tr>
<td>Male:Female</td>
<td>52:34</td>
<td>44:30</td>
<td>0.35</td>
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<tr>
<td>Etiology n (%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td>42 (48.8)</td>
<td>40 (54.1)</td>
<td>NS</td>
</tr>
<tr>
<td>HBV</td>
<td>18 (20.9)</td>
<td>13 (17.6)</td>
<td></td>
</tr>
<tr>
<td>HCV</td>
<td>6 (7)</td>
<td>5 (6.8)</td>
<td></td>
</tr>
<tr>
<td>Cryptogenic</td>
<td>15 (17.4)</td>
<td>13 (17.6)</td>
<td></td>
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<tr>
<td>Others</td>
<td>5 (5.8)</td>
<td>3 (4.1)</td>
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</tr>
<tr>
<td>CTP class, A:B:C</td>
<td>16:26:44</td>
<td>9:25:40</td>
<td>0.76</td>
</tr>
<tr>
<td>CTP score</td>
<td>9.78 ± 2.53</td>
<td>9.68 ± 3.16</td>
<td>0.78</td>
</tr>
<tr>
<td>MELD score</td>
<td>19.85 ± 5.18</td>
<td>18.94 ± 6.24</td>
<td>0.80</td>
</tr>
<tr>
<td>Bilirubin (mg%)</td>
<td>2.3 ± 1.8</td>
<td>2.6 ± 1.7</td>
<td>0.63</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>59.3 ± 28.4</td>
<td>52.8 ± 25.9</td>
<td>0.56</td>
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<tr>
<td></td>
<td>Mean ± SD 1</td>
<td>Mean ± SD 2</td>
<td>p-value</td>
</tr>
<tr>
<td>----------------</td>
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<td>---------</td>
</tr>
<tr>
<td>ALT (IL/L)</td>
<td>61.9 ± 31.2</td>
<td>56.2 ± 27.8</td>
<td>0.72</td>
</tr>
<tr>
<td>Na (mmol/L)</td>
<td>132.7 ± 4.2</td>
<td>134.9 ± 3.7</td>
<td>0.51</td>
</tr>
<tr>
<td>Art ammonia (µmol/L)</td>
<td>74.3 ± 18.6</td>
<td>78.4 ± 15.6</td>
<td>0.41</td>
</tr>
<tr>
<td>CFF (Hz)</td>
<td>40.4 ± 8.8</td>
<td>41.8 ± 9.9</td>
<td>0.88</td>
</tr>
<tr>
<td>PHES</td>
<td>-5.4 ± 3.6</td>
<td>-5.6 ± 3.5</td>
<td>0.55</td>
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<tr>
<td>MHE, n (%)</td>
<td>42 (48.8)</td>
<td>33 (44.6)</td>
<td>0.76</td>
</tr>
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</table>

Table 2 – Changes in parameters at 3 months

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
<th>Group 1</th>
<th>Group 2</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (n=86)</td>
<td>3 months (n=76)</td>
<td></td>
<td>Baseline (n=74)</td>
<td>3 months (n=62)</td>
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<tr>
<td>Art ammonia (µmol/L)</td>
<td>74.3 ± 18.6</td>
<td>61.2 ±15.2</td>
<td>0.04</td>
<td>78.4 ± 15.6</td>
<td>81.3 ± 17.8</td>
<td>0.88</td>
</tr>
<tr>
<td>SIBO, n (%)</td>
<td>33 (38.4)</td>
<td>14 (17.7)</td>
<td>0.006</td>
<td>26 (35.1)</td>
<td>21 (33.9)</td>
<td>0.91</td>
</tr>
<tr>
<td>OCTT (min)</td>
<td>138.6 ±22.9</td>
<td>112.3 ±18.8</td>
<td>0.05</td>
<td>145.6 ±21.2</td>
<td>141.7 ±21.4</td>
<td>0.85</td>
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<tr>
<td>PHES</td>
<td>-5.6 ± 3.6</td>
<td>-2.2 ± 1.9</td>
<td>0.01</td>
<td>-5.3 ± 3.1</td>
<td>-5.1 ± 2.9</td>
<td>0.76</td>
</tr>
<tr>
<td>CFF (Hz)</td>
<td>40.4 ± 8.8</td>
<td>49.9 ± 10.4</td>
<td>0.02</td>
<td>41.8 ±9.9</td>
<td>39.2 ± 13.1</td>
<td>0.62</td>
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<tr>
<td>MHE, n (%)</td>
<td>42 (48.8)</td>
<td>18 (22.8)</td>
<td>0.001</td>
<td>33 (44.6)</td>
<td>25 (40.3)</td>
<td>0.74</td>
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</table>

Art: arterial, SIBO: small intestinal bacterial overgrowth, OCTT: orocecal transit time, PHES: psychometric hepatic encephalopathy score, CFF: critical flicker frequency, MHE: minimal hepatic encephalopathy
Table 3 – Precipitating factor for overt hepatic encephalopathy in each group

<table>
<thead>
<tr>
<th>Precipitating factors</th>
<th>Group 1</th>
<th>Group 2</th>
<th>P</th>
</tr>
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<tr>
<td>Variceal bleed</td>
<td>2</td>
<td>3</td>
<td>0.67</td>
</tr>
<tr>
<td>SBP</td>
<td>1</td>
<td>4</td>
<td>0.21</td>
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<tr>
<td>Constipation</td>
<td>3</td>
<td>3</td>
<td>0.91</td>
</tr>
<tr>
<td>UTI</td>
<td>1</td>
<td>2</td>
<td>0.78</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0</td>
<td>2</td>
<td>0.88</td>
</tr>
</tbody>
</table>

SBP: spontaneous bacterial peritonitis, UTI: urinary tract infection

Table 4 – Multivariate analysis for development of hepatic encephalopathy

<table>
<thead>
<tr>
<th>Parameter</th>
<th>aOR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHE</td>
<td>3.1</td>
<td>1.32 – 13.21</td>
<td>0.016</td>
</tr>
<tr>
<td>CTP score</td>
<td>1.6</td>
<td>1.05-2.03</td>
<td>0.029</td>
</tr>
<tr>
<td>SIBO</td>
<td>2.1</td>
<td>1.11-7.45</td>
<td>0.012</td>
</tr>
<tr>
<td>OCTT</td>
<td>1.01</td>
<td>0.72-1.12</td>
<td>0.221</td>
</tr>
<tr>
<td>CFF</td>
<td>1.44</td>
<td>0.67-1.28</td>
<td>0.541</td>
</tr>
</tbody>
</table>

aOR: adjusted odds ratio, MHE: minimal hepatic encephalopathy, CTP: Child Turcotte Pugh score, MELD: model for end stage liver disease, SIBO: small intestinal bacterial overgrowth, OCTT: orocecal transit time, CFF: critical flicker frequency
Figure 1 - Consort flow chart for the study

290 patients with cirrhosis screened

Excluded (n=130)

Recent alcohol intake (n=20)
H/o overt HE (n=40)
Lactulose therapy (n=25)
Recent antibiotic use (n=20)
Hepatocellular carcinoma (n=3)
Spontaneous shunt (n=3)
Significant systemic illness (n=19)

160 patients randomized

Group 1 (Probiotics) n=86
Lost to follow up (n=6)
Patients analysed (n=80)
Overt HE (n=7)
Death (n=6)

Group 2 (Control) n=74
Lost to follow up (n=5)
Patients analysed
Overt HE (n=14)
Death (n=7)
Figure 2 - Probability of developing hepatic encephalopathy in patients receiving probiotics (dotted line) and control group (continuous line)