ORIGINAL ARTICLE

Multistrain probiotic and lactulose in the treatment of minimal hepatic encephalopathy

Ahmad Shavakhi, Huriyeh Hashemi, Elham Tabesh, Zhaleh Derakhshan, Somaye Farzamnia, Shirin Meshkinfar, Sara Shavakhi[1], Mohammad Minakari, Ali Gholamrezaei[1]

Department of Internal Medicine, [1]Medical Students' Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Background: Some evidence has shown benefits of probiotics in the management of minimal hepatic encephalopathy (MHE). We evaluated the efficacy of a multistrain probiotic compound, alone and in combination with lactulose, in the treatment of MHE. **Materials and Methods:** This study has two parts. First, consecutive adult patients with MHE were randomized to receive lactulose (30–60 mL/day) + probiotic (200 million colony forming units of seven bacteria species/day) (Gp-LPr) or lactulose + placebo (Gp-L). In second part, a non-randomized group of patients received probiotic alone (Gp-Pr). Medication duration was for 2 weeks and patients were followed-up for another 8 weeks. Improvement in MHE status was assessed by psychometric hepatic encephalopathy score (PHES). Development of overt encephalopathy, hospitalization, and death were considered as secondary outcomes. **Results:** Sixty patients (80% male, mean age 38.4 ± 9.6 years) completed the intervention. PHES significantly improved after medication in all the three groups (Gp-LPr: −3.8 ± 3.9 to −1.6 ± 3.0; Gp-L: −4.8 ± 4.1 to −1.6 ± 2.9; and Gp-Pr: −4.9 ± 3.7 to −2.1 ± 2.5, $P < 0.001$). After 8 weeks follow-up, improvement was maintained in Gp-LPr and Gp-Pr, but there was deterioration in those who did not receive probiotics (Gp-L: PHES score reversed to −4.8 ± 4.2). Two patients (one each in Gp-L and Gp-Pr) experienced overt encephalopathy. One patient was hospitalized due to worsening of ascites (Gp-LPr) and one due to spontaneous bacterial peritonitis (Gp-L). Side effects were mild and not significantly different among the groups. **Conclusion:** Lactulose and probiotics are effective for the treatment of MHE; however, probiotics, but not lactulose, have long-term effects. More studies are required before suggesting probiotics for the standard treatment of MHE.

Key words: Hepatic encephalopathy, lactulose, prebiotics, probiotics, synbiotics

How to cite this article: Shavakhi A, Hashemi H, Tabesh E, Derakhshan Z, Farzamnia S, Meshkinfar S, Shavakhi S, Minakari M, Gholamrezaei A. Multistrain probiotics and lactulose in the treatment of minimal hepatic encephalopathy. J Res Med Sci 2014;19:703-8.

INTRODUCTION

Hepatic encephalopathy (HE) is a common and usually reversible neurocognitive syndrome occurring in patients with cirrhosis. It manifests as a spectrum of changes from minimal HE (MHE), which is a state of low-level cognitive dysfunction detectable in up to 70% of the patients, to an overt HE, which has the risk of cerebral edema and death.[1] Although MHE is an underdiagnosed problem, it can affect daily functioning and impair attention span and reaction time.[2] Studies have shown that MHE increases the risk of motor vehicle accidents[3] and falls.[4]

The pathogenesis of MHE is multifactorial and the exact mechanisms causing brain dysfunction are still unknown. Increased blood ammonia is present in about 90% of the patients and is considered to play a major role.[5] Ammonia is produced mainly in the gastrointestinal tract by enterocytes from glutamine and by colonic bacterial catabolism of nitrogenous sources. Then, it enters the circulation via the portal vein and interferes with brain function at different sites.[6,7] Another possible source of ammonia is the urea digested by *Helicobacter pylori* in the stomach, although the role of *H. pylori* in HE and effects of its eradication are still under investigation.[8,9]

Regarding the role of ammonia in the pathophysiology of HE, several therapeutic strategies aimed to lower plasma ammonia for improving HE. These strategies include reducing ammoniagenic substrates, inhibiting ammonia production, and increasing ammonia removal.[5,10] Besides restriction of dietary protein intake and administration of antibiotics and disaccharides (e.g. lactulose), alteration of the gut flora with probiotics/prebiotics has gained substantial attention in the management of HE.[11,12] Studies on different probiotics have shown that modification of gut flora is associated with improvement in HE by lowering blood ammonia concentrations. Possible mechanism in this regard might be favoring the gut flora to colonization of acid-resistant, non-urease producing bacteria.[11,12] However, there is still a lack of data for endorsing probiotics as

Address for correspondence: Dr. Mohammad Minakari, Department of Internal Medicine, Isfahan University of Medical Sciences, Hezar Jarib Street, Isfahan, Iran. E-mail: minakari@med.mui.ac.ir

Received: 11-03-2013; **Revised:** 19-05-2013; **Accepted:** 02-06-2013

effective therapy for MHE. Few reports are available on the efficacy of multistrain probiotics, and few studies have investigated the efficacy of probiotics in comparison/in combination with other treatments such as lactulose.[11,12] Therefore, in a clinical trial, we aimed to evaluate the efficacy of a multistrain probiotic compound, lactulose, and combination of these therapies in the management of MHE. We hypothesized that probiotic compound is as effective as lactulose in the management of MHE and combination of these therapies is more effective than each of them alone.

MATERIALS AND METHODS

Patients and settings

This study has two parts; a randomized, placebo-controlled, comparative study with two arms; and an open-label study with one arm which are described as follow. The study was conducted on adult patients with MHE who referred consecutively to the gastroenterology clinic of a university hospital in Isfahan city (Iran) between June and October 2012. Cirrhosis was diagnosed histologically (unless biopsy was contraindicated) and on clinical and radiological grounds. Diagnosis of MHE was based on the Conn's modification of the Parsons-Smith classification (grade 1 and above).[13] Patients with overt HE, known brain lesions, active gastrointestinal bleeding, active ongoing infection, renal impairment (serum creatinine >2 mg/dL), electrolyte abnormalities (serum sodium <130 or >150 meq/dL, serum potassium <3.0 or >5.5 meq/dL), and those who received HE treatments such as lactulose and antibiotics or consumed benzodiazepines, narcotics, opioids, or alcohol in the preceding 8 weeks were not included into the trial. Considering type I error (α) = 0.05, study power = 0.8, and expecting 0.8 difference in psychometric hepatic encephalopathy score (PHES) between the two groups, sample size was calculated as 20 patients in each group. The study was approved by the ethics committee of Isfahan University of Medical Sciences and informed consent was obtained from the patients or their families. Also, the study was registered at Iranian Registry of Clinical Trials (IRCT201211012417N9).

Intervention

Using a table of random numbers generated by random allocation software,[14] patients were randomized into two groups of lactulose + probiotic (Gp-LPr) and lactulose + placebo (Gp-L). Another non-randomized group of patients who received probiotic alone (Gp-Pr) were separately included for further comparisons; this group did not receive placebo of lactulose. All patients received routine treatment for cirrhosis, including diuretics, β-blockers, endoscopic treatment, and a salt-restricted diet but not protein-restricted diet in those with ascites. For Gp-LPr and Gp-L, lactulose syrup was administered as 30–60 mL/day in divided doses for a stool frequency of two to three soft defecations per day. For Gp-LPr and Gp-Pr, a multistrain probiotics compound, the Balance® (Protexin Co., Somerset, UK), was administered twice daily after meal. Balance capsules contains seven bacteria species including *Lactobacillus* strains (*L. casei, L. rhamnosus, L. acidophilus,* and *L. bulgaricus*), *Bifidobacterium* strains (*B. breve* and *B. longum*), and *Streptococcus thermophiles*. Total viable count is 1×10^8 colony forming units (CFU)/per capsule. Other Ingredients are fructo-oligosaccharides (FOS) as prebiotic, magnesium stearate, and hydroxypropyl methyl cellulose. These mentioned interventions were continued for 14 consecutive days and compliance was assessed with pill and bottle count.

Assessments

All patients were assessed by an experienced gastroenterologist throughout the study. Demographic data and disease characteristics including causes of cirrhosis were gathered from patients' documents. Education level was considered as years of being on education. Severity of cirrhosis was assessed with the Child–Pugh classification which is a scoring system widely used to determine the severity and prognosis of liver diseases, mainly cirrhosis: Grade A = 5-6, Grade B = 7-9, and Grade C = 10-15.[15]

Primary endpoint was improvement in MHE status and was assessed by applying the PHES at baseline, 14 days after starting the intervention (14th day), and then at 8 weeks follow-up (10th week). The PHES is a set of neuropsychological tests including the line tracing test, digit symbol test, serial dotting test, and the number connection test. These tests are used in the diagnosis and grading of MHE and examine visual perception, visuo-spatial orientation, visual construction, motor speed and accuracy, concentration, attention, and memory. Subjects could achieve between +6 and −18 points. The PHES has a high sensitivity and specificity to detect MHE[16,17] and overt HE[18] and is proposed by the 11th World Congress of Gastroenterology for diagnosis and monitoring of MHE.[19]

Secondary outcomes were development of overt HE, admission in hospital for any other complication of cirrhosis, or death. Participants, attending physician, and the outcome assessor were blinded to the Gp-L and Gp-LPr arms, but were aware of the Gp-Pr arm.

Statistical analyses

Data were analyzed with the SPSS software for Windows, version 16.0. Descriptive data are presented as mean ± SD or number (%). All continuous variables had normal distribution; therefore, analysis of variance (ANOVA) was applied for comparing continuous variables among the three groups and paired *t*-test was applied for evaluating

changes in each group. Chi-square test was used for comparison of categorical variables. A P value of <0.05 was considered significant in all analyses.

RESULTS

A total of 97 patients were evaluated during the study period. Twenty patients did not enter the trial according to the criteria of inclusion and eight patients were not willing to participate. After randomization, two patients from the Gp-L, four patients from the Gp-LPr, and three patients from the Gp-Pr declined to receive intervention. Finally, 60 adult patients with cirrhosis (80% male, mean age 38.4 ± 9.6 years) started the trial and completed the intervention [Figure 1]. Etiologies of cirrhosis included viral hepatitis (73.3%), autoimmune hepatitis (18.3%), and other etiologies (8.3%). Patients among the three groups were similar regarding demographic data and disease characteristics [Table 1].

The PHES score at baseline, 14th day, and 10th week are presented in Table 2. Patients among the three groups were similar regarding baseline PHES scores. At 14th day, a significant improvement in HE status was observed in all the three groups (paired t-test $P < 0.001$). During the follow-up period, one patient from each of the Gp-LPr and Gp-L was lost to follow-up. After 8 weeks follow-up, there was a deterioration in the Gp-L (−1.6 ± 2.9 to −4.8 ± 4.2), while the status of the Gp-LPr and Gp-Pr arms did not change significantly [Figure 2].

Secondary outcome variables and reported side effects are presented in Table 3. None of the patients died during the study period. Variceal bleeding plus overt HE was observed in one patient of the Gp-L and one patient of the Gp-Pr. In the Gp-LPr, one patient was hospitalized for worsening of ascites and in the Gp-L, one patient was hospitalized for spontaneous bacterial peritonitis. Common side effects included abdominal pain and bloating, but in none of the patients, these side effects resulted in discontinuing the trial. Patients among the three groups were not significantly different regarding secondary outcomes and side effects [Table 3].

DISCUSSION

Studies have shown high prevalence of small intestinal bacterial overgrowth (SIBO)[20] and also alteration in the composition of the gastrointestinal bacterial flora among

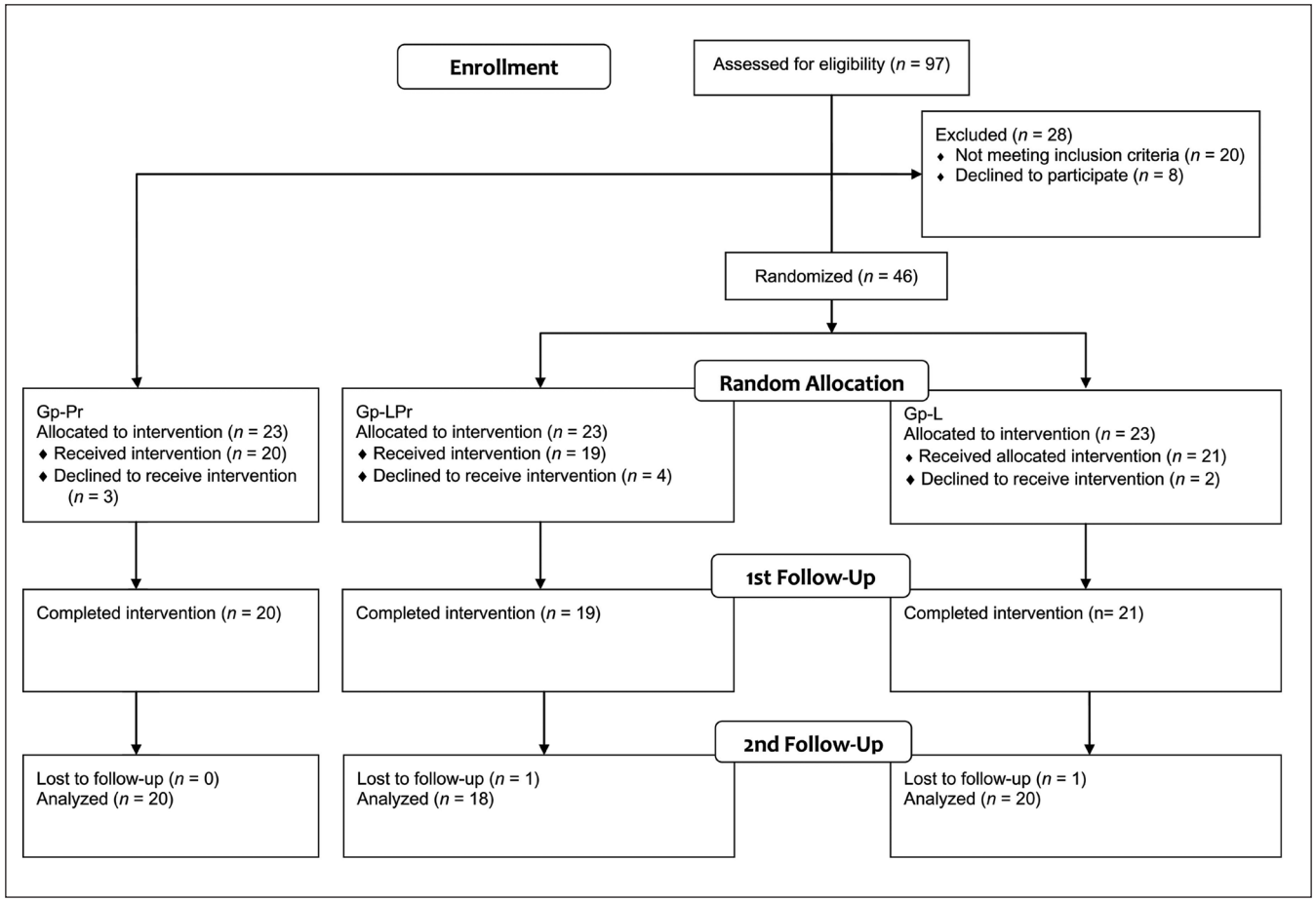

Figure 1: Study flowchart demonstrating patient flow throughout the study procedures

cirrhotic patients with MHE.[21] Probiotics are effective in the treatment of SIBO[22] and also can reduce the prevalence of harmful ammonia-producing bacteria in the gastrointestinal system[12] which provides rationale for the use of probiotics in the treatment of MHE. Our study showed that a multistrain probiotics compound containing *Lactobacillus* and *Bifidobacterium* strains and *S. thermophiles* can improve the neuropsychological status of patients with MHE.

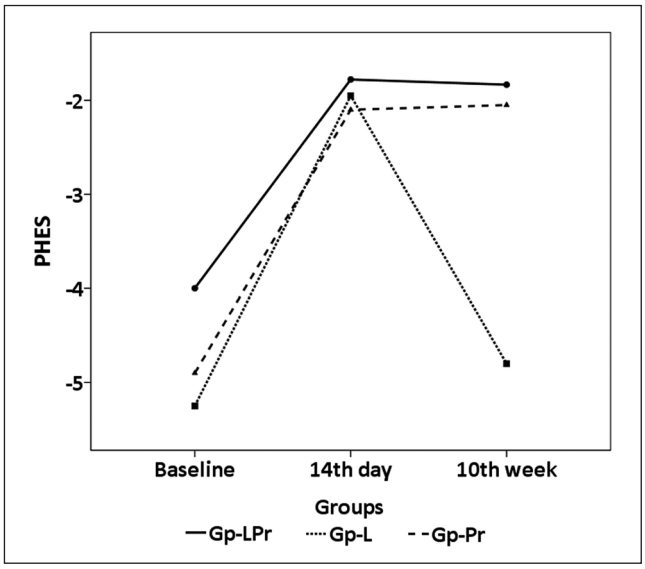

Figure 2: Trend of changes in psychometric hepatic encephalopathy scores from baseline to 10th week among the three groups. PHES, psychometric hepatic encephalopathy score

We found that combination of probiotics and lactulose does not increase the short-term efficacy of lactulose (or probiotics) while it might increase the side effects. However, administration of probiotics (alone or in combination with lactulose) was associated with maintenance of the achieved improvements. Indeed, the neuropsychological status deteriorated after discontinuing lactulose in patients who received lactulose alone. These results support the efficacy of probiotics for treatment of MHE.

Considering the role of gastrointestinal bacterial flora in the generation of ammonia and pathogenesis of MHE, several studies investigated if modulating the flora using prebiotics, probiotics, and synbiotics has beneficial effects on MHE. Lactulose is one of the most frequently used agents in the treatment of HE, and is effective as well as safe.[5] The underlying mechanisms by which lactulose improves HE is not clear. Lactulose converts to lactic and acetic acids and results in acidification of colonic contents. The low colonic pH then decreases passive non-ionic diffusion of ammonia and its systemic concentration. Also, lactulose is a kind of prebiotic that encourages the growth of beneficial bacteria (probiotics).[11] A meta-analysis by Shukla and colleagues on nine studies (with 349 patients) of prebiotics, probiotics, and synbiotics in the treatment of MHE found that administration of these agents was associated with significant improvement in MHE. This meta-analysis found lactulose as the most beneficial therapy, followed by probiotics and synbiotics. It also found that plasma

Table 1: Comparison of demographic data and disease characteristics among the three groups

	Gp-LPr (*n*=19)	Gp-L (*n*=21)	Gp-Pr (*n*=20)	*P**
Age, years	36.1±10.5	40.4±10.5	38.6±7.6	0.367*
Male/Female	14 (73.6)/5 (26.3)	18 (85.7)/3 (14.2)	16 (80)/4 (20)	0.637**
Education, years	8.3±4.9	8.1±5.4	7.4±4.7	0.837*
Cause of cirrhosis				
Viral	13 (68.4)	15 (71.4)	16 (80)	0.760**
Autoimmune	4 (21.0)	5 (23.8)	2 (10)	
Other	2 (10.5)	1 (4.7)	2 (10)	
Child-Pugh score	8.1±1.6	8.5±1.5	8.3±1.5	0.772*
Child-Pugh classification				
A	3 (15.7)	3 (14.2)	2 (10)	0.918**
B	11 (57.8)	12 (57.1)	14 (70)	
C	4 (21.0)	6 (28.5)	4 (20)	

Data are presented as mean±SD or number (%); Gp-LPr, lactulose+probiotic; Gp-L, lactulose+placebo; Gp-Pr, probiotics alone; *ANOVA test; **Chi-square test

Table 2: Comparison of psychometric hepatic encephalopathy scores among the three groups

	Gp-LPr (*n*=19)	Gp-L (*n*=21)	Gp-Pr (*n*=20)	*P**
Baseline	−3.8±3.9	−4.8±4.1	−4.9±3.7	0.640
14th day	−1.6±3.0	−1.6±2.9	−2.1±2.5	0.847
P**	<0.001	<0.001	<0.001	
	n=18	*n=20*	*n=20*	
10th week	−1.8±3.2	−4.8±4.2	−2.5±2.1	0.011
P***	0.868	<0.001	0.874	

Data are presented as mean±SD; Gp-LPr, lactulose+probiotic; Gp-L, lactulose+placebo; Gp-Pr, probiotics alone; *ANOVA; **Paired *t*-test of baseline and 14th day data; ***Paired *t*-test of 14th day, 10th week data

Table 3: Comparison of secondary outcome variables and side effects among the three groups

	Gp-LPr (*n*=19)	Gp-L (*n*=21)	Gp-Pr (*n*=20)	*P*
Mortality	0	0	0	–
Overt encephalopathy	0	1 (4.7)	1 (5)	0.619
Hospitalization due to other complications	1 (5.2)	1 (4.7)	0	0.594
Abdominal pain	2 (10.5)	1 (4.7)	0	0.320
Bloating	4 (21.0)	2 (9.5)	5 (25)	0.411

Data are presented as number (%); Gp-LPr, lactulose+probiotic; Gp-L, lactulose+placebo; Gp-Pr, probiotics alone; *Chi-square test

ammonia level is decreased by probiotics/synbiotics, though there was heterogeneity in the results.[11] However, the results of this meta-analysis are affected by limitations of the included trials. Heterogeneity was present more in the studies on probiotics than in the studies on lactulose, and trial duration was longer in trials on lactulose. On the other hand, authors found that probiotics and synbiotics are better tolerated by patients than lactulose.[11] In contrast, the meta-analysis by Holte and colleagues on seven trials (393 patients) found that in comparison with placebo or lactulose, probiotics and synbiotics significantly improve HE, though they have no effects on major clinical outcomes such as preventing the progression of MHE to overt HE.[23] Another recent meta-analysis by McGee *et al.* on seven trials (with 550 patients) found that probiotics can reduce plasma ammonia level in MHE patients (the same as lactulose), but the efficacy of probiotics in altering clinically relevant outcomes such as mortality and quality of life was not approved.[12] Differences among these studies are mainly related to the included trials. Compared with these meta-analyses, we found that probiotics are as effective as lactulose in the short-term improvement of MHE, with the beneficial effects maintained for long term (unlike lactulose). Similar to previous studies, we found no difference among lactulose, lactulose in combination with probiotics, and probiotics with regard to major clinical outcomes such as mortality, overt encephalopathy, and hospitalization. These available data are in favor of using probiotics for the treatment of MHE. However, regarding the few studies available on the efficacy of probiotics in the treatment of MHE and a considerable heterogeneity among them with regard to the type of probiotics, duration of intervention (10-180 days), and outcome assessments, a clear conclusion cannot be obtained yet.[12]

An important finding of our study is that in contrast to lactulose, improvement achieved by 14 days of administration of probiotics was stable for up to 8 weeks. This finding is similar to the study by Loguercio *et al.*, which showed that a 10-day administration of *Enterococcus* SF68 is as effective as lactulose in lowering blood ammonia, and contrary to lactulose, these effects persist after treatment withdrawal.[24] This finding highlights the modulation of gastrointestinal bacterial flora as one of the main underlying mechanisms by which probiotics can have long-term effects on MHE.[11,12]

It must be noted that the probiotics compound that we used in our study was not a pure probiotic but indeed a synbiotic compound because it also contained FOS which is a prebiotic. In two separate studies, Malaguarnera *et al.* compared *Bifidobacterium longum* + FOS and placebo[25] or lactulose[26] and found significant improvement of MHE in terms of biochemical and neuropsychological tests. Studies have shown that FOS can enhance the effects of probiotics micro-organisms in the large intestine.[27]

As with many other conditions, the probiotic dose and duration needed to confer a health benefit is unknown for MHE. Studies showed that probiotics are well tolerated for long-term administration[28,25] and it is possible to use them in the form of yogurt, which is supposed to be more tolerated by patients.[29] Thus, it is reasonable for future studies to assess the efficacy of probiotics at different dosages and with long-term administration.

There are some limitations of our study. We did not include a no-treatment or a pure placebo arm, and therefore, we cannot attribute the improvements in MHE exactly to the lactulose/probiotics. However, deterioration of MHE after discontinuing lactulose and maintenance of improvement in those who received probiotics can justify the role of treatments. Because we could not provide an appropriate placebo for lactulose, our study was not completely randomized and double blinded, which could affect our results. More importantly, our study sample size and duration were not enough to investigate clinically important outcomes such as mortality and incidence of overt HE.

CONCLUSIONS

This study supports the efficacy of multistrain probiotics in the treatment of MHE. Probiotics are as effective as lactulose in the short-term improvement of MHE. However, the achieved improvement is maintained by probiotics and not by lactulose. Further well-designed studies with larger sample of patients and longer follow-ups on different probiotic types, dosages, and durations are warranted in this regard.

ACKNOWLEDGMENTS

This study was supported by Isfahan University of Medical Sciences by the project No. 390542. We are thankful to Mojtaba Akbari (PhD, Isfahan University of Medical Sciences) who helped us in data analyses.

AUTHOR'S CONTRIBUTION

ASh, MM, and HH generated the study idea and designed the study. HH, ET, ZhD, SF, and ShM gathered data. AGh conducted data analysis. SSh and AGh wrote manuscript draft. All authors studied, revised, and approved the manuscript.

REFERENCES

1. Khungar V, Poordad F. Hepatic encephalopathy. Clin Liver Dis 2012;16:301-20.
2. Bajaj JS. Minimal hepatic encephalopathy matters in daily life. World J Gastroenterol 2008;14:3609-15.
3. Bajaj JS, Saeian K, Schubert CM, Hafeezullah M, Franco J, Varma RR, *et al.* Minimal hepatic encephalopathy is associated with motor vehicle crashes: the reality beyond the driving test. Hepatology 2009;50:1175-83.
4. Roman E, Cordoba J, Torrens M, Torras X, Villanueva C, Vargas V, *et al.* Minimal hepatic encephalopathy is associated with falls. Am J Gastroenterol 2011;106:476-82.
5. Prakash R, Mullen KD. Mechanisms, diagnosis and management of hepatic encephalopathy. Nat Rev Gastroenterol Hepatol 2010;7:515-25.
6. Plauth M, Roske AE, Romaniuk P, Roth E, Ziebig R, Lochs H. Post-feeding hyperammonaemia in patients with transjugular intrahepatic portosystemic shunt and liver cirrhosis: Role of small intestinal ammonia release and route of nutrient administration. Gut 2000;46:849-55.
7. Lemberg A, Fernandez MA. Hepatic encephalopathy, ammonia, glutamate, glutamine and oxidative stress. Ann Hepatol 2009;8:95-102.
8. Zullo A, Hassan C, Morini S. Hepatic encephalopathy and Helicobacter pylori: A critical reappraisal. J Clin Gastroenterol 2003;37:164-8.
9. Gubbins GP, Moritz TE, Marsano LS, Talwalkar R, McClain CJ, Mendenhall CL. Helicobacter pylori is a risk factor for hepatic encephalopathy in acute alcoholic hepatitis: The ammonia hypothesis revisited. The Veterans Administration Cooperative Study Group No. 275. Am J Gastroenterol 1993;88:1906-10.
10. Rose CF. Ammonia-lowering strategies for the treatment of hepatic encephalopathy. Clin Pharmacol Ther 2012;92:321-31.
11. Shukla S, Shukla A, Mehboob S, Guha S. Meta-analysis: The effects of gut flora modulation using prebiotics, probiotics and synbiotics on minimal hepatic encephalopathy. Aliment Pharmacol Ther 2011;33:662-71.
12. McGee RG, Bakens A, Wiley K, Riordan SM, Webster AC. Probiotics for patients with hepatic encephalopathy. Cochrane Database Syst Rev 2011;(11)CD008716.
13. Conn HO, Leevy CM, VlahcevicZR, Rodgers JB, Maddrey WC, Seeff L, *et al.* Comparison of lactulose and neomycin in the treatment of chronic portal-systemic encephalopathy. A double blind controlled trial. Gastroenterology 1977;72:573-83.
14. Saghaei M. Random allocation software for parallel group randomized trials. BMC Med Res Methodol 2004;4:26.
15. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophagealvarices. Br J Surg 1973;60:646-9.
16. Amodio P, Campagna F, Olianas S, Iannizzi P, Mapelli D, Penzo M, *et al.* Detection of minimal hepatic encephalopathy: normalization and optimization of the Psychometric Hepatic Encephalopathy Score. A neuropsychological and quantified EEG study. J Hepatol 2008;49:346-53.
17. Duarte-Rojo A, Estradas J, Hernandez-Ramos R, Ponce-de-Leon S, Cordoba J, Torre A. Validation of the psychometric hepatic encephalopathy score (PHES) for identifying patients with minimal hepatic encephalopathy. Dig Dis Sci 2011;56:3014-23.
18. Goldbecker A, Weissenborn K, HamidiSG, Afshar K, Rumke S, Barg-Hock H, *et al.* Comparison of the most favoured methods for the diagnosis of hepatic encephalopathy in liver transplantation candidates. Gut 2013 [In Press].
19. Ferenci P, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy--definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. Hepatology 2002;35:716-21.
20. Gupta A, Dhiman RK, Kumari S, Rana S, Agarwal R, Duseja A, *et al.* Role of small intestinal bacterial overgrowth and delayed gastrointestinal transit time in cirrhotic patients with minimal hepatic encephalopathy. J Hepatol 2010;53:849-55.
21. Bajaj JS, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, *et al.* Linkage of gut microbiome with cognition in hepatic encephalopathy. Am J Physiol Gastrointest Liver Physiol 2012;302:G168-75.
22. Quigley EM, Quera R. Small intestinal bacterial overgrowth: roles of antibiotics, prebiotics, and probiotics. Gastroenterology 2006;130:S78-90.
23. Holte K, Krag A, Gluud LL. Systematic review and meta-analysis of randomized trials on probiotics for hepatic encephalopathy. Hepatol Res 2012;42:1008-15.
24. Loguercio C, Del Vecchio BC, Coltorti M. Enterococcus lactic acid bacteria strain SF68 and lactulose in hepatic encephalopathy: A controlled study. J Int Med Res 1987;15:335-43.
25. Malaguarnera M, Greco F, Barone G, Gargante MP, Malaguarnera M, Toscano MA. Bifidobacterium longum with fructo-oligosaccharide (FOS) treatment in minimal hepatic encephalopathy: A randomized, double-blind, placebo-controlled study. Dig Dis Sci 2007;52:3259-65.
26. Malaguarnera M, Gargante MP, Malaguarnera G, Salmeri M, Mastrojeni S, Rampello L, *et al.* Bifidobacterium combined with fructo-oligosaccharide versus lactulose in the treatment of patients with hepatic encephalopathy. Eur J Gastroenterol Hepatol 2010;22:199-206.
27. Bomba A, Nemcova R, Gancarcikova S, Herich R, Guba P, Mudronova D. Improvement of the probiotic effect of micro-organisms by their combination with maltodextrins, fructo-oligosaccharides and polyunsaturated fatty acids. Br J Nutr 2002;88Suppl 1:S95-9.
28. Mittal VV, Sharma BC, Sharma P, Sarin SK. A randomized controlled trial comparing lactulose, probiotics, and L-ornithine L-aspartate in treatment of minimal hepatic encephalopathy. Eur J Gastroenterol Hepatol 2011;23:725-32.
29. Bajaj JS, Saeian K, Christensen KM, Hafeezullah M, Varma RR, Franco J, *et al.* Probiotic yogurt for the treatment of minimal hepatic encephalopathy. Am J Gastroenterol 2008;103:1707-15.

Source of Support: Isfahan University of Medical Sciences, **Potential competing interests:** None declared.