Original Article

THAI J GASTROENTEROL 2010
Vol. 11 No. 3
Sept. - Dec. 2010

Probiotic for the Treatment of Minimal Hepatic Encephalopathy: Preliminary Report

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ABSTRACT

Objectives: Minimal hepatic encephalopathy (MHE) is a preclinical stage of overt hepatic encephalopathy (OHE). All MHE therapies modify gut microflora but consensus regarding MHE treatment is lacking. The aim of this study is to determine the effect of probiotic is reversing MHE in cirrhotic patients.

Patients and Methods: A total of 58 cirrhotic patients were enrolled (44 males, 14 females) aged 42-60, 87.7% in Child-Pugh class A. The diagnosis of MHE was based on the number connection test-A (NCT-A), line-tracing test (LTT) and critical flicker frequency analysis (CFF). Diagnostic criteria for MHE were met when abnormalities in at least two out of the three tests were present.

Results: MHE was diagnosed in 16 patients (27.6%). Non-detectable ascites and Child A were the only significant differences between the MHE positive and MHE negative groups (p <0.01). Of the 16 patients, only 10 were recruited in the study, and were randomized, 5 patients in the probiotic group and 5 in the non-treatment group. Probiotic (Infloran®) was prescribed for 8 weeks in the probiotic group. Psychometric test and CFF were performed in both groups after 4 and 8 weeks of treatment. MHE was reversed in none. No significant improvement in NCT was seen in both groups. Improvement of LTT was seen in 2 patients (40%) in the probiotic group and 3 (60%) in the non-treatment group. CFF <39 Hz was found in 4 patients (80%) before treatment and in none after treatment.

Conclusions: Probiotic treatment for 8 weeks was not associated with reversal of MHE nor with improvement in the psychometric test and CFF.

Key words : probiotic, minimal hepatic encephalopathy

[Thai J Gastroenterol 2010; 11(3): 129-136.]

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INTRODUCTION

Along with the increased survival in cirrhotic patients, the neurocognitive complications of liver cirrhosis such as overt hepatic encephalopathy (OHE) and minimal hepatic encephalopathy (MHE) have also been recognized more frequently.[1] MHE is the preclinical stage of OHE. Routine detection of MHE is difficult and specialized diagnostic testing is required[2]. MHE has thus been reported in approximately 60-80% of all cirrhotic patients[3-5]. MHE has been shown to be associated with a poor quality of life, decreased work performance, impaired driving skills[6-8] and increased progression to OHE[9].

The treatment of MHE so far remains undefined. However, a recent consensus statement advocated lactulose as a first-line therapy, while concerns over the palatability of lactulose and patient compliance were acknowledged[2,10,11]. Lactulose can improve psychometric testing and quality of life in MHE patients, but have frequent gastrointestinal adverse effects such as bloating and diarrhea limited its long-term use[12,13]. A study in Thai patients has shown that lactulose in cirrhotic patients with subclinical hepatic encephalopathy was unable to improve psychometric test and EEG[14].

A proposed pathogenic mechanism for hepatic encephalopathy is the production by intestinal flora of many noxious substances such as ammonia and benzodiazepine-like compounds[15]. Probiotic, through manipulation of intestinal flora, has emerged as a therapeutic option for MHE and OHE treatment without significant adverse effects[16-19]. In a recent study, probiotic yoghurt demonstrated a significant rate of MHE reversal and excellent adherence in cirrhotic patients[3].

In this study, Infloran®, a probiotic containing at least 1,000 million each of lyophylized live *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, was chosen, as the preparation is easy to ingest and is available at the Hospital pharmacy. The aim of this study is to determine the effect of probiotic (Infloran®) on reversal of MHE in cirrhotic patients.

PATIENTS AND METHODS

From March 2009 to February 2010, a total of 61 cirrhotic outpatients attending GI clinic at Phramongkutklao Hospital were screened for MHE. Diagnosis of liver cirrhosis was based on clinical history as appeared in OPD files, blood chemistry and hepatic imaging. Inclusion criteria were liver cirrhosis from any cause, age between 18-60 and absence of previous history of hepatic encephalopathy. Exclusion criteria were including: 1) clinically overt hepatic encephalopathy, 2) history of alcohol consumption within 3 months prior to the study, 3) presence of factors interfering with the intestinal flora such as infection, GI bleeding (within the preceding 4 weeks), history of antibiotic usage (within the preceding 4 weeks), 4) presence of precipitating factors for hepatic encephalopathy: serum creatinine >1.5 mg/dL, hypokalemia (K^+ <3.5 mEq/L), hepatocellular carcinoma, 5) current psychoactive / antiepileptic / benzodiazepine medication(s) or current therapy for prevention or treatment of OHE, 6) regular consumption of taking yoghurt, and 7) patient's inability to perform neuropsychometric tests.

Diagnosis of MHE

MHE was assessed by using number connection test-A (NCT-A), line-tracing test (LTT) and critical flicker frequency analysis (CFF). An abnormal test was defined as: 1) NCT-A >30 seconds[14], 2) line-tracing test >150 seconds, or number of errors more than 2 times[21], and 3) critical flicker frequency analysis (CFF) <39 Hertz[22].

MHE was defined as the presence of at least 2 out of the 3 abnormal tests. Primary end point was a complete reversal of MHE, defined as absence of all three previously abnormal tests.

Study protocol

Cirrhotic patients with MHE as defined by the study criteria were randomly divided into two groups: the treatment group and the non-treatment group. In the treatment group, patients were given probiotic (Infloran®) three capsules per day (one capsule after meal, three times per day). Concomitant medications (including drugs used for the treatment of complications of liver cirrhosis or other non-cirrhosis conditions) were continued with minimal dosage changes, but the use of drugs considered to have direct effects on hepatic encephalopathy (HE), such as branched-chain amino acid preparation, non-absorbable antibiotics and lactulose, would not be prescribed. Patients were also not allowed to take yoghurt or drinking yoghurt during in the period of the study, in order to avoid additional probiotic effect. All MHE patients were also advised to avoid driving.

The study period was 8 weeks, involving 3 visits.

In the first visit, the following steps were taken: (a) an interview to evaluate baseline yogurt and drinking yogurt consumption, and to reinforce the avoidance of yoghurt intake, (b) performance of the tests for MHE, (c) the estimation of liver disease severity score (Child score and model-for-end-stage liver disease (MELD) score), and (d) prescription of probiotic for 8 weeks in the treatment group. At the end of wk-4 and the end-of-treatment protocol at wk-8, all patients visit GI clinic for physical examination to detect clinical manifest of OHE. All diagnostic tests were repeated carefully to detect MHE. Drug compliance and medication side effects were assessed in each patient from telephone interview at wk-2 and wk-6 of treatment. Pill counting was also made at each visit.

Statistical analysis

The results were expressed as the mean ± SD. All statistic analyses were performed using Chi-square test, student *t*-test and Rank sum test to compare proportion, mean and median between the probiotic and the non-treatment groups. *P*-value <0.05 was considered as statistically significant.

RESULTS

A total of 58 cirrhotic patients (44 males and 14 females), 87.7% of who were in CTP class A and age 42-60, were eligible for the study. MHE was diagnosed in 16 patients (27.6%). Six patients refused to participate in the study. The remaining 10 patients were randomized into the probiotic and the non-treatment group (Figure 1).

The etiology of liver cirrhosis in the MHE positive group included alcohol (50%), HBV (25%), HCV with alcohol (12.5%), HCV (6.25%) and others (6.25%). In the MHE negative group, the causes of liver cirrhosis were HBV (47.6%), HCV (19%), alcohol (16.7%), HCV with alcohol (9.5%), HBV with HCV (4.8%) and HBV with alcohol (2.4%) (Figure 2). Occurrence of ascites and Child A did not differ significantly between the MHE positive and MHE negative groups (p <0.05). There were no significant differences (p >0.05) regarding age, gender, history of variceal bleeding, MELD and drugs used among two groups (Table 1). MHE negative group contained higher number of patients with history varices bleeding and a number of beta-blocker and antiviral used than MHE positive group.

Baseline characteristics in the probiotic group and the non-treatment group did not differ significantly (Table 2). Subjects in the non-treatment group were older (p = 0.154), with higher education (p = 0.253), and with a lower MELD score (p = 0.913). Ten patients

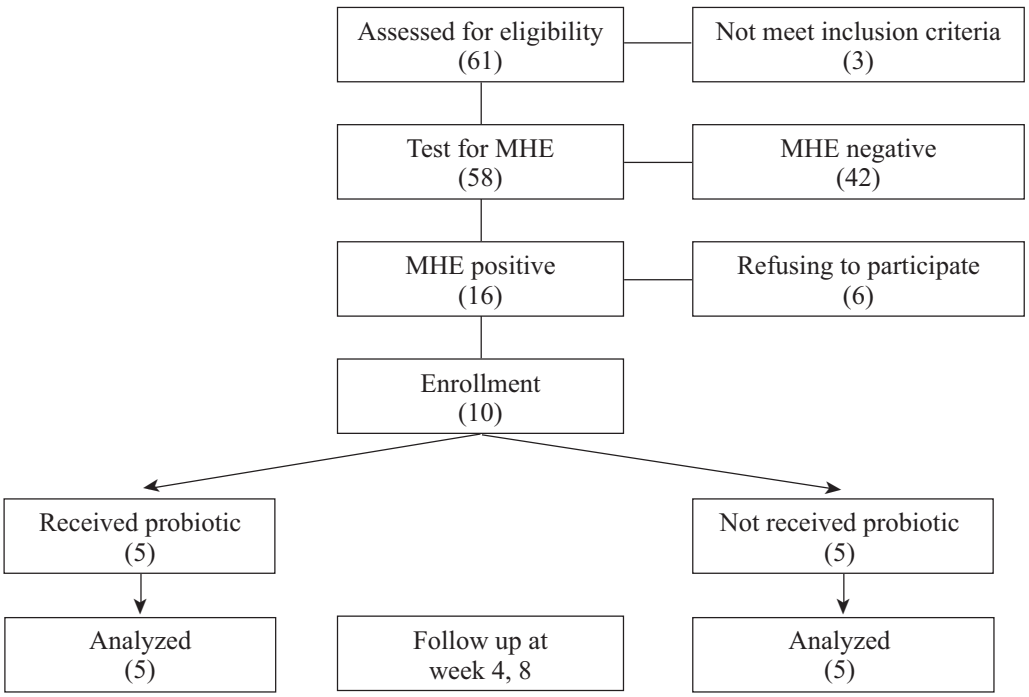

Figure 1. Study flow chart

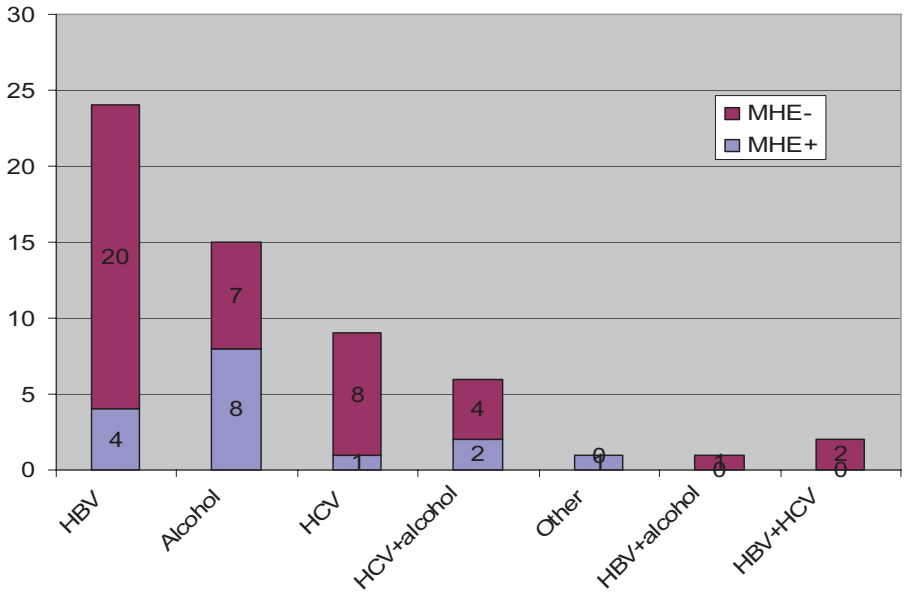

Figure 2. Etiology of liver cirrhosis in the MHE positive and MHE negative groups.

Table 1. Demographic data in the MHE positive and MHE negative groups.

	MHE positive n (%)	MHE negative n (%)	*p*-value
Gender			
male	12 (26.7)	33 (73.3)	0.771
female	4 (30.8)	9 (69.2)	
Age (yrs), mean ± SD	51.63 ± 5.3	50.21 ± 6.45	0.439
History of rupture varices	1 (14.3)	6 (85.7)	0.401
Ascites			
no	11 (20.8)	42 (79.2)	<0.001
moderate	5 (100)	0 (0)	
Child Pugh score			
A	10 (19.6)	41 (80.4)	<0.001
B	5 (100)	0 (0)	
C	1 (50)	1 (50)	
MELD score	9.81 ± 3.47	8.05 ± 2.63	0.042
Drugs used			
Diuretic	8 (57.1)	6 (42.9)	0.004
Beta-blocker	11 (39.3)	17 (60.7)	0.054
Antiviral	5 (23.8)	16 (76.2)	0.628

completed the study. None of the patients developed overt hepatic encephalopathy nor reversed MHE.

Psychometric tests and CFF

The psychometric test such as the NCT-A and LTT are employed to assess several cognitive aspects adversely affected in MHE. In performing the NCT-A, the subject has to trace numbers from 1 to 25 consecutively and the time taken is recorded. This test is used to assess psychomotor, tracking, and attention deficits[23,24]. LTT is used to determine the accuracy of a person's fine motor control. The subject has to rapidly follow the original 5 mm wide track from beginning to end, without going over the edges. This test is used to assess visual-motor co-ordination and psychomotor slowing[25,26].

Table 2. Baseline characteristics of the two groups.

	Probiotic group n (%)	Non-treatment group n (%)	Total	*p*-value
Age (yrs.)	49.4 ± 3.58	53.6 ± 4.77	51.5 ± 4.55	0.154
Sex				
Male: Female	4:1	4:1	8:2	1.000
History of variceal bleeding	0 (0)	1 (100)	1 (10)	0.292
Co-morbid disease	0 (0)	2 (100)	2 (20)	0.114
Current ascites	1 (20)	1 (20)	2 (20)	1.000
Child A:B	4:1	4:1	8:2	1.000
MELD	10 ± 3.67	8.2 ± 1.1	9.1 ± 2.73	0.913
Drugs				
Diuretic	2 (50)	2 (50)	4 (40)	1.000
Beta-blocker	3 (50)	3 (50)	6 (60)	1.000
Antiviral	2 (100)	0 (0)	2 (20)	0.114

Table 3. Changes in psychometric tests and CFF before and after treatment (mean ± SD).

	Probiotic group (n = 5)		Non-treatment group (n = 5)	
	before	after	before	after
NCT (sec)	54.71 ± 15.38	41.92 ± 4.88	44.97 ± 9.07	39.86 ± 9.82
LTT (sec)	108.2 ± 85.7	132.4 ± 38.25	93.78 ± 52.32	95.26 ± 55.1
LTT (time)	2.2 ± 2.17	0 ± 0	1.2 ± 1.64	2.4 ± 2.51
CFF (Hz)	37.86 ± 0.89	42.51 ± 3.35	38.64 ± 2.31	41.49 ± 4.85

None of the study patients had reversal of MHE. The mean times used in NCT, pre-and post-treatment in the probiotic group were 54.71 ± 15.38 sec and 41.92 ± 4.88 sec, compared with 44.97 ± 9.07 sec and 39.86 ± 9.82 sec in the non-treatment group. No significant improvement in the time used for NCT was found in probiotic group at the end of treatment (Figure 3). In LTT, both groups spent a long time to complete the test, although in the probiotic group, there were fewer error times. Three patients in each group exhibited impaired LTT at baseline, and in 1 patient in each group that LTT was impaired at the end of treatment.

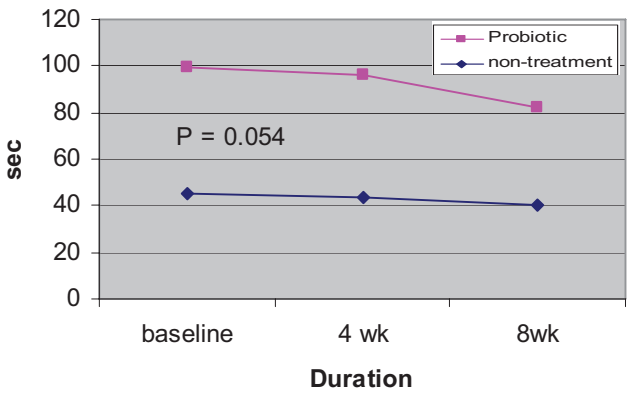

Figure 3. Time to complete NCT (sec).

CFF, a neurophysiologic test, is used to measure visual discrimination and general arousal[13]. CFF threshold is measured by intrafoveal stimulation with a luminous diode. HEPAtonorm analyzer is a CFF equipment. When the HEPAtonorm analyzer is used, the red spot generated by light pulse frequency is seen. The frequency of the light pulses decreases from 60 Hz downward. After that, the CFF threshold is determined as the frequency when the impression of fused light turned to a flickering one. CFF is considered abnormal when the value is <39 Hz[27,39]. In this study, The CFF thresholds and psychometric measurements were checked on the same day.

There were 4 patients in the probiotic group, and

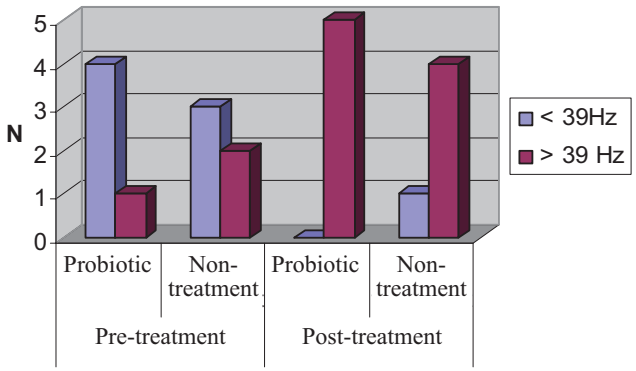

Figure 4. CFF pre-treatment and post-treatment in both groups.

Table 4. Outcomes of patients after 8 weeks of treatment.

	Probiotic group (n = 5)	Non-treatment group (n = 5)
Ascites (n)	0	0
CTP score A:B:C	5:0:0	5:0:0
MELD score	8.6 ± 1.82	9.4 ± 1.67

3 in the non-treatment group with CFF <39 Hz. At the end of treatment, none of the patients in the probiotic groups and only one patient in the non-treatment group had CFF <39 Hz (Figure 4).

Child score distribution and MELD score change

Only 2 Child class B patients were included, one in each group. Both had ascites at baseline. At the end of study, all patients become in Child A and their ascites disappeared. There was no significant difference in MELD scores in the probiotic group and the non-treatment group (Table 4).

DISCUSSION

The prevalence of MHE has been shown in a recent study to be about 60-80% in cirrhotic patients[3]. In our study, the prevalence of MHE was 27.6%. MHE prevalence has been reported to vary between 30 to 80%. This variation in prevalence is due to differences in diagnostic methods used, patient studies, and the definitions of MHE in the various studies. Some western studies have shown a high prevalence of MHE in patients who were predominantly non-alcoholic and without any psychoactive drug use[28,29]. The mentioned prevalence is similar to that of 27.3% reported from Ramathibodi Hospital, Bangkok[14].

Traditionally, the diagnosis has been limited to the presence of neurologic impairment as demonstrated by neuropsychological or neurophysiologic tests[2]. Two psychometric tests, namely the number connection test-A (NCT-A), and the line-tracing test (LTT) and critical flicker frequency analysis (CFF) are employed in the diagnosis of MHE. NCT-A and LTT are psychometric tests in which subjects must have good coordination between the eyes and the hands to complete the tests. Therefore, patients included in the study should <60 years old. CFF is employed because it is simple to use, reliable, easy to follow and requires only a little training to the patient. It also does not show a learning effect. The diagnostic accuracy of CFF is 73%[30,31]. The sensitivity and specificity of CFF for diagnosing MHE are 65% and 91%[32]. To enhance the sensitivity of CFF in our study, we defined the presence of MHE in at least two testings.

In the MHE-positive and MHE-negative groups, absence of ascites and Child class A were the only significant differences between the two groups. This was in line with previous studies in which the prevalence of MHE increased from Child A > B > C[33-35]. Consequently patients with worse liver functions as assessed by Child-Pugh's grading had a higher occurrence of MHE. Although the etiology of liver cirrhosis in the MHE-positive group was mostly alcohol (50%), this etiology was recognized in 16.7% in the MHE-negative group. The higher prevalence of MHE in patients with alcoholic cirrhosis was reported mostly from those studies using the electroencephalogram (EP), implying a diffuse neurological disturbance in alcoholic cirrhotic subjects[36].

Probiotics are defined as live microbiological dietary supplements that have beneficial effects to the host beyond their nutritive value. As bacterial intestinal flora-derived ammonia and neurotoxic substances are held as leading culprits in the development of hepatic encephalopathy, manipulation of the intestinal bacterial flora has been the mainstay of treatment for hepatic encephalopathy[15]. Bajaj et al. noted a significant reversal of MHE in yogurt-treated patients over 60 days[3].

In our study, we chose Infloran®, the probiotic that is available at Phramongkutklao Hospital. Infloran® contains live *Lactobacillus acidophilus* and *Bifidobacterium bifidum*. *L. acidophilus* produces lac-

tic acid without carbon dioxide and occupies the small bowel, whereas Bifidobacteria resides in the colon and produces lactic acid and acetic acid as a result of fermentation[37]. We conducted a study to determine the efficacy of this probiotic in cirrhotic patients with MHE. At the end of 8-week treatment, we found that none of the patients in the probiotic group had reversal of the MHE. The NCT was improved in both groups, but not significantly. There were no significant changes in MELD score and Child grading after the probiotic treatment. Tan HH et al. investigated MHE patients for 3 years, and found that MHE could revert to a normal stage in a significant proportion of patients[38]. Their study also showed that MHE need not be permanent.

In conclusion, in our preliminary study, there was a trend for efficacy of probiotic in the treatment of cirrhotic patients with MHE, although there was no significantly different improvement in both the psychometric and the CFF test between the probiotic and the non-treatment group. This, however, may be related to the small number of study patients and the rather short treatment duration. Larger numbers of subjects and longer treatment periods are recommended for future study.

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