The Effect of Branched Chain Amino Acids and L-Ornithine L-Aspartate Combination as the Late Evening Snacks on Nutritional Status and Minimal Hepatic Encephalopathy in Liver Cirrhosis

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ABSTRACT

Background: *Minimal hepatic encephalopathy (MHE) in liver cirrhotic patients is critical manifestation of low grade hepatic encephalopaty (HE), is caused quality of life decrease and risk of deteriorating into distinct HE. The study was conducted to asses the effect of combination of branched-chain amino acids (BCAA) and L-ornithine L-aspartate (LOLA), which was given late evening, on nutritional status and degree of HE.*

Method: *This single-blind randomized study was conducted on liver cirrhotic outpatients in hepatology clinic of Cipto Mangunkusumo Hospital period June 2011-June 2012. The study subjects were divided into two groups, group of late evening snack (LS) and day snack (DS). Each group was supplemented with combination of milk of BCAAs and LOLA (3.7 g/serving). Evaluation based on history, physical examination, laboratory tests and critical flicker frequency (CFF) was performed one month after the intervention. Data were statistically analyzed with SPSS 15.*

Results: *Thirty-two patients whose the inclusion criteria were divided into 16 subjects for each group, LS and DS. After one month of the intervention, the average level of prealbumin for DS group was increased statistically significant ($p < 0.001$), but not significant for LS group ($p = 0.259$). The increase of average body weight,mid-arm muscle circumference (MAMC) and CFF test result in both groups. There was no improvement on subject global assessment (SGA) score after one month intervention in both groups.*

Conclusion: *This study proved that giving combination of BCAAs and LOLA may improve the condition of MHE, however for nutritional status can not be assessed.*

Keywords: *minimal hepatic encephalopathy, BCAAs, LOLA, prealbumin, SGA score, nutritional status*

ABSTRAK

Latar belakang: *Ensefalopati hepatikum minimal (EHM) pada pasien sirosis hepatis adalah manifestasi ensefalopati hepatikum (EH) derajat ringan yang penting karena mengurangi kualitas hidup dan berisiko jatuh ke dalam EH yang nyata. Penelitian ini dilakukan untuk menilai pengaruh pemberian kombinasi asam amino rantai cabang (AARC) dan L-ornitin L-aspartat (LOLA) larut malam terhadap status gizi dan derajat EH.*

Metode: *Penelitian acak tersamar tunggal dilakukan terhadap pasien sirosis hepatis rawat jalan Rumah Sakit Cipto Mangunkusumo periode Juni 2011-Juni 2012. Subyek penelitian dibagi dalam dua kelompok yaitu kelompok makanan selingan siang hari (MSSH) dan kelompok makanan selingan malam hari (MSMH). Tiap kelompok diberikan suplementasi susu kombinasi LOLA dan AARC (3,7 g/saji). Evaluasi berdasarkan anamnesis,*

pemeriksaan fisik, uji laboratorium dan critical flicker frequency (CFF) dilakukan satu bulan setelah intervensi. Data dianalisis secara statistik dengan SPSS 15.

Hasil: *Tiga puluh dua pasien yang memenuhi kriteria inklusi terbagi menjadi 16 subyek untuk tiap kelompok, MSSH dan MSMH. Setelah 1 bulan intervensi didapatkan peningkatan rerata kadar prealbumin pada kelompok MSSH yang signifikan (p < 0,001), namun tidak signifikan pada kelompok MSMH (p = 0,259). Kenaikan rerata berat badan, LLA dan hasil CFF yang signifikan ditemukan pada kelompok MSSH dan MSMH. Tidak didapatkan perbaikan derajat SGA setelah satu bulan intervensi pada dua kelompok.*

Simpulan: *Penelitian ini membuktikan bahwa pemberian kombinasi AARC dan LOLA dapat memperbaiki kondisi EHM, namun belum dapat dinilai untuk status gizi.*

Kata kunci: *ensefalopati hepatikum minimal, AARC, LOLA, prealbumin, skor SGA, status gizi*

INTRODUCTION

Minimal hepatic encephalopathy (MHE) is the mildest manifestation of hepatic encephalopathy (HE) where there are no any clinical sign of mental disorder, yet found by psychometric tests. MHE is important because it reduces the quality of life and the risk of falling into distinct HE. HE is the one of liver cirrhosis complication which has high impact on morbidity and mortality. The incidence of HE on liver cirrhosis in western countries varies from 30-45% (US) and 50-70% (UK), where most of them are MHE.[1] Asian data is also varied, i.e. 62.4% in India, while in the outpatient clinic of Cipto Mangunkusumo Hospital and Koja Hospital showed that the incidence of MHE among the patients with liver cirrhosis were 75.75% and 94.4%.[2]

Liver cirrhosis patients need more protein to maintain nitrogen balance compared with healthy individuals. This is due to increase of synthesis and turn over of protein in patients with liver cirrhosis.[3] Many studies have shown that the nutritional status, particularly protein malnutrition condition, determines life expectancy of patients with liver cirrhosis. Supplementation with branched-chain amino acids (BCAAs) and L-ornithine-L-aspartate (LOLA) is indicated to improve protein malnutrition. Many studies have been conducted to investigate the efficacy of therapy BCAA and LOLA separately on nutritional status and degree of MHE. These kind of therapy is for better in improving the nutritional status, degree of MHE and quality of life.

The part of the intervention that has long been recommended is routine diet or late evening snack.[4] Late evening snack affects on the energy metabolism includes liver cirrhosis-associated metabolic disorder. Frequent intake of food in small portions can prevent early onset of starvation and also maintain nutritional status of liver cirrhosis patients.[5] It is believed late evening snack can increase the formation of new protein, while snack during the day will only be used as an energy reserve for the activity.[6] This study focused on the appropriate time for administration the combination of LOLA and BCAAs. BCAAs and LOLA combination therapy which had given as snack in the late evening were expected to improve the nutritional status and the degree of MHE in patients with liver cirrhosis measured using modified subjective global assessment (SGA) and prealbumin level as nutritional status parameters as well as the critical flicker frequency (CFF) test result to see improvement of hepatic encephalopathy.

METHOD

Upon written approval of the Research Ethics Committee of Faculty of Medicine University of Indonesia/Cipto Mangunkusumo Hospital, this study was conducted between June 2011-June 2012, at hepatology outpatient clinic of Cipto Mangunkusumo Hospital, Jakarta. Forty adult patients aged over 18 years who came to the Hepatology outpatient clinic of Cipto Mangunkusumo Hospital, which has been diagnosed as liver cirrhosis based on supporting data from liver biopsy, or meet at least two criterias from the typical feature by ultrasonography (USG), the existence of esophageal varices, ascites, or increase of international ratio (INR), which is not caused by another condition[5], were included in this study.

Inclusion criteria were based on the consciousness level of compost mentis, i.e patients EHM degree 1 or 2, had value of CFF < 38 Hz, not taking drugs that affect liver function (lactulose, LOLA, BCAAs and hepato-protector) for one week before treatment, patients with impairmentof oral intake and diarrhea, pregnancy or lactation, major complications of portal hypertension, such as gastrointestinal bleeding, hepatorenal syndrome and spontaneous bacterial

peritonitis, and hepatoma were excluded in this study. The sampling method is non-probability consecutive sampling. Randomization was undertaken to determine the group receiving treatment LS and DS.

All of subjects were given supplementation of milk contain with combination of LOLA and BCAAs (3.7 g/serving). Patients were asked to drink the supplement in accordance with the randomization group, i.e DS group, time consumption of supplements at 07.30 and 12.30, while LS group at 21:30 and 07:30, respectively. Anamnesis of diet evaluation performed every 2 weeks, according to the suggestion of a study conducted by Fukushima.[7]

Analysis of the data distribution was undertaken before further statistical analysis. Comparative associative hypothesis testing of data with numerical scale on dependent group used paired T test for normal data distribution and the Wilcoxon test for abnormal data distribution, while on independent groups with normal data distribution, used unpaired T-test and Mann-Whitney U test when the distribution the data is abnormal. Chi-square test was used for the ordinal scale data analysis. If $p < 0.05$, then it was considered significant. Data from this study were analysed using the software SPSS for Windows version 15.0.

RESULT

During the period of June 2011 to February 2012, 40 cases of liver cirrhosis had met the selection criteria recruited for the study subjects. Of the 40 patients were then undertaken block randomization, so 20 patients for each group assigned to the intervention snack during the day (DS) and late evening snack (LS). A total of 8 patients were excluded from the study because of absent on the second visit, financial problem and worsening clinical condition. So in the end, we collected 32 patients who completed the study program, consisting of 16 patients in the DS treatment group and 16 patients LS treatment group.

The total number of participant was 32 people, consisted of 12 (37.5%) female and 20 (62.5%) male. The youngest participant in this study was 40 years old and the oldest was 77 years of age. The most common etiology of liver cirrhosis was hepatitis B, 22 (68.8%) cases, and hepatitis C 10 (31.3%) cases. Table 1 summarizes the basic data of patients before intervention, while post-intervention results are summarized in Table 2.

Comparing the baseline with the post-intervention results, there was a significant change of the mean prealbumin levels in DS group, but it was not statistically significant in LS group ($p < 0.001$ and $p = 0.259$, respectively).

Table 1. The baseline characteristics of patients

Variables	The intervention group	
	DS Mean (SD)	LS Mean (SD)
Sex (n/%)		
Male	10 (62,5)	10 (62.5)
Female	6 (37.5%)	6 (37.5%)
Age (years)	57.4 (11.0)	57.8 (9.2)
Age group (n/%)		
40-60	9 (56.3)	10 (62.5)
> 60	7 (43.8)	6 (37.5)
Body weight (kg)	61.09 (10.9)	60.13 (11.4)
Mid-upper arm circumference (cm)	23.503 (3.6)	25.00 (6.8)
Hepatitis (n/%)		
B	12 (75.0)	10 (62.5)
C	4 (25.0)	6 (37.5)
Child-Pugh (n/%)		
A	5 (31.3)	11 (68.8)
B	9 (56.3)	5 (31.3)
C	2 (12.5)	0
SGA (n/%)		
A	6 (37.5)	12 (75.0)
B	8 (50.0)	3 (18.8)
C	2 (12.5)	1 (6.3)
Minimal hepatic encephalopathy with CFF (Hz)	35.57 (2.1)	35.84 (2.4)
Prealbumin level (mg/dL)	7.105 (3.4)	13.106 (8.3)
Urea nitrogen level (mg/dL)	11.5 (18.3)	14.5 (6.4)
Ureum level (mg/dL)	25.0 (11.2)	28.0 (12.2)
Creatinine level (mg/dL)	0.9 (0.4)	0.8 (0.4)

DS: day snack; LS: late evening snack; SD: standard deviation; MAMC: mid-upper arm circumference; SGA: subjective global assessment; CFF: critical flicker frequency

Table 2. Levels of albumin, SGA, weight, LLA, CFF after intervention

Variables	DS group	LS group	p
Prealbumin level (mg/dL)	10.325 (5.93)	12.146 (6.75)	0.02 *
SGA			
Mild (A)	8 (50)	12 (75)	
Medium (B)	4 (25)	3 (18.8)	NS **
Severe (C)	4 (25)	1 (6.3)	
Body weight (kg)	62.45 (12.1)	64.38 (9.4)	NS *
Mid-upper arm circumference (cm)	25.53 (3.9)	26.12 (7.3)	NS *
Critical flicker frequency	40.04 (4.0)	40.72 (3.7)	0.001 *

DS: day snack; LS: late evening snack; SGA: subjective global assessment; NS: not significant; *Mann-Whitney test; **Chi-square test

There was an increase of the average body weight in the DS and LS group, which was statistically significant ($p < 0.001$). A significant increase of the average length of mid-arm muscle circumference (MAMC) was found for both groups ($p < 0.001$ and $p = 0.003$, respectively). The same went on CFF test results, there was a significant increase of the average results in both groups ($p < 0.001$ and $p = 0.001$, respectively).

DISCUSSION

After the nutritional supplementation with BCAAs and LOLA for 30 days, the results for nutritional status and degree of EH has been obtained. From the total of subjects studied, only 16 patients in each group who continued the treatment. This was because the subjects of the study were taken from outpatients. The difficulty found in this study was the patient compliance. Plank et al suggested that there are difficulties related with patient compliance regarding of supplement consumption, especially in outpatients.[8]

In accordance with the recommendation from European Society for Parenteral and Enteral Nutrition (ESPEN) 2006, liver cirrhosis patients are suggested to get 30-45 kcal/kg/day and protein intake of 1.2-1.5 g/kg/day. Administer BCAAs enriched-formula (level of evidence B) may improve clinical condition, especially on the condition of hepatic encephalopathy.[9]

From the available data, it was known that since the beginning of the study, both groups did not have the equal basic characteristics prealbumin values, this was thought due to the failure of randomization because of the relatively small sample size. Thus, the interpretation of the results of this study was only based on post-treatment prealbumin levels in both groups.

Prealbumin levels improvement were seen in the day-time rather than night, this was not consistent with the hypothesis of the study. The examination of prealbumin level early before the intervention, showed the DS 7.105 mg/dL (SD = 3.35) and LS 13.106 mg/dL (SD = 8.27). The distribution of prealbumin levels in this study was consistent with the Child-Pugh degree. In this study, we included the patients who were in the state of advanced liver cirrhosis, the Child-Pugh B and C, and get the same interventions as other subjects.

Based on the after intervention prealbumin levels result, we found an increase in the mean of prealbumin level of DS group which was statistically significant, $p < 0.001$. This was predicted because the supplementation was consumed by the study participants as study instruction. While in the LS group, the average level of prealbumin decreased after intervention, but not statistically significant ($p = 0.259$). Decreased level of prealbumin in this group was thought because blood sampling procedure was performed during the day (13:00 to 15:00), where the participants of LS group had not received second portion of daily supplementation yet, while the DS group had received the second portion of daily supplementation.

The difference level of prealbumin after the intervention was statistically significant between groups.It was thought because of short half-life of prealbumin, which was 2-3 days, and the interval between supplementation and blood sampling schedule after intervention were different in both groups. In the LS group, the interval between supplementation of first portion (07:30) to the second portion (21:30) is 14 hours, while in the DS group,the interval between the supplementation of the first portion (07:30) to the second portion (12:30) is 5 hours. In addition, there was a great variation in the prealbuminlevel of LS group because of one patient who had initial prealbumin level far above the average of the 38 mg/dL, who then did not perform second sampling (excluded) and then considered as outliers.

The decrease average level of prealbumin in this study also considered for reliability of examination tools and storage process of blood samples. It was based on our findings when repeating random check by taking a sample of one patient from each group, then obtained a different result than the previous examinations. Re-examination in one subject from DS group after intervention, the result obtained was 8.9 mg/dL for prealbumin level (initial examination 10.7 mg/dL). While LS group after intervention, the result obtained 8.3 mg/dL for prealbumin level (initial examination 8.1 mg/dL). Repeated examination was within approximately six months of the initial examination. Prealbumin samples were stored in freezing temperatures of -80^0 C in a research laboratory.

Prealbumin, known as thyroxine-binding prealbumin or transthyretinis a stable circulating glycoprotein synthesized in the liver. Prealbumin levels will further decline, along with liver disorder from mild state to liver cirrhosis. Hence, prealbumin is currently used as one of important prognostic factor in liver cirrhosis.[10] Serum prealbumin level improvement mechanism associated with the administration of substitution BCAAs considered as result of improved prealbumin turn over. In patients with liver cirrhosis, body protein turn over is disturbed, thus the synthesis

of prealbumin and albumin become slow, therefore BCAAs supplementation may improve this disorder. Other studies also suggest that, BCAAs provided direct regulatory effect at cellular level, to the sensors of leucin intracellular as the molecular target of BCAAs in the regulation of protein synthesis and degradation.

In the study by Fukushima et al,[7] who observed the efficacy of the evening snack administration compared to the day in hospitalized patients, concluded that the evening snack at 21:00, correlated with high level of albumin, particularly at 23:00, where the level of albumin could maintain Fischer ratio on physiological conditions, especially at night. In present study, it was not possible to perform blood test at night, although at the end of the intervention was found that prealbumin level of LS group was better than DS.

Many studies investigated the effects of the late evening snack on the condition of the protein and energy malnutrition which was common in patients with liver cirrhosis. In a study conducted by Koreeda et al, giving substitution BCAAs at night might prevent starvation condition overnight and improve liver parenchymal cells as the evidence of improved nutritional status.[11]

Increased prealbumin were consistent with other studies that investigated the role of BCAAs in nutritional status improvement.[12] It was also consistent to another studies conducted by Muto[13] and Nakaya[14] which concluded that there were improvement of albumin in patients with liver cirrhosis.

Based on the results of statistical tests on both groups DS and LS, showed that, in the DS group after intervention hadmore patients with low-grade SGA. Meanwhile, in the LS group, the number of low-grade SGA did not change significantly after the intervention. Based on the basic characteristics of the study subjects, it was concluded that both groups could not be compared with each other.

In our results, there was no significant improvement of SGA scores in both groups. DS and LS. It was thought that many factors that influenced the increasing of SGA scores, such as albumin and total lymphocytes count (TLC). Improvement of these factors were difficult to obtain during the short intervention period (30 days). In addition, the relatively small sample size, might also influence these results.

The average result of CFF after supplementation of BCAAs and LOLA significantly increased compared with before intervention inboth groups DS and LS, $p < 0.001$ and $p = 0.001$, respectively. In LS group there was no deterioration in CFF result, it indicated that the normal protein intake of 1.5 g/kg was safe in MHE. CFF which was not deteriorated showed: 1). The supplementation of normal protein did not worsen EH condition, as feared in earlier theories;[1,15] 2). Supplementation of nutrition might overcome MHE condition in malnourished liver cirrhosis patients. Consistent with present study hypothesis, it has shown the efficacy of the supplementation of food substitution with BCAAs and LOLA was better in LS group than DS group, which was statistically significant ($p < 0.001$).

The influence of BCAAs to MHE caused controversy in many studies. False neurotransmitter hypothesis was originally used as a basic role of BCAA in the improvement of HE. It was said that the reduced concentration of BCAAs (leucine, isoleucine, and valine) and increased aromatic amino acids (AAA; phenylalanine, tyrosine, and tryptophan) might yieldfalse neurotransmitter that trigger HE.[1] Because of that, administration of BCAAs-enriched formula was initially thought could improve HE.[16] However, many studies have not been able to prove that BCAAs could improve EH.

This present study also proved the efficacy of LOLA administration could improve MHE, and the administration of LOLA combined with BCAAs especially at late evening, is needed to address malnutrition in liver cirrhosis.

In present study, we found several weaknesses. For example, a relatively small size of samples that likely gave impact on the significance of statistical tests result. Additionally, high dropout rate, that was thought because this study was done on outpatient. To overcame this, we had educated and motivated patients routinely and periodically by contacting patients by telephone to evaluate complaint, compliance and nutritional intake monitoring at home. ESPEN 2006 recommended the examination of body cell mass using bioelectrical impedance analysis (BIA) to assess the nutritional status of liver cirrhosis patients. The parameter of nutritional status in this study using SGA and prealbumin, due to limited funds.

CONCLUSION

The present study showed the significantly increase of prealbumin average level for DS group. There was also significant increase of average body weight, MAMC and CFF test result in both groups. There was no improvement on SGA score after 1 month intervention in both groups. We concluded that dietary

administration 35-40 kcal/kg/day with protein 1.5 g/kg with the supplementation of late evening combination of BCAAs and LOLA can improve the condition of MHE. However, the nutritional status can not be assessed, due to the relatively small sample size and the present study subjects were from outpatients setting. It is considered necessary to conduct further research to assess the efficacy supplementation of BCAAs and LOLA on the nutritional status and quality of life with a larger sample size and better study design.

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