The Role of Changes in the Proportion of Fecal Short-Chain Fatty Acids on the Severity of Hepatic Encephalopathy in Cirrhosis Patients

Mochamad Anief Ferdianto[1], Juferdy Kurniawan[1]*, Hasan Maulahela[2], Cleopas M. Rumende[3], Imam Subekti[4], Ikhwan Rinaldi[5], Hamzah Shatri[6], Cosmas Rinaldi A Lesmana[1]

[1]Hepatobiliary Division, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[2]Gastroenterology Division, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[3]Division of Pulmonology, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[4]Division of Endocrine, Metabolism, and Diabetes, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[5]Haematology and Medical Oncology Division, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.
[6]Psychosomatic Division, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia.

Corresponding Author:
Juferdy Kurniawan, MD., PhD. Hepatobiliary Division, Department of Internal Medicine, Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia. Jl. Diponegoro No. 71, Jakarta, 10430, Indonesia. Email: juferdy.k@gmail.com.

ABSTRACT

Background: *Short-chain fatty acids (SCFA) are the main metabolites of the intestinal microbiota, which play a role as colonocyte trophic factors and maintain the integrity of the gastrointestinal tract and blood-brain barrier. Microbiota dysbiosis that occurs in cirrhosis reduces SCFA production and plays a role in the pathogenesis of hepatic encephalopathy (HE). This study aims to compare the amount and composition of fecal SCFA in patients with cirrhosis, with and without HE.* ***Methods****: This research is a cross-sectional study at the Hepatobiliary Clinic and Integrated Procedure Room, Dr. Cipto Mangunkusumo Hospital, Jakarta, in 2023. Patients with cirrhosis underwent a flicker or Stroop test, fecal SCFA examination (acetate, butyrate, and propionate), and a questionnaire with a food recall technique to assess dietary patterns.* ***Results:*** *A total of 86 patients with cirrhosis participated in this study, with a mean age of 53 ± 8.10 years, and the majority were male* (68.6%). Hepatic encephalopathy (HE) was identified in 20 patients (23.25%). Multivariable analysis of SCFA profiles showed no statistically significant associations with HE. The absolute SCFA proportion had an adjusted prevalence ratio (*PR) of 1.98 [95% CI: 0.75–5.24; p = 0.171], the absolute acetate proportion had an adjusted PR of 2.06 [95% CI: 0.40–10.62; p = 0.388], and the butyrate proportion had an adjusted PR of 2.02 [95% CI: 0.76–5.39; p = 0.158].* ***Conclusion:*** *Changes in SCFA composition may be associated with the presence of HE in patients with cirrhosis. Although no statistically significant relationships were found, these findings suggest that SCFA profiles warrant further investigation concerning dysbiosis and HE in cirrhosis.*

Key words: *SCFA, cirrhosis, hepatic encephalopathy, feces, dysbiosis*

INTRODUCTION

Hepatic encephalopathy (HE) is a reversible central nervous system dysfunction caused by liver insufficiency and/or portosystemic shunting, characterized by a wide range of neurological and psychiatric manifestations, from subclinical symptoms to coma.[1,2] HE occurs in 50-70% of patients with cirrhosis and is a poor prognostic indicator, with a one-year survival rate of 42% and a three-year survival rate of 23% in the absence of liver transplantation. Data on HE in Indonesia is limited, with an estimated 30-84% of patients with cirrhosis affected. In 2009, it was found that 63.2% of patients with cirrhosis at Cipto Mangunkusumo Hospital had minimal HE.[3]

Gastrointestinal dysbiosis occurs in patients with cirrhosis. This is evident by the presence of small intestinal bacterial overgrowth (SIBO), which is common in cirrhotic patients and is associated with the severity of cirrhosis. In patients with cirrhosis, especially those with portal hypertension, there is a decrease in intestinal motility, which contributes to the development of SIBO and dysbiosis. The severity of cirrhosis correlates with an increased presence of potentially pathogenic bacteria such as Enterobacteriaceae and Streptococcaceae, and negatively correlates with beneficial taxa such as Lachnospiraceae and Ruminococcaceae. Dysbiosis is associated with reduced levels and altered proportions of short-chain fatty acids (SCFAs) in the feces.[4,5] Jin et al. found that cirrhotic patients have a decreased capacity to produce SCFAs, particularly butyrate. Decreased SCFAs indicate gastrointestinal dysbiosis, impaired gut integrity, and disrupted blood-brain barrier integrity, all of which play a role in the occurrence of HE.[6] A study by Bloom et al. showed that fecal SCFA levels are reduced in cirrhotic patients with HE.[7,8]

The composition of gut microbiota varies significantly among individuals and is influenced by various factors such as age, gender, genetics, geography, and diet, necessitating population-specific research.[9,10] Current therapies for hepatic encephalopathy (HE) primarily focus on modulating the gut microbiota, with lactulose and rifaximin being widely used. Additionally, fecal microbiota transplantation (FMT) and probiotics have shown promising results, making research on the role of microbiota in HE particularly intriguing.[1,11] SCFAs, products of gut microbiota, are believed to reflect dysbiosis conditions in the gut and have both direct and indirect roles in the severity of HE.[6] Therefore, investigating the relationship between SCFAs and the severity of HE is of significant interest. In this study, we aim to assess the relationship between changes in fecal SCFA proportions and the severity of HE in patients with cirrhosis at Cipto Mangunkusumo Hospital, as this has not been previously investigated in Indonesia.

METHODS

This study was a cross-sectional observational study aimed at determining the prevalence of hepatic encephalopathy (HE) and the relationship between fecal short-chain fatty acid (SCFA) proportions and HE severity (normal vs. HE) in patients with liver cirrhosis. The study was conducted at the Hepatobiliary Clinic and Integrated Procedure Room at Dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, in 2023.

Study Participants

Patients with cirrhosis who were aged between 18 and 70 years old and visited the Hepatobiliary Clinic at Dr. Cipto Mangunkusumo National Central General Hospital, Jakarta, in 2023 were included in this study. The exclusion criteria were patients diagnosed with stage four or five chronic kidney disease, patients with a history of alcohol consumption exceeding one standard drink per day, patients with a history of antibiotic use for at least one week within the last two months, patients with a history of narcotic and psychotropic drug use, patients with a history of certain psychiatric disorders as per the operational definition, and patients who have used probiotics for at least one week within the last two months.

Ethical Statement

All procedures performed in this study that involve human participants complied with the 1964 Helsinki Declaration and its later

amendments or comparable ethical standards. This study was conducted according to the study protocol number 23-09-1497 that has been reviewed and approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Indonesia Cipto Mangunkusumo Hospital, with ethical clearance number KET-1367/UN2.F1/ETIK/PPM.00.02/2023. Informed consent was obtained from all patients before they participated in this study.

Data Collection

The study enrolled patients with cirrhosis based on predefined inclusion and exclusion criteria, with informed consent obtained from those who were willing to participate. Baseline characteristics were recorded, followed by an assessment of HE and SCFA analysis. HE was evaluated using the West Haven criteria, classifying patients into two groups, i.e., no HE (grade 0) and HE (grade I or higher). The severity of HE was further categorized as no abnormality (grade 0), covert HE (grade 1), and overt HE (grades 2–4). Fecal samples for SCFA analysis were collected within a maximum of three days after determining the patient's HE status, considering logistical constraints. Samples were delivered to the laboratory within two hours of collection to prevent degradation or microbial changes that could affect the composition of short-chain fatty acids. SCFA composition in feces was analyzed using gas chromatography-mass spectrometry (GC-MS). SCFA levels were classified as normal if all three of the following conditions were met: acetate proportion of 40–75%, propionate proportion of 9–29%, and butyrate proportion of 9–37%. If any of these conditions were not met, the SCFA composition was classified as abnormal.

Statistical Analysis

Using SPSS 26 software, the study conducted descriptive analyses presenting categorical data as percentages. Normally distributed numerical data were reported with mean and standard deviation, while non-normally distributed data were presented with median and interquartile range. Bivariate analyses used independent t-tests or Man Mann-Whitney test and the chi-squared test. A multivariate Cox regression model with a constant time assumption was applied to assess the association between SCFA levels and hepatic encephalopathy (HE) in cirrhotic patients. All variables with $p < 0.250$ are included in the initial model. Variables are eliminated sequentially from the full model using the backward method, with p-values <0.05 considered as significant.

RESULTS

Baseline Characteristics and SCFA Profile in Patients with Cirrhosis

Eighty-six patients with cirrhosis were consecutively enrolled in this study. The flowchart of subject selection is shown in **Figure 1**. The majority of patients with cirrhosis who participated in this study were males (68.6%). The mean age of patients with cirrhosis in this study was 53 years (SD: 8.10), with 24.4% being older adults. The mean BMI of the patients was 25.57 kg/m² (SD: 5.43). The predominant etiology among patients with cirrhosis in this study was hepatitis B (51.2%), followed by hepatitis C (29.1%), non-B non-C (16.3%), and a combination of hepatitis B and hepatitis C (3.5%). The baseline characteristics of these patients are presented in **Table 1**.

Fecal samples were collected from 86 patients with cirrhosis for SCFA analysis. The median acetate level was 3.85 mg/mL (IQR: 2.76–4.82), propionate was 1.40 mg/mL (IQR:

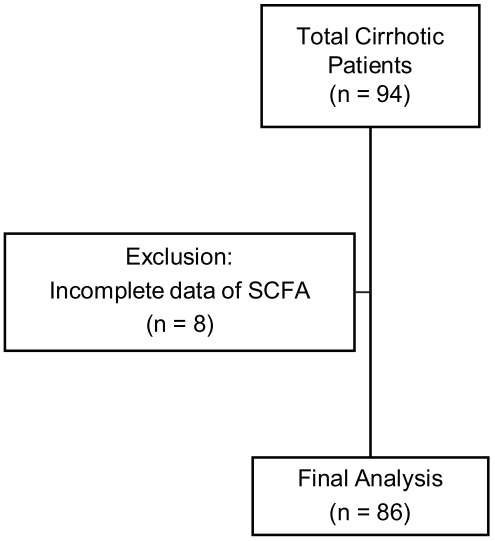

Figure 1. Flowchart of subject selection

Table 1. Baseline Characteristics of Patients with Cirrhosis

Variables	Total (n = 86)
Sex, n (%)	
Female	27 (31.4%)
Male	59 (68.6%)
Age, years, mean (SD)	53 (8.10)
BMI (kg/m2), mean (SD)	25.57 (5.43)
Overweight, n (%)	
No	46 (53.5%)
Yes	40 (46.5%)
Etiology, n (%)	
Non-B Non-C	14 (16.3%)
Hepatitis B	44 (51.2%)
Hepatitis C	25 (29.1%)
Hepatitis B and C	3 (3.5%)
Child-Pugh Score, median (IQR)	6 (5 – 8)
Child-Pugh, n (%)	
A	51 (59.3%)
B	32 (37.2%)
C	3 (3.5%)
Absolute Acetate (mg/mL), median (IQR)	3.85 (2.76 – 4.82)
Absolute Propionate (mg/mL), median (IQR)	1.40 (0.96 – 1.96)
Absolute Butyrate (mg/mL), median (IQR)	1.07 (0.64 – 1.57)
Total Absolute SCFA (mg/mL), median (IQR)	4.28 (3.05 – 5.45)
Acetate (%), median (IQR)	61 (55 – 66)
Propionate (%), median (IQR)	18.0 (16.0 – 22.0)
Butyrate (%), median (IQR)	12 (9 – 15)
Acetate proportion, n (%)	
Normal	83 (96.5%)
Abnormal	3 (3.5%)
Propionate proportion, n (%)	
Normal	76 (88.4%)
Abnormal	10 (11.6%)
Butyrate proportion, n (%)	
Normal	69 (80.2%)
Abnormal	17 (19.8%)
SCFA proportion, n (%)	
Normal	61 (70.9%)
Abnormal	25 (29.1%)
Energy (kcal)	1556 (1525 – 1625)
Energy Adequacy (%)	72.8 (69.9 – 84.6)
Energy Consumption, n (%)	
Adequate	30 (34.9%)
Insufficient	56 (65.1%)
Protein (g)	45.7 (40.4 – 53.0)
Protein Adequacy (%)	73.4 (63.2 – 84.4)
Protein Consumption, n (%)	
Adequate	30 (34.9%)
Insufficient	56 (65.1%)
Fat (g)	51.4 (43.3 – 60.9)
Fat Adequacy (%)	91.7 (73.1 – 105.6)
Fat Consumption, n (%)	
Adequate	55 (64%)
Insufficient	31 (36%)

Variables	Total (n = 86)
Carbohydrate (g)	232.7 (205.8 – 248.6)
Carbohydrate Adequacy (%)	69.1 (58.4 – 77.5)
Carbohydrate Consumption, n (%)	
Adequate	16 (18.6%)
Insufficient	70 (81.4%)
Fiber (g)	7.8 (6.7 – 9.2)
Fiber Adequacy (%)	26.6 (21.1 – 32.4)
Fiber Consumption, n (%)	
Adequate	0 (0%)
Insufficient	86 (100%)
Saturated Fat Consumption, n (%)	
Adequate	14 (16.3%)
Insufficient	72 (83.7%)
Simple Sugar Consumption, n (%)	
Adequate	8 (9.3%)
Insufficient	78 (90.7%)
Artificial Sweetener Consumption, n (%)	
Adequate	0 (0%)
Insufficient	86 (100%)

0.96–1.96), and butyrate was 1.07 mg/mL (IQR: 0.64–1.57). The median total SCFA level was 4.28 mg/mL (IQR: 3.05–5.45). As percentages, the median acetate was 61% (IQR: 55%–66%), propionate 18% (IQR: 16%–22%), and butyrate 12% (IQR: 9%–15%). Most patients had normal SCFA levels for acetate (96.5%), propionate (88.4%), and butyrate (80.2%).

The dot plot shows that most cirrhosis patients have SCFA levels within the normal range. Median proportions were 61% for acetate (normal: 40–75%), 18% for propionate (normal: 9–29%), and 12% for butyrate (normal: 9–37%). While SCFA levels were generally normal, some patients had increased propionate and decreased butyrate (**Figure 2**).

Comparison of SCFA levels between HE and non-HE groups showed no significant differences. The median acetate level was 3.78 mg/mL (IQR: 2.79–5.20) in the HE group and 3.85 mg/mL (IQR: 2.61–4.82) in the non-HE group. Propionate levels were 1.49 mg/mL (IQR: 0.92–1.82) in the HE group and 1.38 mg/mL (IQR: 0.96–1.07) in the non-HE group.

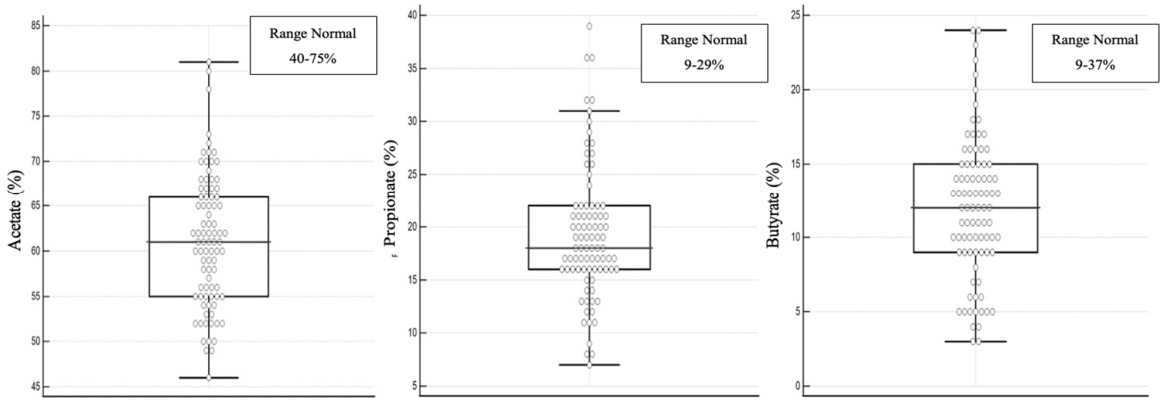

Figure 2. Dot Plot of SCFA Proportion Distribution (Acetate, Propionate, Butyrate) in Cirrhosis Patients

Butyrate levels were 0.93 mg/mL (IQR: 0.61–1.44) in the HE group and 1.07 mg/mL (IQR: 0.68–1.74) in the non-HE group. The median total SCFA level was 4.58 mg/mL (IQR: 3.39–5.40) in the HE group and 4.26 mg/mL (IQR: 2.97–5.73) in the non-HE group. Statistical tests showed no significant differences between the HE and non-HE groups ($p > 0.05$) (**Figure 3**).

Prevalence of Hepatic Encephalopathy in Patients with Cirrhosis

Among all patients with cirrhosis, the prevalences of covert HE and overt HE were 17 (19.8%) and 3 (3.5%), respectively, in this study. Overall, the prevalence of HE in patients with cirrhosis in this study was 23.3%. (**Figure 4**)

Bivariate Analysis of Demographic, Diet, and SCFA Parameters on the Occurrence of Hepatic Encephalopathy

Bivariate analysis did not reveal any significant differences in HE occurrence based on sex, overweight status, hepatitis etiology, cirrhosis status, or SCFA levels. There were also no significant differences in the consumption of energy, protein, fat, carbohydrates, fiber, saturated fat, simple sugars, or artificial sweeteners between the HE and non-HE groups. Only Child-Pugh showed a significant association with HE ($p<0.05$) (**Table 2**).

Final Model of Multivariate Analysis of SCFA on the Occurrence of Hepatic Encephalopathy

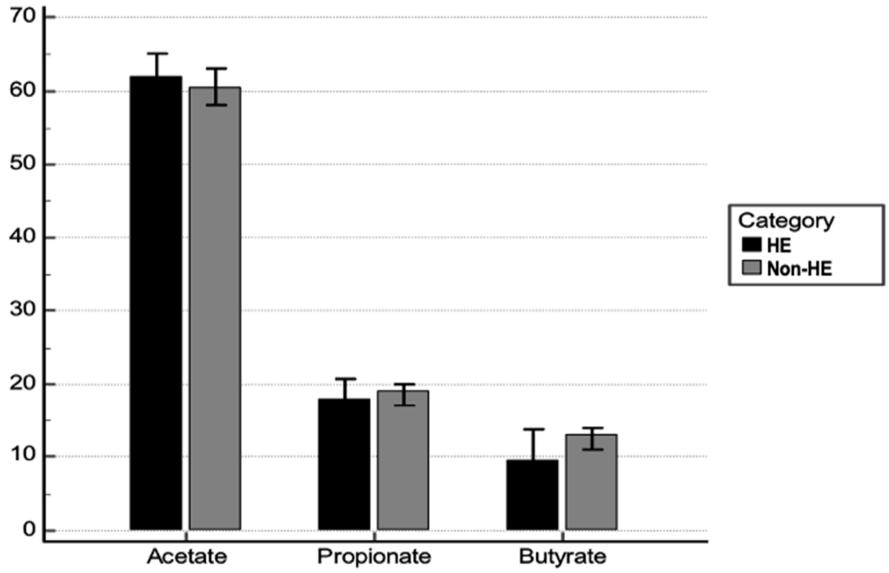

Figure 3. Proportion of SCFA among HE and non-HE groups

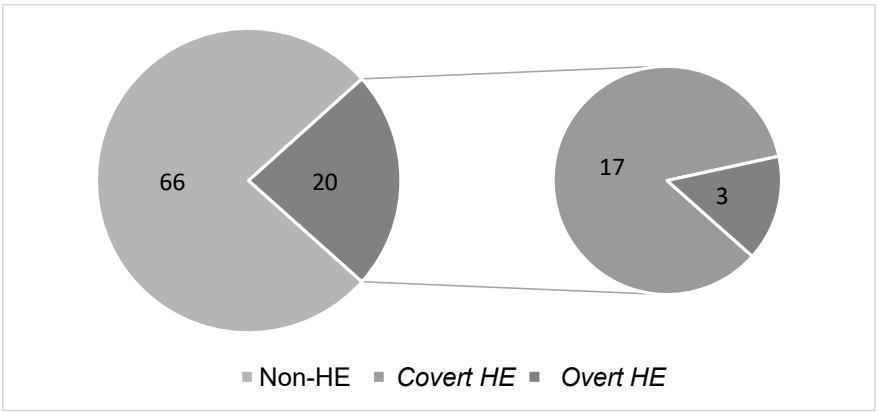

Figure 4. Proportion of Severity Grades of Hepatic Encephalopathy in Patients with Cirrhosis

Table 2. Bivariate Analysis of Demographic, SCFA, and Diet Parameters on the Occurrence of Hepatic Encephalopathy.

Variables	Non-HE (n = 66)	HE (n = 20)	Crude Prevalence Ratio (CI 95%)	p
Proportion of acetate, n (%)				
Normal	65 (78.3%)	18 (21.7%)	-	-
Abnormal	1 (33.3%)	2 (66.7%)	3.07 (0.71 – 13.25)	0.132
Proportion of propionate, n (%)				
Normal	59 (77.6%)	17 (22.4%)	-	-
Abnormal	7 (70.0%)	3 (30.0%)	1.34 (0.39 – 4.58)	0.639
Proportion of butyrate, n (%)				
Normal	55 (79.9%)	14 (20.3%)	-	-
Abnormal	11 (64.7%)	6 (35.3%)	1.74 (0.67 – 4.53)	0.257
Proportion of SCFA, n (%)				
Normal	48 (78.7%)	13 (21.3%)	-	-
Abnormal	18 (72.0%)	7 (28.0%)	1.31 (0.52 – 3.29)	0.560
Child-Pugh, n (%)				
A	45 (88.2%)	6 (11.8%)	-	-
B	20 (62.5%)	12 (37.5%)	3.19 (1.19 – 8.49)	0.020
C	1 (33.3%)	2 (66.7%)	5.67 (1.14 – 28.08)	0.034
Protein Consumption, n (%)				
Adequate	26 (86.7%)	4 (13.3%)	-	-
Insufficient	40 (71.4%)	16 (28.6%)	2.14 (0.72 – 6.41)	0.173
Fat Consumption, n (%)				
Adequate	39 (70/9%)	16 (29.1%)	-	-
Insufficient	27 (87.1%)	4 (12.9%)	0.44 (0.15 – 1.33)	0.146
Carbohydrate Consumption, n (%)				
Adequate	10 (62.5%)	6 (37.5%)	-	-
Insufficient	56 (80.0%)	14 (20.0%)	0.53 (0.21 – 1.39)	0.198
Fiber Consumption, n (%)				
Adequate	0 (0%)	0 (0%)	-	-
Insufficient	66 (76.7%)	20 (23.3%)	-	-
Saturated Fat Consumption, n (%)				
Adequate	11 (78.6%)	3 (21.4%)	-	-
Insufficient	55 (76.4%)	17 (23.6%)	1.10 (0.32 – 3.76)	0.877
Simple Sugar Consumption, n (%)				
Adequate	6 (75.0%)	2 (25.0%)	-	-
Insufficient	60 (76.9%)	18 (23.1%)	0.92 (0.21 – 3.98)	0.914
Artificial Sweetener Consumption, n (%)				
Adequate	0 (0%)	0 (0%)	-	-
Insufficient	66 (76.7%)	20 (23.3%)	-	-

Table 3. Multivariate Analysis of SCFA on the Occurrence of Hepatic Encephalopathy.

Final Model	Adjusted Prevalence Ratio (95% CI)	p
Proportion of SCFA outside the reference range	1.98 (0.75 – 5.24) Adjusted with Child-Pugh	0.171
Proportion of acetate outside the reference range	2.06 (0.40 – 10.62) Adjusted with Child-Pugh	0.388
Proportion of butyrate outside the reference range	2.02 (0.76 – 5.39) Adjusted with Child-Pugh	0.158

The multivariate analysis in the final model of this study did not show any association between SCFA and the occurrence of HE in patients with cirrhosis after controlling with the Child-Pugh. The p-values for SCFA variables were above the threshold of significance ($p > 0.05$) (**Table 3**).

DISCUSSION

Baseline Characteristics of Patients with Liver Cirrhosis

This study found that hepatitis B was the dominant etiology (51.2%) among patients with cirrhosis, followed by hepatitis C (29.1%), non-hepatitis B and C (16.3%), and a combination of hepatitis B and C (3.5%). Similar findings were reported in studies in Indonesia, where hospitalized patients with cirrhosis had reactive HbsAg in 53.7% of the study subjects and positive anti-HCV in 22%.[12] A study examining the profile of patients with cirrhosis who underwent esophageal variceal ligation at Cipto Mangunkusumo Hospital in 2016-2017 also showed a similar etiology pattern, i.e., 51.8% was hepatitis B, 27.8% was hepatitis C, 20.1% was non-hepatitis B and C, and 1% was hepatitis B and C.[13]

The percentages of patients with cirrhosis in this study with Child-Pugh A, Child-Pugh B, and Child-Pugh C scores were 59.3%, 37.2%, and 3.5%, respectively. This distribution differs from a study examining the characteristics of patients with cirrhosis in the Czech Republic, which found Child-Pugh A, Child-Pugh B, and Child-Pugh C scores of 41%, 34%, and 25%, respectively.[14] Another study in China assessing patients with cirrhosis due to hepatitis infection found approximately one-third (30.6%) of patients had Child-Pugh A cirrhosis, while 53.8% had Child-Pugh B, and 15.6% had Child-Pugh C cirrhosis.[15] These differences arise due to different study settings, i.e., the respective studies conducted examinations of patients with cirrhosis in inpatient settings, while our study examined patients in outpatient setting, and hence, higher level of severity was observed in the inpatient group. In patients with HE, a higher Child-Pugh score was found compared to patients without HE. Research by Maggi et al. also showed that Child-Pugh C scores were independently associated with progressive HE development.[16]

Characteristics of SCFA in Patients with Liver Cirrhosis

In this study, the median absolute SCFA value was found to be 4.28 (3.05-5.45) mg/mL, with the largest components being acetate with a median of 3.85 (2.76-4.82) mg/mL, propionate with a median of 1.40 (0.96-1.96) mg/mL, and butyrate with a median of 1.07 (0.64-1.57) mg/mL, respectively. A study by Cao et al.[16] showed that fecal SCFA levels in patients with cirrhosis, whether due to alcohol consumption or metabolic disorders, exhibited a similar pattern where SCFA composition was arranged in descending order of acetate, propionate, and butyrate. A similar pattern was also observed in the SCFA composition of feces from patients without cirrhosis. The study that was conducted by Bloom et al.,[7] which examined six types of SCFA in feces from patients with alcohol-induced cirrhosis (45%) and non-alcoholic steatohepatitis (26%), also found that the three largest groups of fecal SCFA were acetate, propionate, and butyrate, respectively.

In this study, the largest proportion found was acetate at 61.0% (55.0-66.0), followed by propionate at 18.0% (16.0-22.0), and butyrate at 12.0% (9.0-15.0). In patients with cirrhosis due to alcohol, the percentages of acetate, propionate, and butyrate were 64.5% (60.2-66.9), 21.0% (18.6-24.7), and 14.8% (11.3-17.8), respectively. Similar percentages were reported in patients with cirrhosis due to metabolic disorders, i.e., 62.8% (56.3-66.8) for acetate, 22.7% (19.8-26.3) for propionate, and 12.9% (11.3-15.0) for butyrate.[17] The physiological proportion of SCFA formation in the human body is typically 60:20:18 for acetate, propionate, and butyrate according to research by Cao et al. (61.8:19.1:17.1).[18] No changes in proportions were observed in this study or previous research, indicating that dysbiosis does not alter the proportions of fecal SCFA but rather reduces the absolute amount of fecal SCFA, as found by Jin et al.[19] and Wu et al.[20] Studies have identified specific microbiota profiles in cirrhosis, independent of etiology, dominated by *Fusobacteria, Proteobacteria, Enterococcaceae,* and *Streptococcaceae,* with a relative decrease in *Bacteroidetes, Ruminococcus, Roseburia, Veillonellaceae,* and *Lachnospiraceae*.[21] This shift disrupts *Firmicutes, Bacteroidetes, Actinobacteria*, and *Proteobacteria,* which are responsible for producing SCFA in adults.[22]

Characteristics of Energy Needs and Diet in Patients with Liver Cirrhosis

This study found that energy fulfillment in the HE groups had a median of 85%, significantly higher than the non-HE groups, which had a median of 72.0%. Energy fulfillment is determined by body weight and daily activity levels.[23] In the HE group, a lower median BMI was found compared to the non-HE groups. Patients with HE tend to have difficulties in daily activities, including eating, thus their energy intake is often closely monitored by caregivers or family members.[24,25] However, it is important to note that nutritional data in this study were collected using a recall questionnaire, and therefore, recall bias may occur in the completion of the respective questionnaire.

This study found that adequate protein consumption occurred in 39.4% of cirrhosis patients without HE and 20.0% of cirrhosis patients with HE. The median protein fulfillment differed significantly between cirrhosis patients without HE and those with HE (74.7% vs. 49.8%), although the median protein intake between patients without HE and with HE was only slightly different (47g vs. 43.9g). The minimum protein requirement needed to maintain nitrogen balance is used to determine protein needs. Nitrogen balance is achieved in alcoholic cirrhosis with an intake of 0.8 g/kg BW/day.[26] Additionally, this study shows that individuals with cirrhosis can utilize protein up to 1.8 g/kg BW/day. There was previously a debate regarding whether patients with HE should temporarily restrict their protein intake to reduce ammonia production and the conversion of protein into aromatic amino acids; however, HE is not triggered by normal to high protein intake and may even be beneficial for mental health.[27] Protein consumption of 1.2–2.0 g/kg BW/day is recommended for patients with liver cirrhosis to avoid muscle loss and reverse muscle loss in patients with sarcopenia. As previously shown, sarcopenia negatively impacts clinical outcomes, with its prevalence increasing alongside the severity of liver disease.[28]

Prevalence of Hepatic Encephalopathy in Patients with Liver Cirrhosis

The prevalence of HE in this study was 23.3%. The prevalence of overt HE (3.5%) was lower compared to covert HE (19.8%). This is consistent with previous studies that found minimum HE incidences ranging from 20.3% to 37% using the Psychometric Hepatic Encephalopathy Score (PHES).[29] The prevalence of overt HE in this study was lower than in other studies that reported an incidence of 21% over a follow-up period of 548±281 days.[30] The proportion of patients with Child-Pugh A cirrhosis experiencing their first episode of overt HE was 10% within 1 year, increasing to 25% in patients with Child-Pugh B cirrhosis.[29] In those studies, patients with cirrhosis were examined in the inpatient care setting, while our study focused on outpatients, which may explain the higher level of severity observed in the inpatient group.

Relationship Between SCFA and Hepatic Encephalopathy in Patients with Liver Cirrhosis

Analysis of SCFA levels in patients with liver cirrhosis revealed no significant difference between the HE and non-HE groups. Previous studies have also shown that fecal SCFA levels, when compared with controlled rifaximin use, were not associated with HE occurrence.[7] Similarly, Wang et al.[31] also found no significant differences in fecal SCFA levels between the HE and non-HE groups.

SCFA, which helps maintain the blood-brain barrier, is expected to be lower in patients with cirrhosis who experience HE due to neurological disturbances.[6] This is consistent with Bloom et al.'s findings that there is an inverse correlation between the severity of cirrhosis and lower concentrations of specific fecal SCFA (acetate, propionate, butyrate, isobutyrate, valerate, and succinate) in patients with a history of overt HE, thus supporting the important role of SCFA in HE.[7] On the other hand, Cao et al. found that absolute fecal SCFA amounts, acetate, butyrate, and isoacid were higher in patients with metabolically related cirrhosis and minimal

HE compared to those without HE.[17] One explanation for the decreased quantity of SCFA in the non-HE group is the difference in median fiber consumption that was found through questionnaires. The HE groups consumed a median of 8.1 (6.7-8.8) g of fiber, while the non-HE groups consumed 7.7 g (6.7-9.2) g. The main precursor of SCFA is non-digestible saccharides, which are reflected in daily fiber consumption.[32,33] Although the total median absolute was higher in the HE groups, abnormalities in the proportion of SCFA, acetate, butyrate, and propionate were higher in the HE groups.

Another possible explanation for the lack of difference between patients with HE and without HE is the exclusion of patients with cirrhosis. Hospitalized patients tend to have more severe cirrhosis and HE, potentially reflecting more severe dysbiosis.[16] Additionally, the role of other microorganisms, such as fungi, parasites, and bacteriophages in the microbiota is not fully understood yet, and their influence on SCFA formation may affect the outcomes of this study. [34]

Abnormal proportions of total SCFA, acetate, and butyrate were found to be higher in the HE group compared to the non-HE groups, although these differences were not statistically significant. Multivariable analysis showed that abnormal proportions of total SCFA were associated with a higher prevalence of HE [adjusted PR 1.98; 95% CI: 0.75–5.24; p = 0.171], as were abnormal acetate proportions [adjusted PR 2.06; 95% CI: 0.40–10.62; p = 0.388] and abnormal butyrate proportions [adjusted PR 2.02; 95% CI: 0.76–5.39; p = 0.158], after adjusting with Child-Pugh as potential confounder. However, none of these associations reached statistical significance.

These findings suggest that differences in the relative proportions of SCFA components, particularly acetate and butyrate, may be associated with the presence of hepatic encephalopathy in patients with cirrhosis. This association appears to be more relevant than the absolute concentrations of each SCFA. Based on the available literature, similar findings have not been previously reported, which highlights the potential novelty of these results. Further research is needed to explore this relationship.

Strengths and Limitations of the Study

This study is the first in Indonesia to investigate the role of SCFA in hepatic encephalopathy complications in patients with liver cirrhosis. Previous studies that were conducted by Fitriakusumah et al.[35] and Jasirwan et al.[36] at the Hepatobiliary Division of Cipto Mangunkusumo Hospital, Faculty of Medicine, Universitas Indonesia, focused on gut microbiota composition without examining SCFA production, while Astari et al.[37] studied SCFA levels in patients with fatty liver disease. Unlike fatty liver disease, liver cirrhosis involves different SCFA composition, production, and metabolism due to more severe inflammation and fibrosis. Although no direct relationship was found, confounding variables such as liver function were controlled. Further research on inpatients with more severe liver dysfunction is needed to have a more comprehensive understanding.

CONCLUSION

The prevalence of hepatic encephalopathy (HE) among patients with cirrhosis at the Hepatobiliary Outpatient Clinic of Cipto Mangunkusumo Hospital is 23.3%, with 19.8% classified as covert HE and 3.5% as overt HE. The proportion of patients with abnormal fecal SCFA levels was higher among those with hepatic encephalopathy compared to those without, although the difference was not statistically significant. Recommendations include conducting further research to assess the relationship between fecal SCFA composition in patients with cirrhosis who experience HE and those undergoing inpatient care, alongside prospective cohort studies that rigorously control dietary aspects when evaluating fecal SCFA in patients with cirrhosis.

AUTHOR'S CONTRIBUTION

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Mochamad Anief Ferdianto. The first draft of the manuscript was written by Mochamad

Anief Ferdianto and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Conceptualization: Juferdy Kurniawan, Hasan Maulahela, Cleopas Martin Rumende, Imam Subekti, Ikhwan Rinaldi, Hamzah Shatri, Cosmas Rinaldi A Lesmana.

Data Availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

ACKNOWLEDGMENTS

The authors would like to thank the patients who participated in this study. The authors also express their gratitude to Gita Aprilicia for her assistance in manuscript preparation. This study was self-funded, and no funds, grants, or other support were received during the preparation of this manuscript.

FUNDING

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

REFERENCES

1. Ferenci P. Hepatic encephalopathy. Gastroenterol Rep. 2017 May;5(2):138–47.
2. Hadjihambi A, Arias N, Sheikh M, et al. Hepatic encephalopathy: a critical current review. Hepatol Int. 2017 Aug 2;12(Suppl 1):135–47.
3. Hasan I, Araminta A. Ensefalopati hepatik: apa, mengapa, dan bagaimana? Medicinus. 2014 Desember;27(3):1–8.
4. de la Cuesta-Zuluaga J, Mueller NT, Álvarez-Quintero R, et al. Higher Fecal Short-Chain Fatty Acid Levels Are Associated with Gut Microbiome Dysbiosis, Obesity, Hypertension and Cardiometabolic Disease Risk Factors. Nutrients. 2018 Dec 27;11(1):51.
5. Tan C, Wu Q, Wang H, et al. Dysbiosis of gut microbiota and short-chain fatty acids in acute ischemic stroke and the subsequent risk for poor functional outcomes. JPEN J Parenter Enteral Nutr. 2021 Mar;45(3):518–29.
6. Silva YP, Bernardi A, Frozza RL. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. Front Endocrinol [Internet]. 2020 [cited 2022 Nov 29];11. Available from: https://www.frontiersin.org/articles/10.3389/fendo.2020.00025
7. Bloom PP, Luévano JM, Miller KJ, et al. Deep stool microbiome analysis in cirrhosis reveals an association between short-chain fatty acids and hepatic encephalopathy. Ann Hepatol. 2021 Nov;25:100333.
8. Clausen MR, Mortensen PB, Bendtsen F. Serum levels of short-chain fatty acids in cirrhosis and hepatic coma. Hepatol Baltim Md. 1991 Dec;14(6):1040–5.
9. Hasan N, Yang H. Factors affecting the composition of the gut microbiota, and its modulation. PeerJ. 2019 Aug 16;7:e7502.
10. Wen L, Duffy A. Factors Influencing the Gut Microbiota, Inflammation, and Type 2 Diabetes. J Nutr. 2017 Jul;147(7):1468S-1475S.
11. Riggio O, Ridola L, Pasquale C. Hepatic encephalopathy therapy: An overview. World J Gastrointest Pharmacol Ther. 2010 Apr 6;1(2):54–63.
12. Darnindro N, Manurung A, Mulyana E, et al. Clinical Characteristics of Liver Cirrhosis Patients in Internal Medicine Inpatient Ward of Fatmawati General Hospital and Factors Affecting Mortality during Hospitalization. Indones J Gastroenterol Hepatol Dig Endosc. 2021 May 12;22(1):3–8.
13. Kalista KF, Lesmana CRA, Sulaiman AS, et al. Clinical profile of cirrhotic patients with esophageal varices who have undergone band ligation in Cipto Mangunkusumo Hospital. J Penyakit Dalam Indones. 2019;6(1):7.
14. Brůha R, Jachymova M, Petrtyl J, et al. Osteopontin: A non-invasive parameter of portal hypertension and prognostic marker of cirrhosis. World J Gastroenterol. 2016 Mar 28;22:3441–50.
15. Xie W, Cao Y, Xu M, et al. Prognostic Significance of Elevated Cholestatic Enzymes for Fibrosis and Hepatocellular Carcinoma in Hospital Discharged Chronic Viral Hepatitis Patients. Sci Rep. 2017 Dec 1;7.
16. Maggi DC, Borgonovo A, Bansho ET, et al. Serial assessment of hepatic encephalopathy in patients hospitalised for acute decompensation of cirrhosis. Ann Hepatol. 2019 Mar 1;18(2):331–7.
17. Cao X, Zolnikova O, Maslennikov R, et al. Differences in Fecal Short-Chain Fatty Acids between Alcoholic Fatty Liver-Induced Cirrhosis and Non-alcoholic (Metabolically Associated) Fatty Liver-Induced Cirrhosis. Metabolites. 2023 Jul;13(7):859.
18. Roy CC, Kien CL, Bouthillier L, et al. Short-chain fatty acids: ready for prime time? Nutr Clin Pract. 2006 Aug;21(4):351–66.
19. Jin M, Kalainy S, Baskota N, et al. Faecal microbiota from patients with cirrhosis has a low capacity to ferment non-digestible carbohydrates into short-chain fatty acids. Liver Int. 2019 Aug;39(8):1437–47.
20. Wu Z, Zhou H, Liu D, et al. Alterations in the gut

microbiota and the efficacy of adjuvant probiotic therapy in liver cirrhosis. Front Cell Infect Microbiol [Internet]. 2023 [cited 2024 Feb 18];13. Available from: https://www.frontiersin.org/articles/10.3389/fcimb.2023.1218552

21. Trebicka J, Macnaughtan J, Schnabl B, et al. The microbiota in cirrhosis and its role in hepatic decompensation. J Hepatol. 2021 Jul 1;75:S67–81.
22. Fusco W, Lorenzo MB, Cintoni M, et al. Short-chain fatty-acid-producing bacteria: key components of the human gut microbiota. Nutrients. 2023 May 6;15(9):2211.
23. Gerrior S, Juan W, Peter B. An easy approach to calculating estimated energy requirements. Prev Chronic Dis. 2006 Sep 15;3(4): A129.
24. Saleh ZM, Salim NE, Nikirk S, et al. Burden of cirrhosis on patients and caregivers. Hepatol Commun. 2022 Jul 6;6(10):2827–35.
25. Volk ML. The emotional burden of caregiving for patients with cirrhosis. Hepatol Commun. 2020 Aug;4(8):1107.
26. Park JH, Kang M, Jun DW, et al. Determining whether low protein intake (<1.0 g/kg) is a risk factor for malnutrition in patients with cirrhosis. J Clin Med [Internet]. 2021 May [cited 2024 Feb 21];10(10). Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8156669/
27. Nielsen K, Kondrup J, Martinsen L, et al. Long-term oral refeeding of patients with cirrhosis of the liver. Br J Nutr. 1995 Oct;74(4):557–67.
28. Dhaliwal A, Armstrong MJ. Sarcopenia in cirrhosis: A practical overview. Clin Med. 2020 Sep;20(5):489–92.
29. Louissaint J, Deutsch-Link S, Tapper EB. Changing epidemiology of cirrhosis and hepatic encephalopathy. Clin Gastroenterol Hepatol Off Clin Pract J Am Gastroenterol Assoc. 2022 Aug;20(8 Suppl):S1–8.
30. Tapper EB, Zhao L, Nikirk S, et al. The incidence and bedside predictors of the first episode of overt hepatic encephalopathy in patients with cirrhosis. Am J Gastroenterol. 2020 Dec;115(12):2017–25.
31. Wang Q, Chen C, Zuo S, et al. Integrative analysis of the gut microbiota and faecal and serum short-chain fatty acids and tryptophan metabolites in patients with cirrhosis and hepatic encephalopathy. J Transl Med. 2023 Jun 17;21(1):395.
32. Tan J, McKenzie C, Potamitis M, et al. The Role of Short-Chain Fatty Acids in Health and Disease. In: Advances in Immunology [Internet]. Elsevier; 2014 [cited 2022 Dec 8]. p. 91–119. Available from: https://linkinghub.elsevier.com/retrieve/pii/B9780128001004000039
33. Van Der Hee B, Wells JM. Microbial regulation of host physiology by short-chain fatty acids. Trends Microbiol. 2021 Aug;29(8):700–12.
34. Garcia-Bonete MJ, Rajan A, Suriano F, et al. The Underrated Gut Microbiota Helminths, Bacteriophages, Fungi, and Archaea. Life. 2023 Aug 18;13(8):1765.
35. Fitriakusumah Y, Lesmana CRA, Bastian WP, et al. The role of Small Intestinal Bacterial Overgrowth (SIBO) in Non-alcoholic Fatty Liver Disease (NAFLD) patients evaluated using Controlled Attenuation Parameter (CAP) Transient Elastography (TE): a tertiary referral center experience. BMC Gastroenterol. 2019 Mar 20;19(1):43. doi: 10.1186/s12876-019-0960-x. PMID: 30894137; PMCID: PMC6427876.
36. Jasirwan COM, Muradi A, Hasan I, et al. Correlation of gut Firmicutes/Bacteroidetes ratio with fibrosis and steatosis stratified by body mass index in patients with non-alcoholic fatty liver disease. Biosci Microbiota Food Health. 2021;40(1):50-58. doi: 10.12938/bmfh.2020-046. Epub 2020 Sep 15. PMID: 33520569; PMCID: PMC7817510.
37. Astari D, Lesmana CRA, Simadibrata MK. Correlation of short-chain fatty acid (SCFA) levels with transient elastography values and controlled attenuation parameters in non-alcoholic fatty liver disease (NAFLD) patients. Jakarta: Internal Medicine Study Program; 2023. T23492fk. xvi, 56 pages; 21x30 cm.