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Original Article

Fecal short-chain fatty acids level and pediatric relapsing nephrotic syndrome

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Abstract

Background The gut microbiota has a potential role in the development of nephrotic syndrome. Fecal short-chain fatty acid (SCFA) levels are representative of gut microbiota activity.

Objective To assess for potential associations of fecal short-chain fatty acid levels in pediatric relapsing nephrotic syndrome.

Methods This cross-sectional study was done on patients at the Pediatric Nephrology Subdivision of Prof. Dr. R.D. Kandou General Hospital, a referral hospital in Manado, Indonesia. Subjects were 25 patients aged less than 18 years with nephrotic syndrome (NS). We compared the levels of fecal acetic acid, propionic acid, butyric acid percentage, absolute butyric acid, and total short-chain fatty acid between children with relapsing and non-relapsing NS. A receiver operating characteristic (ROC) curve analysis was conducted to determine the significant SCFA cut-off level to diagnose NS.

Results Comparison of fecal SCFAs between relapsing and non-relapsing NS groups showed significantly lower butyric acid percentages, absolute butyric acid level, and total SCFAs levels in the relapsing NS group, but not in acetic acid or propionic acid levels. Further multivariate analysis did not show a significant difference in total SCFA levels between relapsing and non-relapsing NS. Absolute butyric acid level had the strongest association with relapsing NS, with the highest predictive score. The absolute butyric acid cut-off value of 0.85 mg/mL had a high sensitivity (90%) and high specificity (93.3%) for predicting relapsing nephrotic syndrome.

Conclusion Fecal acetic acid, propionic acid, and total short-chain fatty acid in stool are not associated with relapsing NS in children. However, fecal butyric acid measurements are inversely associated with relapsing NS. **[Paediatr Indones. 2024;64:332-8; DOI: 10.14238/pi64.4.2024.332-8**].

Keywords: *butyric acid; children; short-chain fatty acids; nephrotic syndrome*

The gut microbiota is a collection of complex microorganisms found in the gastrointestinal tract.[1] Gut microbiota plays many important roles in maintaining normal digestive function and overall body health.[2] Gut function is associated with kidney function, in which disturbances in gut microbiota (dysbiosis) may affect kidney function, and *vice versa*. This relationship has been extensively studied and referred to as the "gut-kidney axis".[3]

Recently, dysbiosis of gut microbiota has been found to play a role in immune regulation and inflammation, contributing to the development of several diseases, including nephrotic syndrome. Idiopathic nephrotic syndrome is one of the most common glomerular diseases that can cause pediatric glomerulopathy.[4] Several studies suggest that nephrotic syndrome may be induced by T regulatory (Treg) cell dysfunction through two possible mechanisms. Treg cells may reduce the oxidative effects of circulating

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cells during the initial immune response, damaging podocytes. Another proposed mechanism is that suppression of Treg cells could lead to structural deformity of podocyte cells, which then induces proteinuria in nephrotic syndrome.[5]

Short-chain fatty acids (SCFAs), produced by gut microbiota, can induce the production and differentiation of Treg cells. Therefore, the gut microbiota has a potential role in the development of nephrotic syndrome.[6] Currently, a liquid chromatography method can be used to measure fecal SCFAs as a gut microbiota activity biomarker test.[7] However, there have been limited studies on the relationship between gut microbiota and nephrotic syndrome, especially in children. As such, we aimed to analyze for possible associations between fecal SCFA levels and relapsing NS in children.

Methods

We conducted a cross-sectional study in children diagnosed with steroid-sensitive nephrotic syndrome treated at the Pediatric Nephrology Subdivision of Prof. Dr. R.D. Kandou General Hospital, Manado between August and October 2022. We included patients aged 1-18 years who were diagnosed with steroid-sensitive nephrotic syndrome for at least six months. The subjects were divided into two groups: relapsing and non-relapsing nephrotic syndrome. We excluded patients with congenital, secondary, or steroid-resistant nephrotic syndrome, chronic kidney disease, diabetes, or autoimmune disease, and patients who consumed antibiotics, prebiotics, probiotics, or symbiotics in the 4 weeks prior to the study. Patients' data relevant to the study including age, sex, nutritional status, type of delivery, and albumin level at onset, were obtained from questionnaires answered by parents or legal guardians. We took anthropometric measurements including height and weight to determine the patients' nutritional status.

Stool specimens from patients in the hospital were sent to the laboratory. A 0.1 gram portion of each stool specimen was put into a test tube, to which 0.1 mM perchloric acid and 3% phenol were added. The specimen was heated to 80^{o}C for 15 minutes, then centrifuged for 10 minutes. The supernatant from the centrifugation process was then filtered. Fecal SCFAs, including butyric acid, acetic acid, and propionic acid, were measured using high-performance liquid chromatography. The results included acetic acid percentage, butyric acid percentage, absolute butyric acid, and total short-chain fatty acid levels.

Questionnaire data, anthropometric measurements, and SCFA levels were analyzed using *SPSS version 25.0* (*IBM*, Armonk, New York). Bivariate analysis was conducted to compare fecal short-chain fatty acid levels between groups. Multivariate analysis with logistic regression was also conducted to measure the influence of the independent variables (acetic acid percentage, butyric acid percentage, absolute butyric acid, and total short-chain fatty acid level) on the dependent variable (relapsing or non-relapsing nephrotic syndrome). ROC curve analysis was conducted to determine the significant short-chain fatty acid cut-off level for predicting relapsing NS.

Results

Of the 25 children included in the study, 15 (60%) had relapsing nephrotic syndrome and 10 (40%) had non-relapsing NS. Most subjects (80%) were male. The characteristics of the subjects are summarized in **Table 1**.

The characteristics of patients according to NS type are shown in **Table 2**. There were no significant differences in terms of gender (P=0.307), age (P=0.376), type of delivery (P=0.096), nutritional status (P=0.819), or albumin levels (P=0.531) between the relapsing and non-relapsing NS groups.

Bivariate analysis was conducted using T-test to compare the fecal SCFA levels between subjects in the relapsing and non-relapsing NS groups. We found no significant mean differences in acetic acid % (95%CI -2,255 to 14,389; P=0.145) OR propionic acid % (95%CI -3,827 to 5,561; P=-0.706) between the two groups. However, mean butyric acid % (95%CI 0,428 to 5,905; P=0.025), absolute butyric acid (95%CI 0.453 to 1.547; P=0.001), and total short-chain fatty acid (95%CI 0.086 to 4.541; P=0.042) were significantly different between the two groups (**Table 3**). Compared to the non-relapsing NS group, these parameters were lower in the relapsing NS group: mean butyric acid % (6.1% *vs.* 9.3%), absolute butyric acid level (0.44 *vs.* 1.44 mg/mL), and total

Table 1. Characteristics of subjects

Characteristics	N=25
Sex, n	
Male	20
Female	5
Mean age (SD), years	8 (3.4)
Type of delivery, n	
Vaginal	10
Caesarean section	15
Nutritional status, n	
Underweight	3
Normal	18
Overweight	3
Obese	1
Mean albumin (SD), g/dL	1.57 (0.53)
Diagnosis, n	
Relapsing nephrotic syndrome	15
Non-relapsing nephrotic syndrome	10
Organic acid levels in stool	
Mean acetic acid level (SD), %	60.5 (10.20)
Mean propionic acid level (SD), %	19.2 (5.4)
Mean butyric acid level (SD), %	7.4 (3.5)
Mean absolute butyric acid level (SD), mg/mL	0.8 (0.8)
Mean total short-chain fatty acid level (SD), mg/mL	4.9 (2.8)

Table 2. Subject characteristics by NS type

Variables	Relapsing NS (n=15)	Non-relapsing NS (n=10)	P value
Sex, n			0.307
Male	11	9	
Female	4	1	
Mean age (SD), years	9.7 (3.4)	5.8 (2.7)	0.376
Type of delivery, n			0.096
Vaginal	4	4	
Caesarean section	11	6	
Nutritional status, n			0.819
Underweight	2	1	
Normal	10	8	
Overweight	2	1	
Obesity	1	0	
Mean albumin (SD), g/dL	1.4 (0.6)	1.7 (0.3)	0.531

SCFA level (4 *vs.* 6.3 mg/dL).

Logistic regression analysis was conducted for variables with P values of <0.25 in the bivariate analysis: acetic acid percentage, butyric acid percentage, absolute butyric acid, and total SCFA. Only butyric acid percentage (P=0.049) and absolute butyric acid (P=0.008) maintained their statistical significance in the multivariate analysis (**Table 4**).

The association between fecal absolute butyric acid level and the probability of relapsing nephrotic syndrome is illustrated in **Figure 1**. The predicted probability of relapsing NS increased as the butyric acid level in stool decreased, at butyric acid levels of 0-1.5 mg/mL. At butyric acid levels above 1.5 mg/mL, the probability of relapsing nephrotic syndrome was close to 0%.

ROC analysis (Figure 2) on absolute butyric acid level yielded an area under the curve (AUC) of 0.917 (95%CI 0.770 to 1.000). The absolute butyric acid cut-off value of 0.85 mg/mL resulted in 90% sensitivity and 93.3% specificity for predicting the incidence of relapsing NS.

Table 3. Bivariate analysis of fecal SCFAs in relapsing and non-relapsing NS

Variables	Relapsing NS (n=15)	Non-relapsing NS (n=10)	95%CI	P value
Mean acetic acid level (SD), %	58.1 (10.1)	64.2 (9.3)	-2.255 to 14.389	0.145
Mean propionic acid level (SD), %	18.9 (6.1)	19.8 (4.4)	-3.827 to 5.561	0.706
Mean butyric acid level (SD), %	6.1 (1.9)	9.3 (4.5)	0.428 to 5.905	0.025
Mean absolute butyric acid level (SD), mg/mL	0.44 (0.2)	1.44 (0.9)	0.453 to 1.547	0.001
Mean total SCFA level (SD), mg/dL	4.0 (1.7)	6.3 (3.5)	0.086 to 4.541	0.042

Table 4. Logistic regression analysis of organic acid levels in stool and types of nephrotic syndrome

Variables	Constant	Odds ratio (OR)	95%CI	Nagelkerke R square	P value
Acetic acid	-4.653	1.072	0.976 to 1.177	0.123	0.149
Butyric acid	-2.918	1.396	1.002 to 1.945	0.268	0.049
Absolute butyric acid	-4.728	1.721	1.153 to 2.569	0.692	0.008
Total SCFA	-2.170	1.424	0.977 to 2.075	0.220	0.066

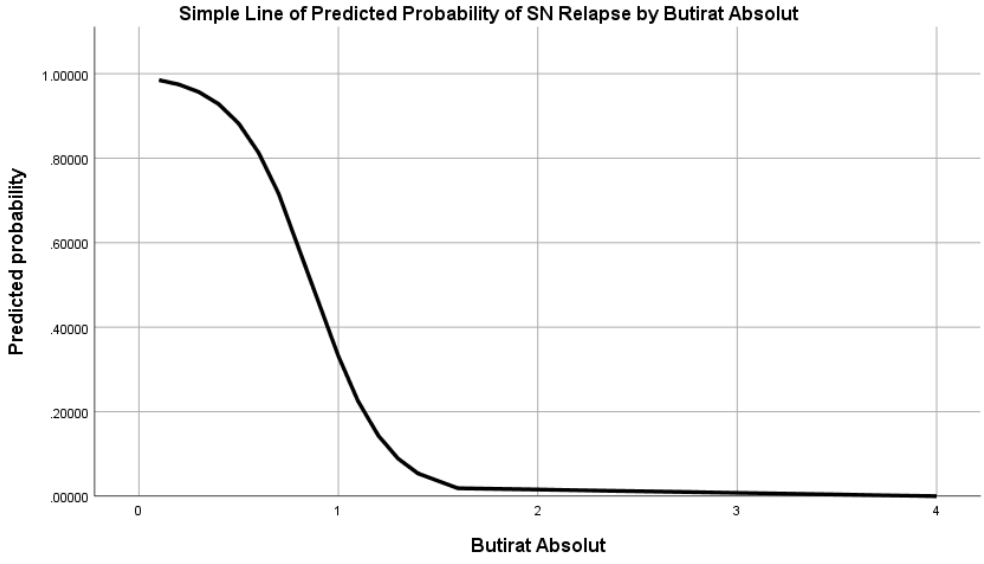

Figure 1. Predicted probability curve of relapsing nephrotic syndrome based on fecal absolute butyric acid level

Discussion

This cross-sectional study was done to determine the associations between fecal SCFA levels and the incidence of relapsing NS in children. Our 25 subjects comprised 15 relapsing NS patients and 10 non-relapsing NS patients. Subjects had a mean age of 8 years and most were male (80%). NS is known to be twice as common in males and is mostly found at the age of 2-6 years.[8] Most of our subjects were older than 5 years old. Previous studies identified several factors associated with relapse: young age at onset (<5 years), male sex, malnutrition, low albumin levels at onset, and slow response to steroids.[9,10] In addition, gut microbiota itself varies and is influenced by several factors, including gestational age, type of delivery, breastfeeding status, use of antibiotics, enterotype, genetics, diet, nutritional status, and exercise.[11] We compared factors that have been reported to influence the incidence of NS relapse (i.e., age, sex, nutritional status, albumin level at diagnosis) as well as factors that might affect the composition of intestinal

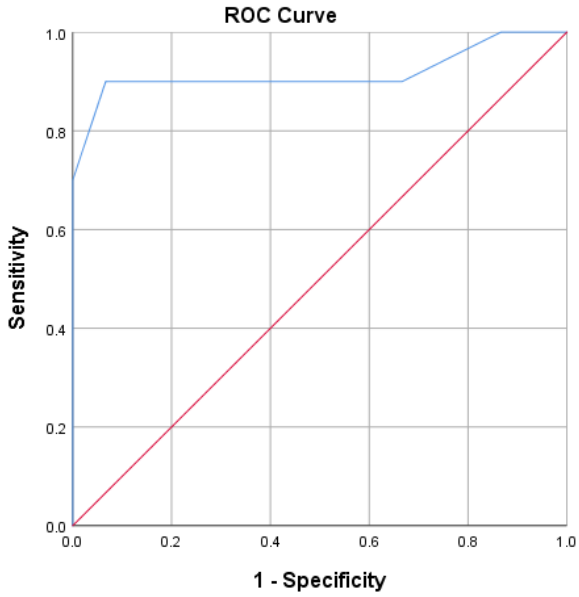

Figure 2. ROC curve of the fecal absolute butyric acid level in predicting relapsing NS

microbiota (i.e., age, sex, nutritional status, and type of delivery) in relapsing and non-relapsing NS groups. We found no significant difference between these factors in the two groups, so it can be assumed that the subjects in the two groups had the same risk factors and can be compared.

The fecal SCFAs studied here were acetic acid, propionic acid, and butyric acid, because these three types of organic acids comprise most of the total SCFA composition in the digestive tract. A balanced and healthy intestinal microbiota has a composition of 40-75% acetic acid, 9-29% propionic acid, and 9-37% butyric acid (absolute value: 0.8-4.8 mg/mL), with total normal SCFA levels of 4-18 mg/mL. Comparison of the mean fecal SCFAs level between the relapsing and non-relapsing NS groups showed significant differences in butyric acid percentage, absolute butyrate levels, and total SCFA, but no significant differences in the levels of acetic and propionic acids. Butyric acid % was significantly lower in the relapsing NS group. The mean difference in butyric acid levels between the relapsing and non-relapsing NS groups was also quite large (3.1% or 1 mg/mL). The total SCFAs in relapsing NS subjects were also lower than those of non-relapsing NS subjects, with a mean difference of 2.3 mg/mL. This finding was consistent with those in a previous study that reported a significant decrease in butyrate-producing bacteria in children with relapsing NS.[12] Another study stated that a decrease in the number of gut bacteria that produce butyrate and lower fecal butyrate levels led to impaired Treg cell induction and differentiation and, thus associated with NS relapse.[13] A different study also showed that Treg cells impaired due to intestinal microbiota dysbiosis played an important role in the worsening or occurrence of NS in children.[14] Still, no studies have stated the differences in the value of mean fecal butyric acid levels in children with relapsing compared to non-relapsing NS.

Our multivariate analysis revealed significant negative associations between the incidence of NS relapse and the three types of fecal SCFAs (acetic, propionic, and butyrate) and total SCFA levels. We noted that both butyric acid % and absolute butyric acid level had significant associations with relapsing NS incidence. The absolute butyric acid cut-off value of 0.85 mg/mL had a high sensitivity (90%) and high specificity (93.3%) in predicting relapsing NS. Research on absolute butyrate in stool cut-offs to predict relapse in NS has not been reported, to the best of our knowledge. This cut-off can be used to identify NS patients with a high risk of relapse so that earlier intervention can be carried out. Acetic acid and total SCFA did not maintain statistical significance in the

incidence of relapsing NS.

Gut microbiota is balanced in composition and amount under normal conditions. In metabolism, gut microbiota produces SCFAs or organic acids excreted in stool. Most of these SCFAs consist of acetic acid, propionic acid, and butyric acid. These three types of acids have various important roles, such as provision of energy, protective function, and immunological function. The relationship between the gut and the kidney (gut-kidney axis) is due to organic acid receptors in the kidney, including GPR41, GPR43, GPR109a, and Olfr78/OR51E2. These receptors are found in the distal tubule, collecting tubule, and glomerular podocytes. This has led to the hypothesis that the composition and/or amount of the gut microbiota, as represented by the levels of organic acids in the feces, can affect kidney function or kidney diseases. The role of gut microbiota in kidney disease, especially pediatric NS, has not been widely studied. The role of the intestinal microbiota in NS is thought to be based on the immunological function of stool organic acids, especially regarding Treg cell regulation.[9,10]

In nephrotic syndrome, Treg cell dysregulation is the underlying pathogenesis of both onset and relapse. It is hypothesized that the low numbers of butyrate-producing bacteria in the gut of patients with NS lead to persistent dysregulation of Treg cells, thereby increasing the risk of NS relapse following precipitating events. The reason intestinal dysbiosis occurs in patients with relapsing NS is still unclear but is thought to be related to genetic and environmental factors. Dramatic changes occur in the human gut microbiota soon after birth.[15] By approximately three years of age, the gut microbiota is similar to that found in adults. Factors that are thought to inhibit the development of abnormal gut microbiota include delivery by cesarean section, formula feeding, and administration of antibiotics during infancy.[16] The type of diet is also an important factor in the formation of a balanced composition of the microbiota.

The limitations of our study were the cross-sectional study design, which precludes the possibility of establishing a temporal relationship between fecal SCFAs level and relapse. Intestinal microbiota dysbiosis was assessed indirectly through fecal SCFA levels, and absolute Treg levels were not measured. We also did not evaluate in detail the diet of subjects, so that we could not exclude the role of diet in our findings.

In conclusion, levels of acetic acid, propionic acid, and total SCFA in stool are not associated with the risk of relapsing nephrotic syndrome in children. However, butyric acid level is inversely associated with the risk of relapsing nephrotic syndrome. The lower the levels of butyric acid in stool, the higher the risk of relapsing nephrotic syndrome.

Conflict of interest

None declared.

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