Butyrate Acid as a Potential Marker for Diversity of Gut Microbiota in Colorectal Cancer Patients

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Abstract: The gut microbiota acts as a real organ and many changes in its composition have been reported in colorectal cancer (CRC). Short-chain fatty acids (SCFA) mainly produced as microbial metabolites, acetate, propionate, and butyrate acids. Butyrate is produced by specific bacteria, mainly in the colon, and is taken up by the host. In our study, we found that CRC patients had lower level of acetate, propionate and butyrate acids than non-CRC. The mean concentration of acetate 8,55 µg/mL, propionate 5,61 µg/mL and butyrate acids 3,79 µg/mL respectively. In three of SCFA, the level of butyrate acids had the best diagnostic properties with area under receiver operating characteristic (ROC) curve of 0.84 higher than acetate (0.71) and propionate (0.75) ($p< 0.05$).

1 INTRODUCTION

Colorectal cancer is the fourth most cancer evident worldwide (Iffrig and Weinber, 2009). The rate of colorectal cancer is 5-10 times higher in the most developed country (Ginsberg *et al*, 2010). In Indonesia, colorectal cancer disease is caused the increasing evidences of cancer-related mortality in recent years Based on epedimiological report during 1996-1999 from Pathology Anatomy Division of Medical Faculty, Indonesia University, wrote the colorectal cancer patients with age under 40 years are around 36.75% (Sudoyo *et al*, 2010). Colorectal cancer is a disease from an accumulation of genetic mutation, epigenetic, disregulate in their communication in signaling pathways, and gut microbial contribution. Microbial involvement in colorectal cancer (CRC) is now well established (Sekirov *et al*, 2010).

SCFA are the main products of anaerobic microbial fermentation in the large intestine and affect colonic health. SCFA mainly produced as microbial metabolites, acetate, propionate, and butyrate acids. The formation of butyrate and other SCFA possibly playing a major role as chemopreventive products of microbial fermentation in the colon (Faujan *et al*, 2010; Topping and Clifton, 2001). Butyrate production is a major source of energy for colonic epithelial cells and affecting to the protection from colitis and colorectal cancer (Rose *et al*, 2007; Vinolo *et al*, 2011). The relation between functional characteristics, such as SCFA especially butyrate acid and CRC has not been extensively investigated. In one study showed butyric acid was significantly higher in the feces of healthy subject than CRC (Simpson and Campbell, 2015). In this study, we evaluated the level of butyrate acid as diagnostic biomarkers for diversity of gut microbiota in colorectal cancer patients.

2 PATIENTS AND METHODS

2.1 Participants and Sample Collection

The study consists of fourteen subjects with CRC and 14 non-CRC were from in the Gastroenterology-Hepatology Department at Dr. Zainoel Abidin General Teaching Hospital Banda Aceh, Indonesia. The patients were selected based on the following inculsion criteria :1) Patients aged 18 years or over ; 2) Indonesian citizen that proved by identity cards; 3) Patients wtih colorectal cancer confirmed by pathological examination; 4) Patients instead of CRC; and 5) Patients who are able to cooperate in

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the study. None of the patients had active antibiotic treatment or within the month prior to the colonoscopy, youghurt consumtion or laxative medicine for the last five weeks and none were treated with chemotherapy and/or radiotherapy in the previous six months. One stool samples was collected from each participant, where stool samples will be labelled and stored at -20ºC freezer service by the researchers. The study was approved by the Ethical Review Committee of Medical Faculty, Syiah Kuala University, Banda Aceh, Indonesia.

2.2 Gas Chromatography Analysis of Fecal SCFA Concentration

Stool samples were analysed for SCFA concentration with gas chromatography (GC) as described from a previous methods (Chen et al, 2013; Tangerman and Nagengast, 1996]. The amounts of acetate, propionate and butyrate acids have been reported as µg/ml and %.

2.3 Statistical Analysis

The exact chi-square test and Student t-test were used for the comparisons between the groups, and nonparametric statistics was used in addition for variables without normal distribution. The diagnostic properties were assessed with Receiver Operating Characteristics (ROC) curves, sensitivity, specificity, positive- and negative Likelihood Ratio (LR) and Diagnostic Odds Ratio (DOR). P-values below 0.05 were judged as statistically significant

3 RESULTS

3.1 Participants

Total 28 participants were included in this study. Table 1 shows the characteristics of participants. There were 10 males and 4 females in the CRC group, and 9 males and 5 female in non-CRC group. The means (±SD) was 53.8± 13.3 years for CRC and 50.0 ± 17.6 years for non-CRC. Haemoglobin, BMI and albumin in the CRC group was lower than non-CRC group. Percentage of cancers based on location: rectum 79% and colon descending 21%.

Table 1 Characteristic of participants in this study

Variable	Colorectal cancer (% or SD)	Non – colorectal cancer (% or SD)
Gender		
Male	10 (72%)	9 (64%)
Female	4 (28%)	5 (36%)
Age (years, mean)	53.8 ± 13.3	50.0 ± 17.6
BMI (kg/m^2, mean)	20.21 ± 2.65	23.6 ± 1.91
Hemoglobin (g/dl, mean)	10.6 ± 2.1	12.3 ± 1.2
Albumin (g/dl, mean)	3.24 ± 0.71	3.91 ±0.53
Colonoscopy		
Ca Rectum	11 (79%)	
Ca Colon Descending	3 (21%)	
Colitis Infection		10 (72%)
ColitisInfection + Hemorrhoid Interna		4 (28%)

The mean faecal concentrations of acetate, propionate dan butyrate were significanty lower in patients with CRC compared non-CRC. From the table 2, results revealed that the mean concentration of acetate 8,55 µg/mL, propionate 5,61 µg/mL and butyrate acids 3,79 µg/mL respectively (all $P < 0.05$).

Table 2. Fecal short-chain fatty acids in subjects with and without colorectal cancer.

Variable	Colorectal cancer patients (N=14)	Non-colorectal cancer patients (N=14)	*P*-value
Acetate Acids	8.55 ± 3.06	11.78 ± 4.61	0.038
Propionate Acids	5.61 ± 1.95	8.61 ± 3.40	0.008
Butyrate Acids	3.79 ± 2.04	6.81 ± 2.59	0.002

The results are given as mean values with SD.

3.2 Diagnostic Properties of SCFA

In three of SCFA, the level of butyrate acids had the best diagnostic properties with area under receiver operating characteristic (ROC) curve of 0.84 higher than acetate (0.71) and propionate (0.75) ($p< 0.05$) **(Table 3, Figure 1).**

Table 3. *Area under curve (AUC) short chain fatty acids.*

	AUC	95%CI
Acetate acids	0.71	0.52 – 0.90
Propionate acids	0.75	0.57 – 0.94
Butyrate acids	0.84	0.68 – 0.99

Butyrate acid value < 5.4 µg/mL was judged as a well-suited cut-off for indicating CRC and was used in the calculation of the diagnostic. According to the reference range employed in this study, the sensitivity, specificity, positive and negative likelihood ratio, and diagnostic odds ratio were 85%, 78%, 4.04, 0.18 and 22.2 respectively.

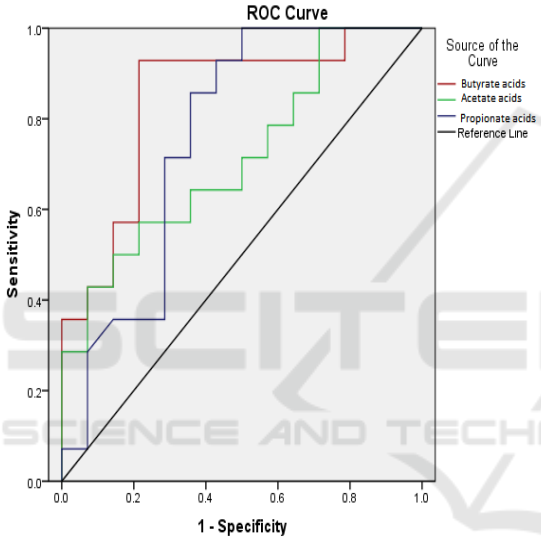

Figure 1: The diagnostic properties of SCFA presented as the Receiver Operating Characteristic curve.

4 DISCUSSION

With the increasing evidence linking gut microbiota and CRC, fecal microbiota has emerged as a promising candidate to non-invasively screen for CRC. The present study demonstrated that the level butyrate acids could be a valuable diagnostic biomarker for CRC than the other SCFA.

Colorectal cancer (CRC) is a leading cause of cancer-related mortality worldwide, and its incidence has increased rapidly in recent years (Jemal et al, 2011; Ji *et al*, 1998). In recent years, the 16S rRNA gene sequencing approach has been widely used as an effective tool to globally analyze the microbial community, and multiple studies have demonstrated that breakdown of the intestinal microbiota structure can promote carcinogenesis and development of CRC (Wei *et al*, 2016). Flanagan et al, 2014 . demonstrated a significant association between *Fusobacterium nucleatum* level and patient outcome and suggested that *F. nucleatum* may have value as a prognostic indicator. Boleij et al, 2015. found that the detection of Bacteroides fragilis toxin (BFT), which was produced by *Enterotoxigenic Bacteroides fragilis* (ETBF), increased in the mucosa of later staged CRC.Recently, the others of our study suggest that the appearance of Bifidobacterium as one of the indicators of detections for colorectal cancer (Yusuf et al, 2016).

The microbiota metabolises non-digestible food constituents into short-chain fatty acids (SCFA) that have extensive immunological and regulatory functions and appear to be the link in the host-microbe interactions Within gut microbiota, several distinct bacterial communities live at a certain ratio under steady state condition (Farup et al, 2016). The level of SCFA content in faecal samples have been shown to be related with some diseases such as IBD, irritable bowel syndrome (IBS), cardiovascular disease (CVD), diarrhoea, and cancer (Faujan *et al*, 2010). There is significant association between levels of SCFAs and composition of the microbiota, with high luminal concentrations resultant of fermentation lowering colonic pH (5.5–6.5 in proximal colon where fermentation is highest, compared to pH 6.5–7.0 in the distal colon) and inhibit growth of Gram-negative Enterobacteriaceae including familiar pathogens Salmonella spp. and Escherichia coli (Simpson and Campbell, 2015). Therefore, a greater increase in SCFA production and potentially a greater delivery of SCFA, specifically butyrate, to the distal colon may result in a protective effect (McOrist et al, 2011)

Fecal levels of butyrate in patients with colonic neoplasia have been investigated by different studies. Vernia et al, 1995. compared 20 patients with colorectal cancer, 8 patients colon polyps, and healthy controls. No significant differences were found, although patients with rectal cancer showed slightly lower levels of propionate and butyrate than those with more proximal cancer. Contrast with the previous study, in our study we found that butyrate was the best properties diagnostic for CRC with AUC (0.84) higher than acetate (0.71) and propionate (0.75).

The results of the present study are limited by the relatively low number of participants and a larger study population would provide enhanced statistical reliability. In addition, we did not study

for identifies bacteria as far as we know, SCFA is the main products of anaerobic microbial fermentation. Another limitation is the fact that we cannot eliminate environment factors such as diet and everything related to microbiota.

5 CONCLUSIONS

In conclusion, this study was the first report demonstrating of the level butyrate acids as useful biomarkers to detect the presence of cancerous lesions. Because the study had an exploratory design and limited number of participants, these results need for more validation study.

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