Microbiota Composition, HSP70 and Caspase-3 Expression as Marker for Colorectal Cancer Patients in Aceh, Indonesia

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ABSTRAK

Tujuan: *menganalisis hubungan komposisi mikrobiota terhadap ekspresi HSP70 dan Caspase-3 pada jaringan kolon pasien yang menjalani kolonoskopi dalam upaya pengembangan kandidat deteksi dini untuk pasien kanker kolorektal di Indonesia.* ***Metode:*** *penelitian potong lintang dilakukan pada 32 responden yang menjalani pemeriksaan kolonoskopi. Selanjutnya diketahui bahwa 16 orang adalah penderita kanker kolorektal, sementara 16 lainnya bukan kanker kolorektal (yaitu kolitis dan hemorrhoid interna). Komposisi mikrobiota pada sampel feses diperiksa dengan menggunakan 16S rRNA Denaturing Gradient Gel Electrophoresis (DDGE) sedangkan pemeriksaan immunohistokimia untuk menilai ekspresi HSP70 dan Caspase-3 diperiksa dengan pewarnaan Haematoxylin-Eosin(HE) untuk mengetahui perubahan morfologis pada jaringan kolon.* ***Hasil:*** *analisis dengan PCR-DGGE menunjukkan perbedaan komposisi mikrobiota yang terdapat pada pasien kanker kolorektal dan bukan penderita kanker kolorektal. Semua pasien dengan kanker kolorektal menunjukkan hilangnya pita dominan pada kelompok Bifidobacterium. Pengamatan histologi yang dihitung berdasarkan uji Inter Class Corelation (ICC) didapati skor yang cukup tinggi (5,2-9,2) pada pasien kanker dan skor yang lebih rendah (1,7-2,4) pada pasien bukan kanker kolorektal. Ekspresi HSP70 mengalami peningkatan secara signifikan pada pasien kanker kolorektal dengan persentase tertinggi yaitu 84%, sebaliknya, ekspresi Caspase-3 mengalami penurunan dengan persentase tertinggi hanya 21%. Hasil analisis statistik menunjukkan bahwa kejadian kanker kolorektal berhubungan dengan ekspresi HSP70 ($p<0,001$) dan berhubungan dengan ekspresi Caspase-3 ($p<0,001$).* ***Kesimpulan:*** *penelitian ini mengindikasikan bahwa Bifidobacterium menjadi indikator penting terhadap pasien kanker kolorektal yang ditunjukkan pada gambaran pita yang menghilang, sedangkan ekspresi HSP 70 mengalami peningkatan dan ekspresi Caspase 3 terjadi penurunan yang signifikan.*

Keywords: *bifidobacterium, Caspase-3, colorectal cancer, HSP70, mikrobiota.*

ABSTRACT

Aim: *to investigate the relationship between microbiota composition with HSP70 and Caspase-3 expressions in colon tissue as an initial study to develop the candidate for early detection of colorectal cancer for Indonesian patients.* ***Methods:*** *this is a cross-sectional study on 32 patients undergoing colonoscopy; 16 patients of colorectal cancer (CRC) while the other 16 patients are not (colitis and internal hemorrhoid). The composition of microbiota in stool samples was examined using 16S rRNA Denaturing Gradient Gel Electrophoresis (DDGE) while expression of HSP70 was examined by immunohistochemistry and Caspase-3 by using Haematoxylin-Eosin(HE) staining to*

determine the morphological changes in colon tissue. ***Results:*** *analysis of PCR-DDGE shows a different composition of microbiota between patients with CRC and non-CRC. All CRC patients showed disappearance of dominant band from Bifidobacterium groups. Histological observation based on Inter Class Correlation (ICC) test from all slide showed a high scores (5.2-9.2) in CRC patients and low scores (1.7-2.4) in non-CRC patients. HSP70 expression was increased significantly in CRC patients with the highest percentage of 84%, while expression of caspase-3 decreased with the highest percentage of 21%. Statistical analysis showed that the incidence of colorectal cancer was associated with the expression of HSP 70 ($p<0.001$), and Caspase 3 ($p<0.001$).* ***Conclusion:*** *bifidobacterium is an important indicator for colorectal cancer patients that show disappearance of dominant band, while expression of HSP70 increased and the Caspase-3 expression decreased significantly.*

Keywords: *bifidobacterium, Caspase-3, colorectal cancer, HSP70, microbiota.*

INTRODUCTION

Colorectal cancer is the fourth most prevalent cancer worldwide.[1] Colorectal cancer incidence in 2000 accounted for around 579,000 deaths. It is equivalent to 1% of total deaths and around 8% of total death related to malignant neoplasms. The rate of colorectal cancer is 5-10 times higher in the developed countries.[2] In Indonesia, colorectal cancer is associated with the increasing evidences of cancer-related mortality in recent years. Medical Service Directorate, Department of Health Collaboration and Indonesian Pathology Anatomy Association in Jakarta reported that colorectal cancer patients aged under 45 years old are around 47.85%. Whereas in an epidemiological report between 1996-1999 from Pathology Anatomy Department of Medical Faculty, Universitas Indonesia, colorectal cancer in patients under 40 years are around 36.75%.[3]

Colorectal cancer is a disease from an accumulation of genetic mutation, epigenetic, disregulation in signaling communications pathways, and gut microbial contribution. Over the last few decades, many studies have explored a disease related to gastrointestinal tracks. Between 1990-2009 periods, there is an increasing number of researches about microbiota, almost five times in every year.[4] The complex gut microbiota community may play an important role in the development of colorectal cancer. One of the interesting discussions related to this research is the important role of microbiota in gastrointestinal tracts that have pathogenic character and the pathomechanism of those bacteria. Colorectal cancer is a disease that has closely related with fiber consumption and microbiota in gastrointestinal tracts. The composition of microbiota on the colon are already reported as a marker in colorectal cancer patients. The microbiota imbalance is reported in several studies related to this disease, such as the increase of *Clostridium spp., Bacteroides* and *Bifidobacterium spp*.[5] Other report is about the population of Fusobacterium which was increased, but Bacteroidetes and Firmicuteswere decreased.[6] Those discoveries were also supported by the Pyro-sequencing assay at region V3 from 16S ribosomal RNA gene and it was found that there are close relationship between colorectal cancers with the imbalance of microbiota compared to the healthy individual.[7]

The imbalance of microbiota composition can be caused by significant decrease of butyrate-producing microbes in gastrointestinal tracts such as Firmicutes (especially Roseburia spp. and Eubacteriumrectale) and Actinobacteria (such as Bifobacterium spp. and Collinselaaerofaciens).[8-10] Butyrate production protects the bowel from colitis and colorectal cancer through lowering of oxidative damage of DNA, triggering the occurrence of apoptosis of DNA cells on the cells that already damage, suppressing the growth of tumor cells, and decreasing the activity of co-carcinogenic enzyme.[8]

Several in vitro studies showed that butyrate induce expression of heat shock protein (HSP) 70 that has function in the beginning of apoptosis.[11] Caspase, which is an aspartate-specific cysteine proteases, has an important function on apoptosis

and inflammation and contribute mainly to the balancing of the gastrointestinal tract. In the rat model with colorectal cancer, it was reported that there is a relation between caspase 3 and the microbiota structure.[12]

In this study, we reported the relationship between the gut microbiota community structures and severity colorectal cancer compared to non-colorectal cancer patients that have several characteristics related to colorectal cancer in Aceh Province, as initial candidate to develop the novel early detection for Indonesian specific patients. Besides that, we also measured the expression of HSP70 and Caspase-3 as a protein marker in correlation with colorectal cancer condition.

METHODS

This is a cross-sectional study on 32 patients undergoing colonoscopy. There were 16 patients of colorectal cancer (CRC) while the other 16 patients are not (colitis and hemmorhoid interna). The biopsy was done on all patients subject to indication with several criteria. Inclusion criteria of colorectal cancer patients were proven to have colorectal cancer by colonoscopy and histopathology; did not get any antibiotics; not in youghurt consumption; or laxative medicine for the last five weeks, and Indonesian citizen that proved by identity cards. Informed consent was obtained from all subjects before participation. One fecal sample was collected from each control, and from each patient before colonoscopy. The study was approved by the Ethical Review Committee of Medical Faculty, Syiah Kuala University, Banda Aceh, Indonesia, registration number: 333/KE/FK/2015.

DNA Chromosome Isolation

Patients were prepared for feces examination with diet limitation. Feces specimens were carefully taken and grown in MRS agar for 48-72 hours. Growth bacteria colonies were taken for DNA isolation. DNA isolation was conducted based on previous method[13] with some modification. DNA was diluted using TE buffer at pH 7.6.

The primer was designed to amplify several bacterial groups that usually found in human gastrointestinal tracts. There were primer sets to amplify universal bacteria, Bacteroides, Bifidobacterium, Enterobacterium, Lachnospiraceae, and Erec groups. Preliminary study was done to test all primer. Results of preliminary study (data not shown) showed a difference between sample only appears on four sets of primer: Universal bacteria, Bifidobacterium, Enterobacterium, Lactobacillus. Therefore, the study was focused on four sets of primer without using primer that amplifying Lachnospiraceae or Bacteroides.

Denatured Gradient Gel Electrophoresis (DGGE) Analysis of Bacterial 16S rRNA in Stool Samples

In this study, primers were used to amplifying region of 16S rRNA based on previous studies by Vanhoutte et al.[14] and Maukonen et al.[15] Every primer that used in this study was listed in **Table 1**. Forward or reverse primer from every primer sets was added with GC-clamp on 5' side to increase detection from PCR products with DGGE. Polymerase chain reaction (PCR) was conducted using GoTaq green master mix (Promega). Program PCR was done as follow: 940C for 3 minutes; 30 cycles of 940C for 30 second, 550C for 30 second and 720C for 1 minute; and final extension of 720C for 5 minutes. Product of PCR was analyzed using DGGE (Biorad). The number of DGGE bands was used as an indicator of fecal bacterial diversity for each participant. Similarities between banding patterns in the DGGE profile were calculated based on the presence and absence of bands and expressed as a similarity coefficient. DGGE analysis was conducted based on manufacturer's protocols in combination with several previous study with some modification.[16-18]

Histopathological Analysis and Immunohisto-chemistry

Observations for colorectal cancer were conducted by making the histology slide. To ensure the representativeness of each area selected in the paraffin blocks for immunohistochemical analysis, two samples were collected from different sections of the same block.A biopsy from patients was fixated on 10% formalin solution, followed by organ cutting and

Table 1. Primer for amplification of 16S rRNA region from bacterial colony

Primer	Primer sequence (5'-3')	Target group	References
V6-V8: U968-GC	GC clamp-TACGGGAGGCAGCAG	Universal groups	14,15
V6-V8: L1401	ATTAACCGCGGCTGCTGG		
g-Bifid F	CTCCTGGAAACGGGTGG	Bifidobacterium	14
g-Bifid R-GC	GC-clamp-GGTGTTCTTCCCGATATCTACA		
Ent. 1017F	CCTTTGACCACTCTAGAG	Enterobacterium	14
Ent. 1263R-GC	GC-clamp-CTTAGCCTCGCGACT		
Lac1	AGCAGTAGGGAATCTTCCA	Lactobacillus	14
Lac2-GC	GC-clamp-ATTYCACCGCTACACATG		

dehydration using serial alcohol (70%, 90%, Absolute), each for 60 minutes. The sample was cleared with xylol for 30 minutes. Then it was continued with impregnation, embedding and sectioning of paraffin blocks. The slide was stained using Haematoxylin-Eosin. Observation was conducted using electronic microscope BX-53 (Olympus). Histology classification was determined based on Lanza et al.[19], which classified the appearance as follow:

- Category 1: Negative for neoplasia (+)
- Category 2: Indefinite for neoplasia (++)
- Category 3: Mucosal low grade neoplasia, low grade adenoma, low grade dysplasia (+++)
- Category 4: Mucosal high grade neoplasia, high grade adenoma/dysplasia, non-invasive carcinoma, suspicious for invasive carcinoma, intramucosal carcinoma, intramucosal carcinoma (++++)
- Category 5: sub mucosal invasion by carcinoma (+++++)

The expression of HSP70 and caspase-3 on the slides were examined and scored with colour proportion by Leake Scoring[20], that direct count of the proportion of stain, stain intensity plus a measure of intensity of stain with two different pathologists

Statistical Analysis

Statistical analysis was conducted using SPSS 16.0 for windows. Data was analyzed using Chi square for the relationship of composition gut microbiota between CRC and non-CRC patients and T test to determine the expression of HSP70 and caspase-3 in relation to different clinicopathological characteristics patients. Tests were considered significant when P-value were less than 0.05.

RESULTS

A total of 16 patients with positive colorectal cancer and 16 non-colorectal cancer were included in this study. Patients demography and clinical features were shown in **Table 2**. All patients from two groups had similar average age. Almost all variable on general clinical observation from two groups were comparable, except for LED in which colorectal cancer group showed almost twice average value than control group. The clinicopathological characteristics of the 32 patients are shown in **Table 3**. The most common chief complain were diarrhea and hematochezia with most result of colonoscopy is carcinoma of rectum. The most frequent histopathogical and CT scan of patients were stage IV.

Analysis of PCR-DGGE from patients and control subject showed a different appearance (**Figure 1**). The result of DGGE from PCR product using primer for universal bacteria (line 1), Enterobacterium (line 3) and Lachnospiraceae (line 4) groups resulted in similar band between colorectal cancer patient and healthy patient. The different appearance was found in PCR products using primer for Bifidobacterium groups (line 2). Patients with colorectal cancer did not show any band appearance. In contrast, control subject showed one clear band of + 500bp.

Results of PCR-DGGE did not show any dominant band from Bifidobacterium groups in all patients with colorectal cancer. In contrast, all

Table 2. Demography and clinical features from each patients

Variables	Colorectal cancer patients mean (SD)	Non-colorectal cancer patients mean (SD)
Age (years)	50.06 (13.37)	53.06 (21.93)
BMI (kg/m^2)	21.06 (4.51)	24.16 (2.70)
Hb (g/dL)	9.39 (2.27)	12.00 (1.44)
Erythrocytes (10^{6}sel/mm^3)	4.21 (0.70)	5.14 (1.25)
Leukocytes (10^3/mm^3)	8.20 (2.11)	8.14 (2.75)
Platelet (10^3/mm^3)	318.69 (101.40)	255.83 (107.47)
Hematocrite (%)	34.47 (5.32)	36.19 (3.82)
LED (mm/1jam)	64.81 (27.47)	33.06 (13.22)
Blood sugar (mg/dL)	107.87 (17.12)	96.00 (36.84)
SGOT (U/L)	31.33 (7.69)	30.44 (5.74)
SGPT (U/L)	31.80 (8.55)	27.00 (7.48)
Albumin (g/dL)	2.77 (0.31)	3.56 (0.38)

Table 3. Clinicopathological characteristics of study participants

Variables	Colorectal cancer patients, n (%)	Non-colorectal cancer patients, n (%)
Sex	9 (56.00)	11 (69.00)
Male	7 (44.00)	5 (31.00)
Female		
Complaint		
Chronic Diarrhea	8 (50.00)	5 (31.00)
Hematochezia	8 (50.00)	11 (69.00)
Colonoscopy		
- Ca Rectum	11 (68.75)	0 (0.00)
- Ca Colon Descending	5 (31.25)	0 (0.00)
Colitis Infection	0 (0.00)	14 (87.50)
- Colitis Infection + Hemorrhoid Interna	0 (0.00)	2 (12.50)
Stadium		
- I	0 (0.00)	
- II	1 (6.25)	
- III	6 (37.50)	
- IV	9 (56.25)	

patients that only have several symptoms related to colorectal cancer showed one dominant band of Bifidobacterium groups. The summaries of dominant band appearance from all samples were shown in **Table 4**.

Histological observation of all samples resulted in various appearances (**Figure 2**). Results from IHC staining were shown between the expression of Caspase-3 and HSP-70. In patients with colorectal cancer, there was a relatively increasing of HSP-70 and decreasing of Caspase-3 expression. In contrast, control subjects that only showed several similar symptoms with colorectal cancer showed a lower expression of HSP-70 compared to Caspase-3.

Quantitative measurement of Caspase-3 and HSP-70 supported the results of IHC observation and correlated with the severity of colorectal cancer (**Figure 3**). Among samples of patients with colorectal cancer, sample P4 showed the highest expression of HSP-70 (84%). Besides that, the lowest expression of caspase-3 was also obtained from sample P4 (21%). Sample P8 and P3 showed significantly lower HSP-70 expression among these groups. Both, P8 and P3 sample showed no significant differences in percentage (53% and 49%, respectively). With a different group of patients that have colorectal cancer, among a group of control patients almost a similar expression of HSP-70 and Caspase-3 were resulted. The expression of Caspase-3 in this group was higher compared to the expression of HSP-70. Opposite expression of Caspase-3 and HSP-70 were showed in comparison between those two groups.

Statistical analysis from three variables (bacterial composition, caspase-3, and HSP-70) showed a corelation between analysis results and disease appearance in **Table 5**. Analysis

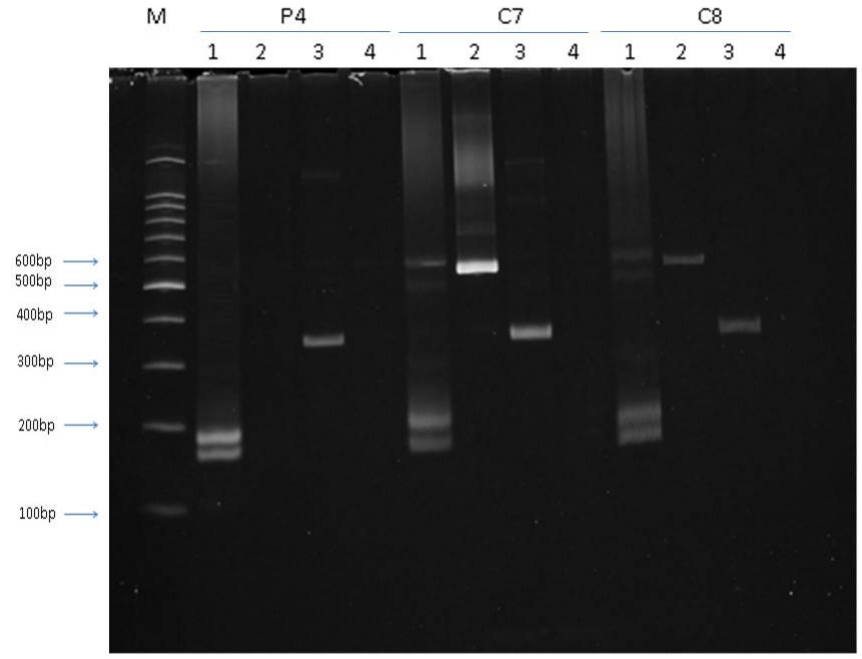

Figure 1. The 16S-RNA profile identified by PCR-DGGE analysis. Different band appearance was obtained from the sample, especially from the PCR product that using primers to amplify bacteroides groups. M=marker; P=patient with positive colorectal cancer; C=control subject with characteristics of colorectal cancer; Line 1=universal bacteria; Line 2=bacteroides; Line 3=bifidobacterium; line 4=lachnospiraceae.

Table 4. Interpretation of 16S-RNAs bands from PCR-DGGE analyses of all samples

Sample	Universal Bacteria		Bifidobacterium	Enterobacterium	Lachnospiraceae	Grade
	± 190 bp	± 200 bp	± 500 bp	± 370 bp		
P1 to 16	+	+	-	+	-	3
C1 to C16	+	+	+	+	-	4

+ = appear (1); - = not appear (0) , P=patient, C=control

showed that colorectal cancer condition affected the HSP-70 and caspase-3 expression (**Table 6**).

DISCUSSION

Bacterial content and number that formed the gut microbiota has been recognized to be able to cause gastrointestinal diseases and disorders such as colorectal cancer.[21,22] Imbalance composition of bacteria can increase the proliferation of carcinogenic bacteria that affect the production of carcinogenic compounds, secondary bile acids and cholesterol metabolites, finally trigger the ephitelium change and pathogenesis of cancer colorectal.[23,24] Analysis of PCR-DGGE showed a significant difference in term of dominant band appearance correlated with Bifidobacterium groups. Patients with colorectal cancer did not show any appearance of the dominant band from Bifidobacterium groups. Bifidobacterium is the normal habitat in healthy human gut beside other species such as *Faecalibacterium prausnitzii, Roseburia intestinalis, Bacteroides uniformis*, and lactobacilli groups.[24] The alteration of this bacteria becomes one of the most common feature related with the disease appearance.[26-29] Dysbiosis condition which cause the change of bacteria complexity and instability such as increasing in Bacteroides and Clostridia and a reduction in Bifidobacterium are present in various gastrointestinal related diseases.[30] Our

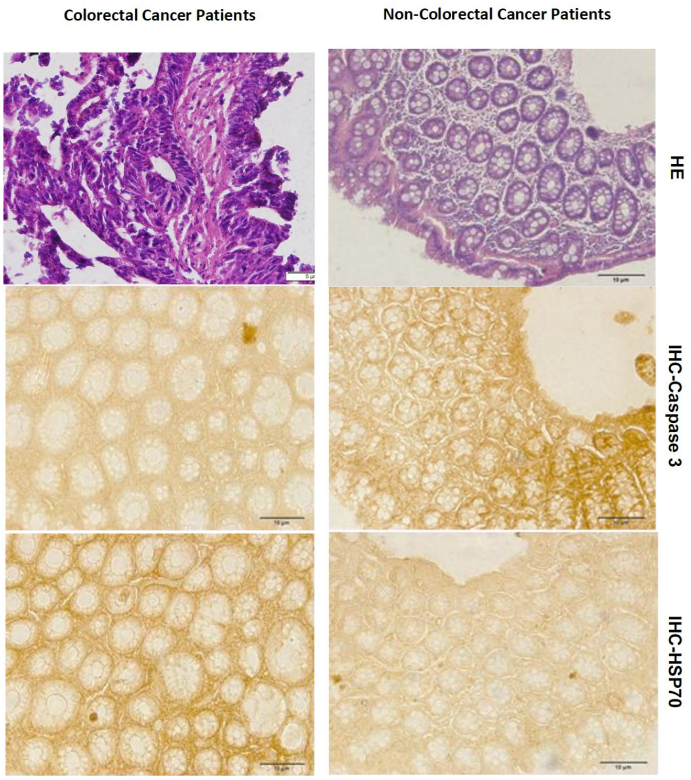

Figure 2. Comparison of histopatological observed between patient with colorectal cancer and non-colorectal cancer with similar symptoms using immunohistochemistry and HE staining. Inflammation area was shown on slides that stained with hematoxylin-eosin from patients. The appearance of inflammation area was observed from HE staining. Result of IHC was showed opposite expression between HSP-70 and Caspase-3on two groups of samples. (Scale: 400x)

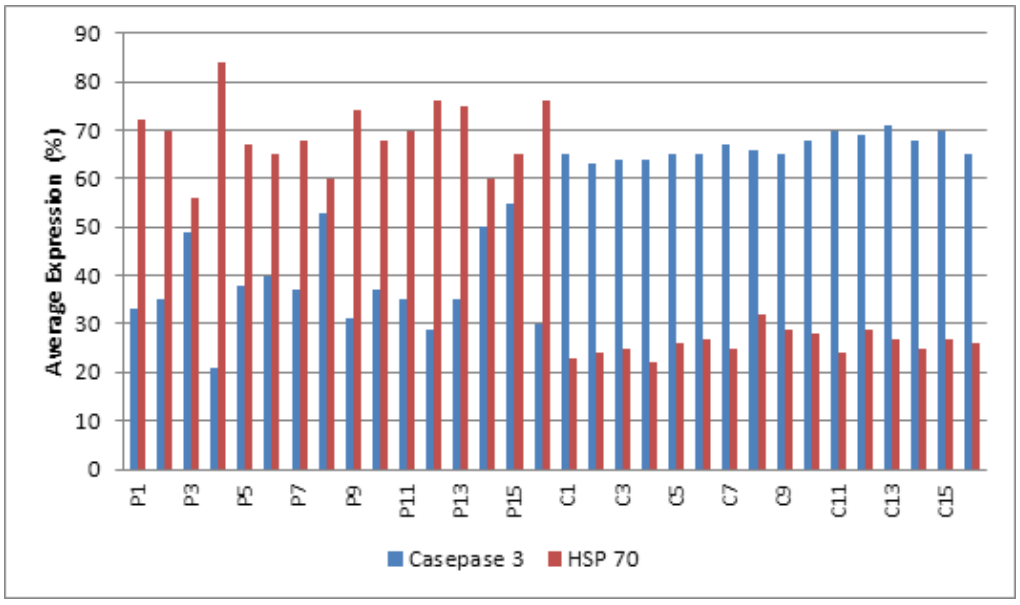

Figure 3. Intensity of HSP-70 and Caspase-3 expression of colorectal cancer were valued from Immunohistochemistry analysis. In patients with colorectal cancer, the expression of HSP-70 was higher compared to caspase-3 expression. On the contrary with control subject patients, the expression of HSP-70 was lower comparing to caspase-3 expression.

Table 5. Bivariate analysis of the relationship composition gut microbiota between CRC and non-CRC patients

Group	Bacterial Composition		Total, n (%)	p
	Enterobacterium (+/- 370 bp), n (%)	Bifidobacterium (+/- 500 bp), n (%)		
CRC patient	16 (100.00)	0 (0.00)	16 (100.00)	<0.001
Non-CRC patient	0 (0.00)	16 (100.00)	16 (100.00)	
Total	16 (50.00)	16 (50.00)	32 (100.00)	

* Chi-square test

Table 6. Distinctive profile of caspase-3 and HSP-70 between CRC and non-CRC patients (N=32)

Group	mean (SD)	p*
Caspase-3		
- CRC patient	80.43 (7.85)	<0.001
- Non-CRC patient	91.37 (5.70)	
HSP-70		
- CRC patient	89.73 (5.55)	<0.001
- Non-CRC patient	79.51 (4.90)	

*T test independent

result related with the loss of Bifidobacterium was almost similar with previous results by Gueimonde et al.[31] They reported a decrease number, or change in species composition of genus Bifidobacterium in patients with colorectal cancer. Other study by Sobhani et al.[32] showed that several species of bacteria were decreased in patient with cancer compared to normal person such as *Bifidobacterium longum, Clostridium clostridioforme, Ruminococcus sp., Ruminococcus bromii*.

Bifidobacterium are bacterial groups that have several characteristics such as non-motile, non-spore-forming, non-gas producing, gram-positive, anaerobic, catalase-negative bacteria with high GC content.[33,34] Generally, morphology of this bacteria is bifid or irregular V-or Y-shaped rods resembling branches. Previous study in infants showed that large number of bifidobacterium was found in breast-fed infants and lower in infantile diarrhea. Bifidobacterium was used for therapy in infants with intestinal diarrhea. Mix between freeze-dried of Bifidobacterium and Lactobacillus are usually used as a treatment for person having gastrointestinal disorders.[35]

Although the specific function of this bacteria is still unclear, but based on its high colonization in infant gut than adult, it is possible that the main function of this bacteria is related with gut microbiota development. This suggestion is supported by several studies which showed the interaction with other microbes in gut, essspecially related with the digestion process of polysaccharides.[36] Other possible functions of this bacteria are production of water-soluble vitamins; controlling certain bacteria that can be harmfull to the host[37]; producing several antimicrobial compounds such as organic acids, iron-scavenging[38] and bacteriocins[39,40]; and also affecting the host innate immune response.[41,42] In term of its function for controlling other bacteria growth, it is related with several clinical studies that showed opposite total number between this bacteria and other bacteria with possiblity to become harmfull in host gut. Besides that, the total number of Bifidobacterium is inversely correlated with the concentration of compounds and its related enzyme that can caused intestinal decay.[35] Therefore, it could be understood that the decreasing number of Bifidobacterium will increase the appearance of other pathogen bacteria and finally affecting the occurrence of colorectal cancer. Our study has also proved that suggestion.

In recent years, several studies have been conducted to identify the potential usage of food with probiotic (such as lactobacilli or bifidobacteria) and/or prebiotic content in affecting the selectively growth of bacteria and in turn change the bacteria composition that could prevent gastrointestinal diseases including

cancer colorectal.[21] Several studies showed the usage of Bifidobacterium either alone or in combination with other bacteria or substrates for cancer treatment and prevention using mouse model[21] and human.[43] Possible mechanisms of probiotic affecting the gut microbiota are through modifying the pH[44] and increasing the short-chain fatty acid (SCFA) (especially acetate, propionate and butyrate); suppress the pathogen bacteria through production of antimicrobial and antibacterial; stimulating the immunomodulatory cells; competing with pathogens for nutrients, receptors and growth factors.[21]

Measurements of HSP-70 expression showed a significantly higher concentration in cancer pantients compare to non-colorectal cancer patients. This protein is a member of heat shock protein family and known as molecular chaperones. Several important role of this protein family are transferring protein into correct subcellular compartments; folding and protein forming; avoid the protein degradation; cell growth, development, differentiation and gene transcription. It is also related to the development and severity of cancer. Overexpression of this protein was observed in tumor tissues and cancer cell lines in which it will affect the protection of tumor cell and suppresing the apoptosis mechanism. It could be considered that the HSPs family is related with the cancer incidents.[45] Zhang et al.[45] reported that transcription factor of HSPs family such as HSP-60, HSP-70 and HSP-90α were elevated significantly on colorectal cancer patient compare to para-cancerous tissue. Other previous study also showed increasing of HSP-70 on esophageal carcinoma and colorectal cancer. In normal cells, HSPs are involved in cell growth and metabolism and strictly regulated by cell cycle. The increasing expression of HSPs is related with the stress condition in cells and affecting several process such as mediate proper holding and transmembrane transport of the oncogen and anti-oncogenes products; regulate the degradation of mismatching protein and harmonize quick metabolism balance of the protein in tumor cells to support the cells growth; and increase the tumor cells résistance to apoptosis.[45]

In our study, the measurement of caspase-3 was inversely related with HSP70. Patients with colorectal cancer showed a significant decrease in expression of caspase-3 compared to non-colorectal cancer patients. Previous study related with caspase-3 measurement also showed in decrease concentration on colorectal cancer patients and affecting the cancer prognosis.[47] The caspase-3 and HSP-70 expression is correlated each other. Several studies show that increasing of HSP-70 could suppress the caspase-3 expression.[48,49] A study of colorectal cancer treated with a cancer related drug, EGCG, has shown the ability of this compound to bind to the HSP-70 and suppress its expression. The decrease in HSP-70 affect the increase in caspase 3 and activation of the apoptosis mechanisms. Caspase-3 is cytokine that related to apoptosis mechanism. This cytokine is found to decrease in patients with colorectal cancer.[50] However, our study has several limitations. Firstly, the small sample size of the colorectal cancer and non-colorectal cancer with less than 16 patients each may have led to chance occurance of statistically significant results. Secondly, although at inclusion criteria we did not include patients with yoghurt consumption and antibiotics using, but we cannot eliminate environment factors such as diet and everything related to microbiota. One of the interesting implications of this work is the potential of Bifidobacterium as a biomarker of cancer risk.

CONCLUSION

The results of our study suggests that the appearance of Bifidobacterium as one of indicator of detections for colorectal cancer. The expression of HSP-70 in patients with colorectal cancer were elevated with declining of caspase 3 expression. Further studies are still needed to search the exact species of Bifidobacterium that abundant in predictive colorectal cancer patients

CONFLICT OF INTEREST

We declared no conflict of interest in this study and the writing of this manuscript.

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