Association Between Patient Characteristics and Diet Profile with Kirsten Rat Sarcoma (KRAS) and Neuroblastoma Rat Sarcoma (NRAS) Gene Mutation in Colorectal Cancer

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ABSTRACT

Background: *Colorectal cancer is the third most common cancer according to American Cancer Society. It is also the third most common cause of death in men and women in US. Colorectal cancer encompasses 5% of all cancer and 29% of gastrointestinal cancer with men and women ratio is about 3:1. More than 1/3 of colorectal cancer occur below the age of 45 years. Mutation in Kirsten rat sarcoma* **(***KRAS) gene was found in 30-50% of colorectal cancer in which it was suggested to associated with increase proliferation and decrease apoptosis. This study aimed to analyze the association between diet profile and KRAS gene mutation.*

Method: *This study was a cross sectional study. Data was collected from medical records of colorectal cancer patient in Dr. Saiful Anwar General Hospital, which included KRAS gene mutation analysis.*

Results: *There were 12 subjetcs included in this study. Four subjects (33.3%) had gene mutation with 3 subjects (75%) had positive KRAS mutation and 1 subjects (25%) had positive neuroblastoma rat sarcoma (NRAS) mutation. In non-mutation group, it had been found a higher frequency of green leaf vegetables diet, in comparison with mutation group (p = 0.023). There was positive correlation between green leaf vegetables diet with gene mutation.*

Conclusion: *Mutation of KRAS and NRAS mutation in colorectal carcinoma were found in 33.3% of subjects. Data analysis showed positive association between low green leaf vegetables diet with KRAS and NRAS mutation.*

Keywords: *Kirsten rat sarcoma* **(***KRAS), gene mutation, colorectal cancer, diet*

ABSTRAK

Latar belakang: *Kanker kolorektal adalah kanker ketiga terbanyak dan merupakan kanker penyebab kematian ketiga terbanyak pada pria dan wanita di Amerika Serikat berdasarkan American Cancer Society. Kanker kolorektal mempunyai frekuensi 5% dari seluruh kanker dan 29% dari keganasan gastrointestinal dengan rasio laki-laki 3:1 perempuan dan lebih dari 1/3 kasus dibawah usia 45 tahun. Mutation in Kirsten rat sarcoma (KRAS) terjadi pada 30-50% dari kanker kolorektal dan diduga berhubungan dengan proliferasi dan penurunan apoptosis. Penelitian ini bertujuan untuk mengetahui hubungan antara faktor diet dengan mutasi KRAS.*

Metode: *Desain penelitian adalah cross-sectional, data diambil berdasarkan data dari rekam medis pasien kanker kolorektal yang menjalani pemeriksaan mutasi gen KRAS di Rumah Sakit Umum Daerah (RSUD) dr. Saiful Anwar Malang.*

Hasil**:** *Dari 12 pasien kanker kolorektal yang menjalani pemeriksaan mutasi gen di RSUD Dr. Saiful Anwar Malang didapatkan hasil sebanyak 4 pasien (33,3%) positif mengalami mutasi gen dengan sebagian besar berupa mutasi KRAS positif yaitu 3 pasien (75%) dan 1 pasien (25%) mutasi NRAS positif. Pada kelompok yang tidak mengalami mutasi didapatkan frekuensi diet sayuran daun lebih tinggi dibandingkan dengan kelompok yang mengalami mutasi gen (p = 0,023) serta terdapat korelasi positif antara konsumsi sayuran daun dengan terjadinya mutasi gen.*

Simpulan: *Mutasi KRAS dan NRAS pada kanker kolorektal terjadi pada 33,3% pasien. Data penelitian menunjukkan adanya hubungan antara diet rendah sayuran daun dengan mutasi KRAS dan NRAS.*

Kata kunci: *Kirsten rat sarcoma* **(***KRAS), mutasi gen, kanker kolorektal, diet*

INTRODUCTION

There are 3 families of rat sarcoma virus (RAS) gene which include H-RAS, K-RAS, and N-RAS. The three families produce 3 kinds of similar proteins with the size about 21 kD. RAS gene produce proteins that bind and hydrolize GTP. Those proteins work as mediator in extracellular signal transduction for growth factor, cytokine, and hormone in cytoplasm and nucleus.[1-6] Thus those proteins have important roles in the process of proliferation, differentiation, and also apoptosis of the cells.[2] Specific mutation in RAS gene will change the formation of the produced protein. The structural change induces continuous activation of RAS protein (GTPase).[6] Continuous activation of the protein bring disruption in the process of extracellular signal transduction which in turn bring disruption of cellular proliferation. In colorectal carcinoma, more than 90% mutation of RAS gene happen in K-RAS family.[2] Mutations of K-RAS are commonly found in codon 12, 13, and 61. Mutation of codon 61 is the least common mutation.[6] Mutation of K-RAS gene causes constant transcription of K-RAS protein. It consequently causes the cancer cells to continue proliferate. The average of colorectal cancer patients which experience K-RAS gene mutation is 36%.[7,8,9,10]

It has been widely accepted that colorectal cancer was an adenoma which progressively became a carcinoma. Metastatic process of the carcinoma is mainly caused by ineffective treatment of carcinoma. One of the mainstay treatment for colorectal carcinoma is chemotherapy. Evaluation of any gene mutation biomarker should be performed before administration of chemotherapy so that the targeted therapy can be delivered accurately. One of the biomarker of gene mutation can be detected on rat sarcoma virus (RAS) oncogene. Oncogene of RAS includes Kirsten rat sarcoma (KRAS) and neuroblastoma RAS viral oncogene (NRAS). Oncogene of KRAS is now the most relevant biomarker for epidermal growth factor receptor (EGFR) targeted therapy in colorectal carcinoma with metastatic process.[6,9]

The KRAS gene is a proto-oncogene located at p-arm of chromosome 12 in the position of 12.1. This gene translates the KRAS protein, one family of protein which is be able to be found in the inner surface of cell membrane, including human cells. RAS protein is a guanine-nucleotide-binding protein that serves the role as binary molecular switches, controlling the intracellular signal transmission.[2,6] Mutation of KRAS is found in about 10-15% of adenoma cases with the size below 1 cm, 30-60% of adenoma with size more than 1 cm, and 30-60% of all colorectal carcinoma. An international study showed 30-40 of 100 colorectal carcinoma patient had mutation in KRAS gene.[9] Gene of KRAS is one of the important factors which influences the treatment and prognosis of colorectal carcinoma. The gene is affected by some environmental factors such as the diet profile of the patient. This study aims to analyze the association between KRAS gene mutation and diet profile of subjects with colorectal carcinoma.

METHOD

This was a cross sectional study. Data was collected from medical records of the patients in Gastroentero-hepatology Division and Clinical Pathology Department of Dr. Saiful Anwar General Hospital. Diet data was taken by history taking based on the frequency of consumption each food per week. The data encompassed any colorectal carcinoma subjects which undergone evaluation for KRAS gene mutation. Study was held for period of May -July 2017.

Total number of subjects which undergone KRAS gene mutation evaluation was 12, with equal ratio of male and female [6 females (50%) and 6 males (50%)].

The range of age of the subjects was 29-73 years old. Demographic and clinical characteristics such as age, sex, tumor histology, history of smoking, etc, were obtained from the medical records. The inclusion criteria were all colorectal cancer patients who was detected in Gastroentero-hepatology division and undergone KRAS gene mutation examination in Saiful Anwar General Hospital.

Human genomic DNA was extracted from the tissue or from formalin-fixed paraffin embedded (FFPE) samples. DNA extraction was performed using AmoyDx KRAS/NRAS mutations detection kit (AmoyDx FFPE DNA Kit, Cat No. Adx-FF01 for specimens with paraffin embedded). Mutation of KRAS/NRAS gene was detected using real-time polymerase chain reaction (PCR). The data was analyzed using Chi-square test with significance value if $p < 0.05$. Statistical analysis was done with SPSS 16.0 for Windows.

RESULTS

There were 12 subjects which had been evaluated for gene mutations with equal ratio of male and female [6 females (50%) and 6 males (50%)]. Range of age was about 29-73 years in which there 9 subjects with age < 60 years old (75%) and 3 subjects with age > 60 years old (25%). The gene mutations were found in 4 subjects (33.3%) of 12. Three subjects (75%) had positive KRAS gene mutation and 1 subject (25%) had positive NRAS gene mutation. It was described in Table 1 that 3 subjects (25%) had history of smoking and 9 subjects (75%) did not have any history of smoking.

From the 4 subjects with gene mutation, 1 subject (8.3%) was male and 3 subjects (25%) was female, with 2 subjects (50%) were aged below 60 years old and 2 subjects (50%) were aged above 60 years old. Histological feature of subjects with gene mutation was well differentiated adenocarcinoma (33.3%). All the subjects with gene mutation did not have any history of smoking.

Three subjects (25%) with gene mutation had symptom of bloody stool. Subjects with the symptoms of diarrhea was the same percentage as subjects with mucous in stool (16.7%). All subjects with mutation (33.3%) never had any complaint of constipation. Three of them (25%) never had any change in bowel habit. Baseline characteristic of subjects with gene mutations was described in Table 1 and Table 2.

Table 1. Baseline characteristic of subjects with colorectal carcinoma

Characteristic	n (%)
Sex	
Male	6 (50)
Female	6 (50)
Age (year old)	
< 60	9 (75)
> 60	3 (25)
Histopathology features	
Well differentiated adenocarcinoma	5 (41.7)
Moderate differentiated adenocarcinoma	2 (16.7)
Mucinosum adenocarcinoma	1 (8.3)
Adenocarcinoma	4 (33.3)
History of smoking	
yes	3 (25)
no	9 (75)

Table 2. Characteristic of colorectal carcinoma subjects with gene mutations

Characteristic	Positive mutation n (%)	Negative mutation n (%)	Total n (%)	p
Sex				
Male	1 (8,3)	5 (41,7)	6 (50)	0,545
Female	3 (25)	3 (25)	6 (50)	
Age				
< 60 years old	2 (16,7)	7 (58,3)	9 (75)	0,236
> 60 years old	2 (16,7)	1 (8,3)	3 (25)	
Histopathological features				
Well differentiated adenocarcinoma	4 (33,3)	1 (8,3)	5 (41,7)	0,491
Moderate differentiated adenocarcinoma	0 (0)	2 (16,7)	2 (16,7)	
Mucinosum adenocarcinoma	0 (0)	1 (8,3)	1 (8,3)	
Adenocarcinoma	0 (0)	4 (33,3)	4 (33,3)	
History of smoking				
Yes	0 (0)	3 (25)	3 (25)	0,491
No	4 (33,3)	5 (41,7)	9 (75)	
Bloody stool				
Yes	3 (25)	6 (50)	9 (75)	1,0
No	1 (8,3)	2 (16,7)	3 (25)	
Mucous in stool				
Yes	2 (16,7)	5 (41,7)	7 (58,3)	1,0
No	2 (16,7)	3 (25)	5 (41,7)	
Diarrhea				
Yes	2 (16,7)	1 (8,3)	3 (25)	0,236
No	2 (16,7)	7 (58,3)	9 (75)	
Constipation				
Yes	0 (0)	5 (41,7)	5 (41,7)	0,081
No	4 (33,3)	3 (25)	7 (58,3)	
Changes of bowel habit				
Yes	1 (8,3)	3 (25)	4 (33,3)	1,0
No	3 (25)	5 (41,7)	8 (66,7)	

Data profiles about the diet had been presented in Table 3 and Table 4. The most significant factor for gene mutation was low green vegetables diet (p = 0.023). It had negative correlation with gene mutation. There were not any association between another diet factors with the gene mutation in colorectal carcinoma subjects.

Table 3. Chi-square test for diet profile in colorectal carcinoma subjects with positive and negative mutations

Diet profile	Positive mutation	Negative mutation	p
Bread	1,50 ± 3,00	3,38 ± 2,50	0,277
Potato	3,50 ± 2,65	3,13 ± 2,42	0,811
Tofu/soybean	4,75 ± 1,26	5,0 ±2,67	0,865
Meat	4,75 ± 0,96	2,50 ± 2,51	0,050
Chicken meat	3,25 ± 1,71	3,25 ± 2,25	1,00
Eggs	4,50 ± 1,73	4,25 ± 2,49	0,862
Saltwater fish	4,0 ± 1,41	4,88 ± 1,46	0,346
Green leaf vegetables	3,50 ± 1,29	5,63 ± 1,30	**0,023**
Fruity vegetables	3,75 ± 0,96	4,38 ± 1,92	0,560
Banana	4,25 ± 0,96	4,25 ± 1,98	1,00
Papaya	4,75 ± 1,50	3,75 ± 2,31	0,456
Orange/apple	2,50 ± 2,38	3,75 ± 1,98	0,356
Milk	2,50 ± 3,0	3,63 ± 2,44	0,500
Oil/coconut milk	2,50 ± 2,89	4,50 ± 1,60	0,267
Tea	5,0 ± 0,82	4,13 ± 2,23	0,474
Coffee	4,0 ± 2,94	2,88 ± 2,53	0,506
Instant noodle	3,0 ± 2,16	2,75 ± 2,55	0,870

Table 4. Correlation between diet profile with gene mutation

Diet	Kirsten rat sarcoma (KRAS) gene mutation	
	p	R (correlation)
Bread	0,277	0,342
Potato	0,811	-0,078
Tofu/soybean	0,865	0,055
Meat	0,120	-0,473
Chicken meat	1,000	0,000
Eggs	0,862	-0,056
Saltwater fish	0,346	0,298
Green leaf vegetables	**0,023**	0,645
Fruity vegetables	0,560	0,187
Banana	1,000	0,000
Papaya	0,456	-0,238
Orange/apple	0,356	0,293
Milk	0,500	0,216
Oil/coconut milk	0,146	0,446
Tea	0,474	-0.229
Coffee	0,506	-0,213
Instant noodle	0,870	0,053

DISCUSSION

The results of this study, from the total of 12 subjects with colorectal carcinoma which had undergone gene mutation evaluation in Dr. Saiful Anwar General Hospital, it had been found 4 subjects (33.3%) with gene mutation. Most of the mutation occurred in KRAS gene (75%). These results corresponded with some literatures in which it was suggested that KRAS gene mutation occurred in 30-50% of colorectal carcinoma and it related to increase proliferation and decrease of apoptosis.[2]

Proto-oncogene of KRAS translates the components of ERK signal transmission. It commonly subjects to mutation in the case of colorectal carcinoma. This ERK signal transmission mediates cellular response to growth factors and regulates some factors which play important roles in the cell cycles, apoptosis, and cell differentiation.[11]

Sexual characteristic was not found to be different between male and female, in which it consistent with results from Adam et al that suggested there were not any significant differences of mutation incident between male and female.[11] The non-significant difference was also found in the history of smoking.

According to Oleg, positive gene mutation was more commonly found in the population below 60 years old.[12] It was rather different with the result of this study in which there were more population above 60 years old. Well differentiated histopathology results were more mostly found in subjects with positive mutation compared to another histopathological result. Those results corresponded with study from Liu et al which suggested positive mutation was mostly found in well to moderate differentiated colorectal carcinoma.[2]

In non-mutation group, the frequency of green leaf vegetables diet was higher than in gene mutation group (p = 0.023). Green leaf vegetables diet had negative correlation with gene mutation (p = 0.023; r = -0.645). It corresponded to the results from Adam et al which showed low green leaf vegetables diet correlated with KRAS mutation (p = 0.02). Diet low in green leaf vegetables and also fruits will increase the prevalence of G transition to A which can produce gene mutation. Vegetables and fruits have some bioactive components such as the flavonols. The flavonols inhibit production of nitrous substances, in which the nitrous substances itself play important role in the alkylation of guanine base. Alkylation of guanine base can lead to G transition to A that will induce gene mutation. Therefore, diet low in vegetables, especially green leaf, and fruits, is consistent with the transition of G to A in tumor.[11]

Subjects with positive mutation had higher frequency of meat consumption compared to the non-mutation subjects. It had positive correlation with mutation although it was not statistically significant (p = 0.05 for Chi-square test; p = 0.120; r = 0.473). It was suitable with results of Adam et al that suggested the KRAS mutation connected to increase consumption of white meat (p < 0.001). Study of Satu Valo et al yielded similar results where it showed diet rich in energy, red meat and preprocessed meat, high alcohol consumption, along with low fiber, low calcium, low vitamin D, low folic acid, and low selenium diet correlated with increased risk of colorectal carcinoma.[12]

Besides, study from Petra et al also showed positive correlation between red meat consumption with KRAS mutation. It was suggested that high fat diet increase proliferation of colonocyte and tumor formation.[12]

The main limitation of this study was the small number of the samples which could make the results of the study become insignificant. Besides, this study lacked accurate measurement of daily diet of the subjects.

CONCLUSION

Mutation of KRAS and NRAS genes was found in 33.3% of subjects with colorectal carcinoma. This study showed an association between low green leaf diet with the mutation of KRAS and NRAS genes. Further study is needed with more sample and specific dietary history recording.

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