The Role of Xenobotic Metabolism MGST1 Gene Polymorphism in Colorectal Cancer Patients

Fardah Akil, H.A.M. Akil, A.M. Lutfie, Wahyu S.Wibowo, Upik Miskad, Irawan Yusuf

Centre of Gastroentero-Hepatology, Department of Internal Medicine, Faculty of Medicine, Hasanuddin University-Wahidin Sudirohusodo Hospital. Jl. Perintis Kemerdekaan KM 11, Makassar, Indonesia. Correspondence mail: dndakil@gmail.com

ABSTRAK

Tujuan: *untuk melihat peranan gen Microsomal Glutathione S-Transferase1 (MGST1) sebagai salah satu gen yang mengkode enzim metabolisme yang bekerja pada faktor lingkungan.* ***Metode:*** *dengan menggunakan studi kasus-kontrol, subjek usia <50 tahun dikumpulkan dari beberapa rumah sakit pendidikan di Makassar antara tahun 2008-2010. Biopsi sampel tumor dari kolonoskopi atau pembedahan serta darah perifer diambil pada 35 kasus dengan 61 orang sebagai kontrol. Kasus kanker kolorektal (KKR) tanpa riwayat keluarga kanker, didiagnosis melalui pemeriksaan klinis dan dikonfirmasi secara histopatologi adenokarsinoma. Sekuensing dilakukan pada 3 kb regio genom DNA MGST1 mengunakan PCR-restriction fragment length polymorphism (PCR-RFLP).* ***Hasil:*** *dari 96 subjek, ditemukan dua varian single nucleotide polymorphisms (SNPs) MGST116454T>G and 16416G>A. Hubungan bermakna dengan KKR (p=0.047) dideteksi pada genotipe GG SNP 16454T>G MGST1 dengan resiko 3.5 kali lipat (95% confidence interval (CI) 0.962-13.191).* ***Kesimpulan:*** *polimorfisme gen MGST1 sebagai salah satu gen lingkungan berkonstribusi untuk resiko KKR pada usia muda (<50 tahun).*

Kata kunci: *kanker kolorektal, mikrosomal glutathione S-transferase (MGST1), usia muda, SNP 16454T>G.*

ABSTRACT

Aim: *to asses the role of Microsomal Glutathione S-Transferase1 (MGST1) gene as one of enzym metabolism that plays in enviromental factor.* ***Methods:*** *using case-control study, subjects with age less than 50 years were collected from teaching hospital Makassar between 2008-2010. Frozen or routinely processed tumour samples biopsy and peripheral blood were obtained from 35 CRC patients undergoing surgery and endoscopic examination with 61 subject as control. CRC cases were diagnosis by clinical examination and confirm by histopathology without familial aggregation of CRC. DNA resequencing was conducted for the 3 kb genomic DNA region MGST1 using PCR-restriction fragment length polymorphism (PCR-RFLP).* ***Results:*** *from 96 subject, two varian single nucleotide polymorphisms (SNPs) 16454T>G and 16416G>A MGST1 were identified. Significant CRC association (p= 0.047) was detected in GG genotipe SNP 16454T>G MGST1 with 3.5 fold risk (95% confidence interval (CI) 0.962-13.191).* ***Conclusion:*** *the results suggest that MGST1 gene polymorphisms as one of environment gene may contribute to CRC risk in younger age (<50 years old).*

Key words: *colorectal cancer, microsomal glutathione S-transferase (MGST1), younger age, SNP 16454T>G..*

INTRODUCTION

Colorectal carcinoma (CRC) is a major human health problem worldwide affecting approximately one million individuals per year and causing 500.000 deaths annually.[1] Data from GLOBOCAN 2008 estimated number of new cases ages less than 65 years are increasing 56.497 compared ≥65 years 40.839. Demographic changes was estimated in 2015 about 12,827 cases. Either in Indonesia, number of new cases of CRC estimated in 2015 are increasing at ages less than 65 years.[2]

Other data from national cancer registry of Ministry of Health in collaboration with Indonesian Pathology Anatomy Association observed tendency of younger age of 40 years old with incidence rate 35.26%.[3] Ridho et al., report CRC incidence in Makassar about 26.8% in 2000-2004 from endoscopic patients. Rates of age for female and male respectively 50-59 and 60-69 years.[4]

Epidemiological evidence indicates that most of our human cancers are originally caused from environmental exposures to genotoxic agents. To be able to protect cells from damage by the exposure of various forms of reactive substances, cellular systems for detoxification are essential. The major systems that strongly regulate the susceptibility to, e.g., cancer have been identified, namely the enzymes involved in drug metabolism and in DNA repair. Since most carcinogens or mutagens need to be altered chemically by drug-metabolizing enzymes before they can exert their genotoxic effect, the importance of these enzymes in regulating the levels of induced genotoxic effect in cells is obvious.

Both in vivo and in vitro studies suggested that the exposure to dietary carcinogens increased CRC risk significantly.[5,6] The metabolism of carcinogen involves both activation (phase I) and detoxification (phase II) reactions. Glutathione S-transferase group (GST) are a superfamily of proteins that perform thephase II detoxification reactions. This detoxification reaction inactivates the compounds and renders them water-soluble, so they can be readily excreted through urine or bile. Microsomal GST1 (MGST1), a member of GST family is an abundant enzyme detected in the liver microsomes and the outer membrane of mitochondria. Polymorphisms MGST1 result in reduced and increased enzyme activity[7,8], but these same polymorphisms have not been implicated in risk for hereditery pancreatitis.[9] An overall increased risk for sporadic colorectal cancer has been described for the MGST1 allele, in a report by Zhang et al.[10]

Study from Sudoyo et al. suggested that environment mechanism maybe involved in the occurance of CRC in youger people other than genetic etiology (microsatellite instability).[11] Therefore, we assessed the association between polymorphisms in genes involved in xenobiotic-metabolism MGST1 and younger age at onset of colorectal cancer to determine if genetic variation of this candidate metabolic genes modifies the age at onset of colorectal cancer.

METHODS

Subject

A total of 35 cases and 61 controls were included in this study, between years 2008 and 2010 at Wahidin Sudirohusodo Hospital and others teaching hospital in Makassar. Young age defined as less than 50 years old, since according epidemiology data, the risk of CRC is increasing at age >50 years. The cases are histopathologically confirmed from sporadic CRC patients. The controls are age-matched healthy individuals visiting the same hospital for routine health check up or treatment for noncancer illness. The exclusion criteria for both controls and cases are self-reported family cancer history, previous or current radiotherapy/chemotherapy for unknown conditions. All subjects gave written informed consent to participate in the study in accordance with the process approved by the Health Research Ethics Committee of Hasanuddin University of Medical Faculty.

Genotyping

All participants provided 10 mL samples of blood from which DNA was extracted using the GFX™ Genomic Blood DNA Purification Kit (Amersham Biosciences,NJ, USA). Primers for MGST1 (Gen Bank accession No.NT_009714) were designed by using the Primer3software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3).

Polymerase chain reaction (PCR) were performed using AmpliTaq@ Gold DNA polymerasekit (Applied Biosystems, CA, USA) and a MJ-PTC 200 thermocycler (Bio-Rad Laboratories, South San Francisco, CA). PCR reaction was carried out in 20 µl

solution containing 1.5 mM Mg2+, 200 µM diethylnitrophenylthiophosphate (dNTP), 0.3 µM each primer, 10 ng genomic DNA as template, and 0.5 U polymerase. Each PCR product was verified by its mobility in 1% agarose gel electrophoresis, purified, and subjected to DNA sequencing. DNA sequencing was conducted by using BigDye@ Terminator v3.0 Cycle Sequencing Kit (Applied Biosystems, CA, USA) and the ABIPRISM@ 3100 Genetic Analyzer (Applied Biosystems, CA,USA). Each sample was sequenced or genotyped at leasttwice to confirm the results, and the SNPs were identified using Bio Edit v 7.0.0 software.

Statistical Analysis

We estimated the genotype frequencies for each variant gene polymorphisms and tested them for Hardy-Weinberg equilibrium. Data were analyzed using a standard $\chi 2$ test. The genotype or allele frequencies in cases and controls were compared under a normal approximation tested association between SNPs and CRC risk. $P<0.005$ was considered to be statistically significant. Odds ratio (OR) given with 95% confidence interval (CI) was computed by logistic regression analyses with adjustment age and sex (SPSS 12.0).

RESULTS

We detected 2 SNPs 16416G>A and 16454T>G in the 3.4-kb DNA region of the partial MGST1locus that covers the 3′ untranslated exon 4 regions. All of the SNPs analyzed were in Hardy-Weinberg equilibrium (exact $P>0.05$; data not shown). A total subjects were 35 (45.5%) in CRC and 61 (66.7%) in controls. The median age cases was 38.83 ±8.10years and 42.26±6.10 for controls. On stratified analysis by gender in case and controls, we found that female 14 (40%) and 25 (41%); male 21 (60%) and 36 (59%) respectively. The common symptoms was found in CRC cases is hematokezia, with endoscopic examination result mostly in rectum localisazation and polypoid morphology. Majority histopathology yield as well differentiation, and combine with radiology imaging, we found negative metastasise as a common findings. (**Table 1**)

Table 1. Characteristics of patient with colorectal cancer and healthy control

Variable	Cases (n=35)	Control (n=61)
Mean (year)	38.83±8.10	42.26±6.10
Gender		
- Male	21 (60%)	36 (59%)
- Female	14 (40%)	25 (41%)
Symptoms		
- GI bleeding	26 (74.2%)	
- Change of defecation	9 (25.8%)	
Tumour localization		
- Rectum	21 (60%)	
- Non-rectum	14 (40%)	
Tumour morphology		
- Polypoid	23(65.7%)	
- Ulcerative	12 (34.3%)	
Histology (differentiation)		
- Well	20 (57.1%)	
- Moderate	14(40%)	
- Poor	1 (2.9%)	
Metastasize		
- Negative	23(65.7%)	
- Positive	12 (34.3%)	

The genotype frequency observed in all groups of the study are presented in **Table 2.** MGST1 analysis has shown majority of the study participants displayed the homozygous GG genotype in SNP 16416G>A. The "AA" genotype was only identified in controls, but

Table 2. Association between MGST1 genotype and colorectal cancer risk

SNP	Genotypic			Odds ratio (95% CI)	*p value
	<50 years				
	Genotipe	Cancer	Control		
16416G>A	GG	23	37	1.243	0.622
	GA+AA	12+0	22+2	(0.523-2.957)	
16454T>G	GG	7	4	3.563	0.047
	TG+TT	11+17	24+33	(0.962-13.191)	

*Chi square. The significant results ($P<0.05$) are in bold

no differences has been found in allelic groups. Homozygous GG genotype in SNP 16454T>G was much lower, but it was slightly higher in cancer group as well. The carriers of GG genotype SNP 16454T>G showed significantly increased CRC risk with p=0.047 (OR=3.563, 95% CI: 0.962-13.191). (**Table 2**)

We did not detect any statistically significant difference in the distributions and risk for CRC at allelic levelfor SNPs 16416G>A and 16454T>G (p>0.05). (**Table 3**)

DISCUSSION

The pathogenesis of colorectal cancer can be ascribed to multiple factors, such as the environment and family history. Metabolic enzymes are responsible for the activation ordetoxification of mutagenic xenobiotics. Cancer susceptibility might result from differences in the expression of metabolic enzymes.

This study showed significant association with CRC risk in statistical analysis for individual GG genotype SNP 16454T>G with OR=3.563, 95% CI: 0.962-13.191, p=0.047. (**Table 2**)

Zhang et al. reported the first time that the xenobiotic metabolism gene MGST1 was associated with sporadic CRC in a Chinese Han population. The combined genotype analysis of two SNPs 102G>A and 16416G>A detected a statistically significant CRC association for individuals carrying GG/GG (102G>A/16416G>A) genotype (adjusted OR, 1.682; 95% CI, 1.177–2.404; P=0.004).Several studies have examined the association between other family of GST and colon cancer risk, however, these results have been conflicting.[12-14]

Two studies about CRC in younger age from Indonesia was published in 2009 and 2010. One study explored the role of inflammation, the expression of NF-κB and COX-2, but did not find any correlation comparing older age 15. Other study was to examine neo-angiogenesis pathway, the expression of VEGF-A and desmoplastic reaction between the young aged and old aged patients, and found comparable correlation between strong positive VEGF-A expression and strong desmoplastic reaction in young patients with CRC.[16]

The literature has shown that associations between colon cancer and some dietary constituents are stronger among those diagnosed at a younger age.[17-19] Associations with other factors, such as family history of colorectal cancer, have also been shown to be stronger among those diagnosed when younger.[20,21]

Being diagnosed with cancer at a young age may imply a higher degree of genetic susceptibility. Genetic susceptibility broadly can take two forms: inheritance of high- or low-penetrance genes. Inherited susceptibility associated with high-penetrance genes (such as the adenomatous polyposis coli gene or APC), carries ahigh level of cancer risk and is associated with a strong family history of colon cancer. Such genes are rare andaccount for less than 5% of colon cancers in the population. Susceptibility associated with inheritance of low-penetrance genes, while carrying a much lower independent risk than high-penetrance genes, is relatively common and may therefore be associated with a much higher population-attributable risk.[22] Dietary constituents may interact with molecular variants of low-penetrance genes that regulatemetabolizing enzymes fase 2, such as gluathione-S-transferases (GST). For instance, meat prepared at high temperatures contains heterocyclic amines and polycyclic aromatic hydrocarbons that are metabolized by enzymes such as MGST1. Other dietary components, such as fats, also contain polycyclic aromatic hydrocarbons and heterocyclicamines, and may interact with variants of these low-penetrance

Table 3. Association between MGST1 allel and colorectal cancer risk

SNP	Allelic			Odds ratio (95% CI)	*p value
	<50 years				
	Allel	Cancer	Control		
16416G>A	G	60	96	1.354	0.431
	A	12	26	(0.636-2.885)	
16454T>G	T	46	87	1.405	0.282
	G	26	35	(0.755-2.613)	

*Chi square

genes.[21] In our study, twenty-one (87.5%) from 24 subject recall about their diets dominant for intake meat/fats.

The MGST1 gene have been associated with colon cancer in one study from China, although different associations were observed between variants of genotypes and colon cancer in this study. This gene as one of family glutathione S-transferases (GSTs) are a family of enzymes widely expressed in mammalian tissues including in colonocyte epitel with more than 50%[23];and have a broad substrate specificity. It has been found that most GST substrates are xenobiotics or products of oxidativestress, including some environmental carcinogen, such as high meat intake, in some studies[24] but not others.[25,26]

MGST1 enzyme is thought to be of particular importance in the detoxification of carcinogens including metabolic electrophile intermediates and lipophilichydroperoxides through its glutathione dependent transferase and peroxidase activities.[27] This variant genotype, homozygous GGSNP 16454T>G has an transversion in exon[4], causing phenyl to cystein amino acid change and have been suggested to decrease enzyme activity. Hence, this could make metabolite electrophilic reactive due to excessive production of reactive intermediates (reactive oxygen species (ROS), reactive nitrogenspecies (RNS), lipid peroxides, free radicals) increasing thatcan bind to DNA and result in adducts that cause mutations if not repaired, thereby initiating carcinogenesis.While it is reasonable to hypothesize that the dietary pattern composed of meat or fats interacts with MGST1 based on the proposed mechanisms, wedid not yet observe interaction between dietary pattern and molecular variants of this gene.

Those diagnosed at a younger age appear to be most affected by diet. Previous studies have shownthat components of diet, such as meat, are associated with colon cancer in populations diagnosed at a relatively young age. This finding could answer a part of COR responding study from Sudoyo et al., and epidemiology data, that the role of genetics in colorectal cancer was not affected mostly in young Indonesian people.

CONCLUSION

Data from this study corroborate the previous findings that MGST1 gene may be an important determinate in the observed colorectal cancer risk associated with younger age. This gene have been found to carry significant risk in conjunction with environmental exposures, especially with homozygous GG genotype SNP 16454T>G. These results indicated the potential of developing MGST1 as CRC a susceptibility marker, but would require validation in further multiethnic studies.

ACKNOWLEDGEMENTS

We acknowledge and gratefully for the SNP examination support from SNP Division of Mochtar Riady Institute for nano technology-Tangerang, Banten.

REFERENCES

1. Alpers DH, Giardiello FM. Malignant tumors of the colon. In: Yamada T, Alpers DH, Kalloo AN, et al., eds. Atlas of gastroenterology. 4th edition.UK: Blackwell Publishing Ltd; 2009. p. 465-90.
2. GLOBOCAN International Union Against Cancer (IUCC) global cancer control. Available at http://www.uicc.org/general-news/globocan-2008-launched,2008.
3. Abdullah M. Tumor kolorektal. In: Aru WS, Setiohadi B, Alwi I, et al., eds. Buku ajar ilmu penyakit dalam. Volume 1. 4th edition. Jakarta:Pusat Penerbitan Ilmu Penyakit Dalam FKUI; 2006. p. 375-80.
4. Ridho F, Tahir H, Akil HAM, et al. Prevalensi kanker kolorektal berdasarkan temuan endoskopi saluran cerna bawah di Makassar. Laporan penelitian. Buku abstrak dan makalah lengkap Pertemuan Ilmiah Nasional XIII PPHI-Kongres nasional PGI-PEGI XII, Makassar; 2005. p. 84.
5. Willett WC. Diet and cancer: an evolving picture. JAMA. 2005;293(2):233-4.
6. Sachse C, Smith G, Wilkie MJV, et al., and the Colorectal Cancer Study Group. A pharmacogenetic study to investigate the role of dietary carcinogens in the etiology of colorectal cancer. Carcinogenesis. 2002;23:1839–49.
7. Johansson K, Ahlen K, Rinaldi R, et al. Microsomal glutathione transferase 1 in anticancer drug resistance. Carcinogenesis. 2007;28:465–70.
8. Maeda A, Crabb JW, Palczewski K. Microsomal glutathione S-transferase 1 in the retinal pigment epithelium: protection against oxidative stress and a potential role in aging. Biochemistry. 2005;44:480-9.
9. Schneider A, Tögel S, Barmada MM, et al. Genetic analysis of the glutathione s-transferase genes MGST1, GSTM3, GSTT1, and GSTM1 in patients with hereditary pancreatitis. J Gastroenterol. 2004;39(8):783-7.
10. Zhang H, LiaoLing-Hong, Liu Shuk-Ming, et al. Microsomal glutathione S-transferase polymorphisms and colorectal cancer risk in a Han Chinese population. Int J Colorectal Dis.2007;22:1185–94.
11. Sudoyo A, Krisnuhoni E, Basir I, et al. Colorectal cancer among young Indonesian a clinicopathology & immunohistochemical study in microsatellite instability among ethnic Javanese, Sundanese, Makassar and Minang patients. Laporan penelitian. Buku abstrak

dan makalah lengkap Pertemuan Ilmiah Nasional XIII PPHI-Kongres nasional PGI-PEGI XII, Makassar. 2005. p. 64.
12. Goodman JE, Mechanic LE, Luke BT, et al. Exploring SNP-SNP interactions and colon cancer risk using polymorphism interaction analysis. Int J Cancer. 2006;118(7):1790–7.
13. Ates NA, Tamer L, Ates C, et al. Glutathione S-transferase M1,T1,P1 genotype and risk for development of colorectal cancer. Biochem Genet. 2005;43(3-4):149-63.
14. Stoehlmacher J, Park DJ, Zhang W, et al. Association between Glutathione S-Transferase P1, T1, and M1 genetic polymorphism and survival of patients with metastatic colorectal cancer. J Natl Cancer Inst. 2002;94:936–42.
15. Abdullah M, Sudoyo AW, Pranowo BW, et al. Expression of NF-kB and COX-2 in young versus older patients with sporadic colorectal cancer. Acta Med Indones-Indones J Intern Med. 2009;41:70-4.
16. Ganggaiswari A,Kresno SB, Krisnuhoni E. VEGF expression and desmoplastic reaction as potential progressive factors in young patients with colorectal cancer. Acta Med Indones-Indones J Intern Med. 2010;42:6-11.
17. Slattery ML, Potter JD, Ma KN, et al. Western diet, family history of colorectal cancer, NAT2, GSTM-1and risk of colon cancer. Cancer Causes and Control. 2000;11:1-8.
18. Pande M, Amos CI, Osterwisch DR, et al. Genetic variation in genes for the xenobiotic-metabolizing enzymes CYP1A1, EPHX1, GSTM1, GSTT1, and GSTP1 and susceptibility to colorectal cancer in Lynch syndrome cancer. Epidemiol Biomarkers Prev. 2008;17(9):2393–401.
19. Mahdavinia M, Bishehsari F, Ansari R, et al. Family history of colorectal cancer in Iran. BMC Cancer. 2005;5:112-9.
20. Andrieu N, Launoy G, Guillois R, Ory-Paoletti C, Gignoux M. Estimation of the familial relative risk of cancer by site from a French population based family study on colorectal cancer (CCREF study). Gut. 2004;53(9):1322-8.
21. de Jong MM, de Nolte IM, te Meerman GJ, et al. Low-penetrance genes and their involvement in colorectal cancer susceptibility. Cancer Epidemiol Biomarkers & Prev. 2002;11:1332-52.
22. Surapureddia S, Svartza J, Magnusson, et al. Colocalization of leukotriene C synthase and microsomal glutathione S-transferase elucidated by indirect immunofuorescence analysis. FEBS Letters. 2000;480:239-43.
23. Chao A, Thun MJ. Meat consumption and risk of colorectal cancer. JAMA. 2005;293(2):172-82.
24. English DR, Mac Innis RJ, Hodge AM, et al. Red meat, chicken, and fish consumption and risk of colorectal cancer. Cancer Epidemiol Biomarkers & Prev. 2004;13(9):1509-14.
25. Little J, Bradley L, Bray MS, et al. Colorectal cancer and genetic polymorphisms of CYP1A1, GSTM1 and GSTT1: a case-control study in the Grampian region of Scotland. Int J Cancer. 2006;119(9):2155-64.
26. Osna NA. Role of MGST1 in reactive intermediate-induced injury. World J Gastroenterol. 2011;17(20): 2552-7.
27. Norat T, Bingham S, Ferrari P, et al. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. J Natl Cancer Inst. 2005;97(12):906-16.