

Genetic pattern of CYP1A1*2A (T>C) gene in patients with colorectal cancer in South Jakarta – Indonesia

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Abstract— The information of relationship between genetics and cancer in Indonesia, especially colon cancer, is still limited. This study aimed to determine the genetic pattern of colon cancer allele in Fatmawati Hospital, Jakarta -Indonesia. We conducted a case-control study of 40 colorectal cancer patients and 133 volunteers as controls. We observed CYP1A1*2A alleles using RFLP-PCR method. Three milliliters of blood was used to isolate DNA. Our observation showed of the genetic patterns CYP1A1*2A with the incidence of colorectal cancer showed 17 patients were variant-type homozygote and 12 patients were heterozygote type. In this research shown the common of genotyping type CYP1A1*2A of colorectal cancer from the patients are a homozygous variant type.

Keywords: CYP1A1*2A; colorectal cancer; Indonesia

I. INTRODUCTION

The information of the relationship between genetics and cancer had been carried out. Cytochrome P450 (CYP450s) is an enzyme that plays a pivotal role in the xenobiotics and drug metabolism. It has been widely reported that mutations in one of the nucleotides in this enzyme gene causing individual to be susceptible to some cancers. The CYP1A1 gene is one of the most widely studied genes, which associated with cancer. Several CYP1A1 polymorphisms had been reported on Human CYP Allele Nomenclature website showing evidences of the link between genetics and cancer incidences. The involvement of this enzyme activity in the activation or de-activation shows the individual's susceptibility to cancer. The CYP1A1 polymorphism especially CYP1A1* 2A (T>C) will produce a cutting site for MspI enzyme; it has been linked to individual sensitivity to colon cancer [1-5].

The study about genetics and cancer in Indonesia, especially colon cancer is still limited. Colorectal cancer is one of the three main types of cancers that commonly found in Indonesia. Epidemiological studies in Europe, Asia, and America have shown this disease is more prevalent in adults aged 45 to 50 years. Furthermore, there is still lack of information about colorectal cancer in Indonesia [6-9].

We screened for colorectal cancer patients from Fatmawati Hospital Jakarta-Indonesia, starting May until the end of 2014. There were 40 colorectal cancer patients who were new cases or old cases patients who routinely control to the hospital.

II. MATERIALS AND METHODS

A. Subjects

A total of 40 patients and 133 volunteers were recruited for this study. The inclusion criteria for cases were patients who were positively diagnosed as colon cancer and have been getting medical treatment (surgery, chemotherapy, etc.). All volunteers as controls should show a negative result for FOB test (Faecal Occult Blood test). Three milliliters of blood used for genetic screening. All patients and volunteers shall agree to participate in this study.

B. Methods: PCR-RFLP

The DNA from patients and controls was extracted from peripheral blood. PCR-based restriction fragment length polymorphism (RFLP) methods were used to analyze CYP1A1*2A.

The genetic examination commenced by isolating genome using a Genome Isolation Kit from Genaid® with catalog numbers: #GB300. The testing was continued by target gene amplification using PCR Kit Kapa2G Fast PCR Kits with catalog number #KK5609. The primers used were 5'-TAGGA GTCTT GTCTC ATGCC T-3' (sense) and 5'-CAGTG AAGAG GTGTA GCCGC T-3' (anti-sense), following experiments by Nisa et al (2012). The PCR steps are: denaturation I 95⁰C for 3 minutes; thirty-five cycles of PCR: denaturation II 95⁰C for three minutes, annealing 60⁰C for 15 seconds and elongation 72⁰C for two seconds; and the final extension 72⁰C for five seconds, with large PCR product is 340 base-pair (bp). The results were observed using electrophoresis techniques in order to 1.5% of agarose for 60 minutes. Electrophoresis results were observed using UV light. The next stage is cutting by enzyme MspI fragment using RFLP technique.

C. Data Processing and Statistical Analysis

Analysis Statistic of Hardy-Weinberg equilibrium calculator with Chi-squared distribution (1 degree of freedom) by Santiago Rodriguez [10].

III. RESULTS

RLFP MspI enzymes showed two kinds of banding pattern with a size of 200 and 140 bp. Individuals with wild-type homozygotes have a band size of 340 bp. Individuals with variant (mutant) type homozygote have band size 140 and 200 bp, whereas heterozygous individuals will pick the pattern of the band are 340, 200 and 140 bp. The observation of all samples, showing the pattern of genotyping *CYP1A1*2A* varied (Fig 1).

Analysis of the genotype distribution and allele frequencies showed that homozygous mutant type is commonly found in patients, whereas heterozygous type is commonly found in controls (Table 1, 2).

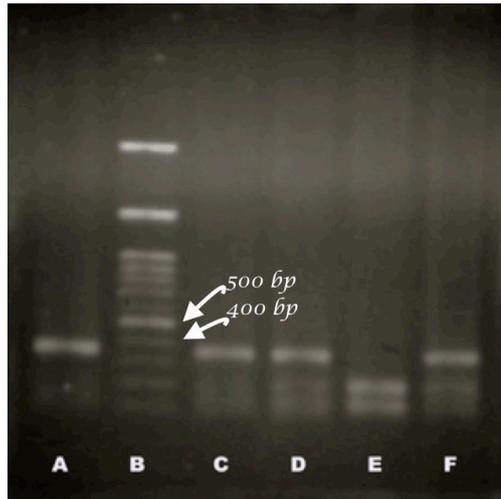


Fig 1. PCR- RFLP result
 A: Band with 340 bp
 B: DNA Marker size 100 bp
 C,D, F: Band with 340, 200 and 140 bp
 E: Band with 140 and 200 bp

IV. DISCUSSION

The earlier study had reported the association of lifestyle (especially cigarette) and genetic with colorectal cancer risk. Nisa et al showed that lifestyle and genetic factors have played in the role in colorectal carcinogenesis. They studied in comparison genetic of smoker and non-smoker colorectal cancer patients in Fukuoka, Japan used RFLP-MspI PCR Method [6].

CYP genes are member of large families of endoplasmic and cytosolic enzyme genes. *CYP1A1*2A*, part of *CYP1A1* gene located on chromosome 15q, is a member of the *CYP1* genes family with a major role in the oxidative activation and or deactivation of a wide range of xenobiotic and drug metabolism. The expression of this gene was consisting of the aryl hydrocarbon receptor and mutation on this gene reported has associated with cancer evidenced [11,12,13,14]. Two common polymorphisms in the *CYP1A1* gene are a T6235C change within the 3' noncoding region of the gene (*2A, also known as the MspI restriction) and an A4889G change in the

hems-binding domain of exon 7 (*2C). This polymorphism of the gene has been examined to cancer susceptibility [4,5,15].

TABLE 1. GENOTYPE (N)

No	Genotype	Control Obs (Exp)	Cases Obs (Exp)
1	Wild-type homozygote (TT)	59 (37.9)	11 (7.23)
2	Heterozygote (TC)	24 (66.2)	12 (19.55)
3	Variant type homozygote (CC)	50 (28.9)	17 (13.22)

Exp = expected
 Obs = observed

TABLE 2. ALLELE FREQUENCIES (%)

No	Frequencies Allele	Control	Cases
1	T or p allele freq	53.38 %	42.5 %
2	C or q allele freq	46.62 %	57.5 %

TABLE 3. SOLUTIONS FOR HWE OF EACH GROUP

Control					
Group affected	Common (T)	Heterozygotes	Rare (C)	p allele freq	Q allele freq
Common (T)	2.88	24	50	0.19	0.81
Heterozygotes	59	108.63	50	0.52	0.48
Rare (C)	59	24	2.44	0.83	0.17
χ^2	54.04 > 3.84; P value 0.05 (3.84)				
Cases					
Group affected	Common (T)	Heterozygotes	Rare (C)	p allele freq	Q allele freq
Common (T)	2.12	12	17	0.26	0.74
Heterozygotes	11	27.35	17	0.45	0.55
Rare (C)	11	12	3.27	0.65	0.35
χ^2	5.97 > 3.84; P value 0.05 (3.84)				

TABLE 4. RISK RATIO, ODDS RATIO ALLELE IN CONTROL AND CASES

	Cases	Control	Total	OR
Allele variant-type (C)	46 (27%)	124 (73%)	170	1.55
Allele wild-type (T)	34 (19%)	142 (81%)	176	
TOTAL	80 (23%)	266 (76%)	346	

Swinney RM et al (5), Cosman G et al [16] and Taioli E et al [17] reported that the *CYP1A1*2A* has been shown to vary among different ethnic groups and has an influence on risk to susceptibilities environmental toxins. Studies in Japan and other country showed the gene has been associated with a risk of colorectal cancer [6,14,18,19,20,21]. The other researcher was reported that *CYP1A1* MspI was contributed to increased cancer susceptibility among Asians. The effect of this polymorphism is diverse according to by ethnicity and cancer types [8].

As described earlier, the *CYP1A1* gene has an important role in the metabolism of xenobiotic and drugs. The presence of *CYP1A1*2A* polymorphism in our samples might be enable the risk of colorectal cancer, where polymorphism *CYP1A1*2A* in the form homozygote variant allele (C) might be increases the risk compared to wild-type form (T). Several studies have reported that the risk of the emergence of colorectal cancer is not only caused by the presence of variant alleles, but the environmental factors that are extrinsic factors are major contributors to the risk of cancer [22].

V. CONCLUSION

The research has shown the common genotyping type CYP1A1*2A of colorectal cancer from 40 samples were a homozygous variant type.

CONFLICT OF INTEREST

The authors report no conflict of interest. The authors alone are responsible for the content and writing of this article.

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