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*The Description of Polymorphism of CYP1A1*2A rs4646903 (T>C) Gene as Colorectal Cancer Risk Factor In Medical Study Programs and Doctor Profession 2012-2014 UIN Syarif Hidayatullah Jakarta*

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***Abstract*—Colorectal cancer is the third leading cause of death from all cancer cases in Indonesia. From previous research it is known that there is a relationship between genetic polymorphism of cytochrome P450 (CYP) with the incidence of colorectal cancer. Therefore, research team of FKIK UIN Syarif Hidayatullah Jakarta and Fatmawati General Hospital work together to evaluate the frequency of SNP CYP1A1*2A rs 4646903 (T> C) allele in Medical Study Program and Doctor Profession of FKIK UIN Syarif Hidayatullah Jakarta. Methods: Blood sampling was taken from healthy student of Medical Study Program and Doctor Profession of FKIK UIN Syarif Hidayatullah Jakarta. Genotype examination was performed by PCR-RFLP method. Results and Conclusions: Based on screening results, most of genotype is a heterozygot , while wild type and mutant are minority. So most of the students has polymorphism SNP CYP1A1*2A rs4646903 (T> C)**

Keywords— Colorectal Cancer; CYP1A1*2A rs4646903; single nucleotide polymorphism

I. INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in the world and its prevalence has been steadily increasing over the last century. It is the fourth most common cancer that cause death in the world.[1] Thus, CRC is the third leading cause of death from all cancer cases in Indonesia, which prevalence at 2013 is 1,4% or about 347.792.[2] Cytochrome P450 (CYP) enzymes are a critical importance for the metabolism of xenobiotic, which function is detoxification. If the process is failed, it can cause carcinogenesis. [3-6] It revealed that these polymorphisms were associated with increased enzyme activity to activate carcinogens. The CYP1A1 gene is located on the long arm of chromosome 15q22-qter.[7] Previous studies shown that mutant homozygous and heterozygous allele of CYP1A1*2A have a risk of colorectal cancer. The frequency of the CYP1A1*2A allele is about 9.4% in Caucasians, 35.8% in Asians, and 23.8% in Africans.[8-9] The aim of the present study was to test for potential association between the CYP1A1*2A and the risk of CRC in student of Medical Study Program and Doctor Profession of Faculty of Medicine and Health Sciences, Syarif Hidayatullah State Islamic University, Jakarta.

II. MATERIALS AND METHODS

A. Sample collection

This study was conducted after review and approval of the Institutional Review Board of the Ethics Committee at FKIK UIN Syarif Hidayatullah Jakarta. Blood samples were collected from 74 healthy subjects (27 males, 47 females, age range 17-23 years; mean age 19,78 years). The control samples were collected from subjects referred to the university.

Tissue samples to be used for RNA analysis were immediately submerged in RNA later solution (Ambion, Courtabeuf, France) to avoid RNA degradation, stored at 4°C for 24 h, and then stored at -20°C until needed.

B. DNA Isolation

Genomic DNA was isolated from blood samples using Geneaid GB100. Concentration, purity, and quality of the isolated RNA were determined using the DeNovix DS-11 Spectrophotometer.

C. Genotyping

Genotyping for the CYP1A1*2A allele (T>C; rs4646903) was achieved by polymerase chain reaction restriction fragment length polymorphism (PCR RFLP). Briefly, a 338 bp DNA fragment containing the polymorphic MspI restriction site, corresponding to the 3' end of CYP1A1, was amplifed

using the following primers: 5'-TAGGAGTCTTGTCTCATGCCT and 5'-CAGTGAAGAGGTGTAGCCGCT.

The PCR was carried out in a final volume of 25 µl containing 12,5 µl KAPA2G Fast ReadyMix + dye (2X) PCR kit (Headquarters, USA), 1 µl of each primer, 5 µl of the extracted genomic DNA, 5,5 µl of dH2O. Cycling conditions were as follows: pre incubation step at 95°C for 2 min, 56 sec; 40 cycles consisting of denaturation at 95°C for 15 sec, annealing at 65°C for 15 sec, and extension (elongation) at 72°C for 20 sec; followed by a final extension step at 72°C for 2 min. PCR products were analyzed using 1 % agarose gel electrophoresis and the size of the products were determined by including 100 bp DNA ladder on the gel and visualization using AttoPrintgraph (Printgraph ATTO AE-6905 CF CCD camera controller).

Finally, 5 µl of each PCR product was digested 4 hours at 37°C with 1,5 µl of MspI restriction enzyme. The digestion products were subjected to electrophoresis on 1.5% agarose and on Agarose LE (Kit Code No.11-685-660-001, Roche Diagnostics) using elektroforesis ATTO My Power II 300 AE-8135 electrophoresis system at 90 V, 1 A and stained with DNA Loading Dye AM29502.

III. RESULT

The PCR result are illustrates in Figure 1 and the CYP1A1*2A allele (T>C; rs4646903) genotypes are illustrated in Figure 2. The distribution of CYP1A1*2A allele (T>C; rs4646903) genotypes among the tested subjects and statistical analysis of the obtained data are detailed in Table 1. The frequency of the CYP1A1 wild type homozygous allele was 23%, mutant homozygous allele was 20.3%, heterozygous allele was 56.7% in healthy subjects.

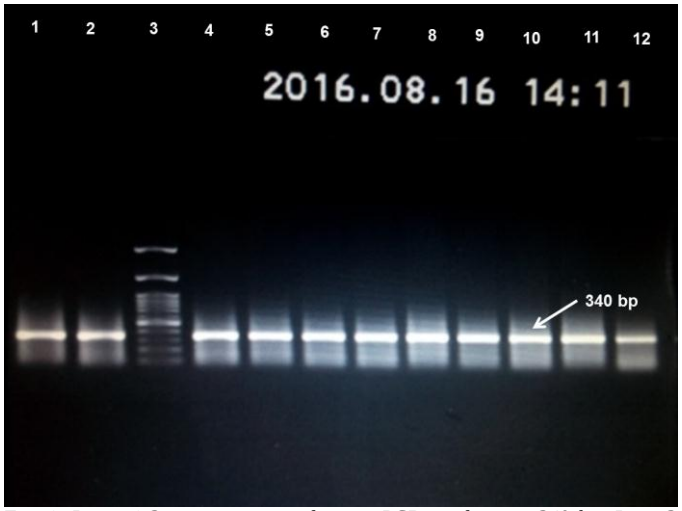

Fig. 1. Lanes 1-3 represent control uncut PCR products at 340 bp. Lane 3 represents DNA ladder molecular weight markers.

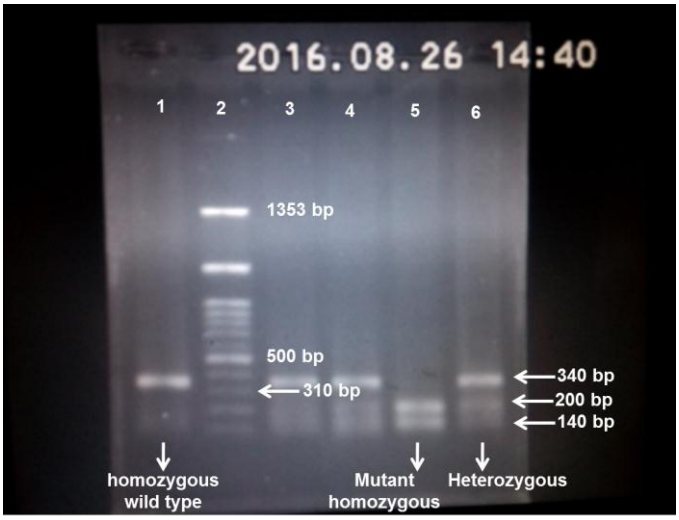

Fig. 2. Agarose gel (2.5%) Electrophoresis for PCR Products of CYP1A1*2A allele (T>C; rs4646903).

The amplicon is subjected to digestion with MspI prior to electrophoresis. The 340 bp uncut amplicon (upper band) reveals CYP1A1*2A allele (T>C; rs4646903) wt/wt homozygous wild type (lane 1), the mutant homozygous (lane 5) shows two fragments (200 and 140 bp), and the heterozygous genotype (lane 3,4,6) presents three fragments (the uncut 340-bp fragment and two restriction fragments of 200 and 140 bp)

TABLE I. ANALYSIS PCR RFLP OF CYP1A1*2A ALLELE (T>C; RS4646903) GENOTYPE

Genotyping	Frequency	Percentage (%)
Wild type homozygous	17	23,0
Mutant homozygous	15	20,3
Heterozygous	42	56,7
Total	74	100,0

IV. DISCUSSION

The results of genotyping CYP1A1*2A rs4646903 (T>C) genotyping using RFLP PCR technique in the students of Medical Study Program and Doctor Profession of FKIK UIN Syarif Hidayatullah Jakarta from 74 samples,wild type homozygous allele was 23%, mutant homozygous allele was 20,3%, heterozygous allele was 56,7% in healthy subjects. Based on screening results, most of genotype is a heterozygot, while wild type and mutant are minority. So most of the students has polymorphism SNP CYP1A1*2A rs4646903 (T> C). This is reinforced by previous studied that CYP1A1*2A is a large variety of alleles owned by residents of the Asian region (Japan) from Caucasians. The frequency of the CYP1A1*2A allele is about 9.4% in Caucasians, 35.8% in Asians, and 23.8% in Africans.[8-9]

CYP1A1*2A allele (T>C; rs4646903) allele might thus exhibit higher rates of carcinogen activation than individuals with the wild-type allele. From the studied gene variations, two genetically linked polymorphisms of CYP1A1, *2A and *2C, conferred at least 3-fold increases in its catalytic

activity.[10] Similar to the four studies carried out in Asian populations, the carriers of the C allele of CYP1A1*2A have a 2.53-fold increased risk for CRC compared with the T allele carriers. [8,9,11,12] Previous studies at Japan shown that heterozygous allele of CYP1A1*2A have a risk of colorectal cancer.[13]

Wes suggest to do other diagnostic criteria such as FOB (Fecal Occult Blood) examination, look for data about smoking habits, consumption of red meat, family history which are some risk factors for colon cancer and educate the students of Medical Study Program and Doctor Profession of FKIK UIN Syarif Hidayatullah Jakarta due to the association between the CYP1A1*2A rs4646903 gene with the risk factor for colorectal cancer,

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