Association of CYP1A1 Gene Polymophism (3801T/C), Smoking, Menstrual Status and Post Hormonal Contraceptive Use with Invasive Breast Carcinoma

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***Abstract*- Background:** CYP1A1 plays a role in the phase I metabolism of polycyclic aromatic hydrocarbon (PAHs) and estrogen metabolism. This gene polymorphism is suspected to be one of predisposition factors contributing to incidence of breast carcinoma. Literatures about CYP1A1 gene polymorphism in breast carcinoma showed contradictory results.
Aim: To analyze the association of CYP1A1 gene polymorphism (3801T/C), smoking, menstrual status and past hormonal contraceptive use with invasive breast carcinoma.
Material and method: Forty six patients of invasive breast carcinoma and 46 healthy women (control) were analyzed with PCR-RFLP method by using Msp1 restriction enzyme.
Results: Overall, there were no significant associations between CYP1A1 gene polymorphism and invasive breast carcinoma (p=0.877). Smoking women with heterozygote T/C genotype apparently had higher risk of suffering from invasive breast carcinoma (p=0.04, OR=2.551 [95%CI 1.304-4.989]), while other variables didn't.
Conclusion: This study showed that CYP1A1 gene polymorphism (3801T/C) is not a risk factor for invasive breast carcinoma. However, smoking women with heterozygote T/C genotype were 2.5 times at risk than wild type and homozygote genotype to suffer invasive breast carcinoma.

***Index Terms*-** polymorphism, CYP1A1 (3801T/C), CYP450 1A1, invasive breast carcinoma

I. Introduction

Breast carcinoma is the most common malignancy among women worldwide, with estimated 911,014 new cases and 137,514 cases in Southeast Asia. Based on GLOBOCAN 2018, this carcinoma is the second most frequently diagnosed carcinoma, with an estimated 2,088,849 cases (11.6%) and 626,279 carcinoma-related deaths.[1] In Indonesia, breast carcinoma is one of the carcinoma with highest prevalence in 2013.[2]

Epidemiologic studies showed that breast carcinoma is a multifactorial and polygenic disease. Combination of genetic and environmental factors play a role in the development of breast carcinoma.[3] About 5-9% of all invasive breast carcinoma is thought be hereditary breast carcinoma.[4] Combination of BRCA1 and BRCA2 gene mutation are found in 30% hereditary breast carcinoma and less than 2% of all breast carcinoma.[5] It is thought that maybe there are other low penetrating genes also increasing individual susceptibility to breast carcinoma.[6] For last few years, several genetic researches have been done to identify genetic variation associated with breast carcinoma.[7]

Cytochrome P450 (CYP450s) are enzymes catalyzing phase I metabolism reactions. Cytochrome P450 1A1 (CYP1A1) is one of the cytochrome P450 superfamily playing an important role in metabolism of xenobiotic and endogenous substance which mostly found in extrahepatic tissues, especially in the epithelial cells, including breast.[8] This CYP1A1 catalyzes the metabolism of polycyclic aromatic hydrocarbons (PAHs) and contributes in the formations of reactive metabolites that are capable to induce DNA damage. If this process is not intervened, it will initate or accelerate carcinogenesis.[9]

CYP1A1 also involves in breast carcinoma through estrogen-related mechanism. Estrogen can initiate and promote the process of breast carcinoma. Estradiol is metabolized through two pathways into inactive 2-hydroxyestrone or active 16α-hydroxyestrone. 2-hydroxyestrone has a weak binding capacity to estrogen receptor, meanwhile 16-hydroxyestrone is increased in breast carcinoma and usually associated with tumorigenesis.[10]
CYP1A1 gene is located in 15q22-q24 and consists of 7 exon and 6 intron spanning 5810 base pair (bp).[25] CYP1A1 Gene polymorphism is divided into 3 groups, such as wild-type T/T, heterozygote T/C and homozygote C/C. If PCR shows only 1 fragmen, such as 340 bp, this will be defined as wild-type; 2 fragments (200bp and 140 bp) as homozygote; and 3 fragments (340bp, 200bp, and 140bp) as heterozygote.[8]

Studies about the association between gene polymorphisms and breast carcinoma is still controversial. Therefore, researchers would like to analyze about the association between cytochrome CYP1A1 gene polymorphisms in invasive breast carcinoma.

II. METHODS

Forty six breast carcinoma patients and 46 healthy women were analyzed in this study. Patients' groups were stored DNA from previous study, but control groups were obtained from community service activities. Control groups had been gathered

since March 2019 after obtaining approval from the Ethics Committee of Medical Faculty of University of Sumatera Utara. The blood was isolated using Promega Wizard Genomic DNA Purification Kit. The isolating procedures were done according to the kit protocol. The isolated samples were kept while waiting for the histopathology results.

The CYP1A1 (3801T/C) polimorphism gene were determined using a PCR-RFLP-based assay method[4]. PCR amplification of a 340-base DNA fragment using the primers 5'-CAGTGAAGAGGTGTAGCCGCT-3' (*Forward*) dan 5' TAGGAGTCTTGTCTCATGCCT-3' (*Reverse*). PCR process was prepare containing 12,5 µl mix solution (Gotaq PCR master mix by Promega), 2 µl DNA Template, 1,0 µl Forward primer 10 pmol, 1,0 µl, Reverse primer 10 pmol, 8,5 µl nuclease-free water, resulting in 25 µl solution in each PCR tube. These mixture was spindown and the PCR amplifications were performed as follows: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 1 minutes and a final extension at 72°C for 10 minutes. 10 µl of PCR product was digested with 1,5 µl MspI (Bench Top 100bp DNA Ladder, Promega Corporation, Madison, USA), then incubated at 37°c for 16 hours. The restricted products were analyzed by electrophoresis in 2% agarose gel containing ethidium bromide and visualized under a UV illuminator. The PCR product was identified by the presence of bands on the gel. Three different genotypes were defined for the individual polymorphism. Wild type T/T showed 1 fragment (340 base bp), heterozygote T/C showed 3 fragments (340 bp, 200bp and 140 bp), and homozygote C/C showed 2 fragments (200 bp and 140 bp).[9]

The data was analysed using statistical software and the results were presented in frequency tables. Menopausal status is determined as cessation of menstruation for ≥ 12 months, excluding cessation of menstruatian caused by pregnancy or breastfeeding.Smoking was categorized as active and passive smokers. Past hormonal contraceptive use was defined as yes if ever used pill, injection, or implant contraception.

III. RESULT

Forty six breast carcinoma patients and 46 controls were analyzed in this study. Mean age of patients was 49.54 years old (median 49.5 years and range 33-68 years), while the mean age of controls was 43.32 years old (median 38.5 years and range 28-64 years). Past smoking and menstrual status is different between cases and controls, but there is no difference in hormonal contraceptive use.

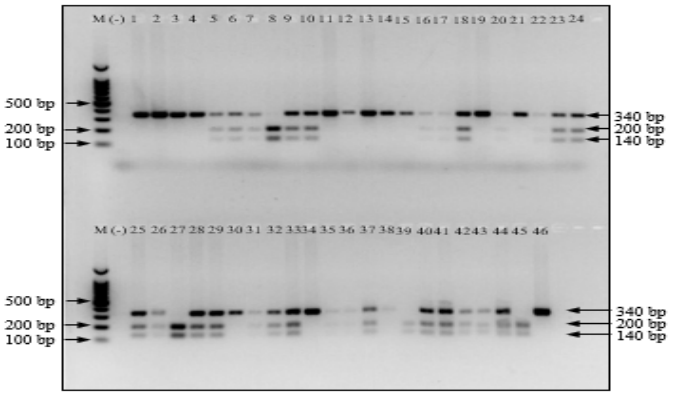

Figure 1. Gel electrophoresis showing PCR-RFLP product of CYP1A1 gene (3801TC) from patients on 32 well-2% agarose gel: Marker (lane1), blanco control (lane 2), samples 1-24 (lane 3-24 line 1), samples 25-46 (lane 3-22 line 2).

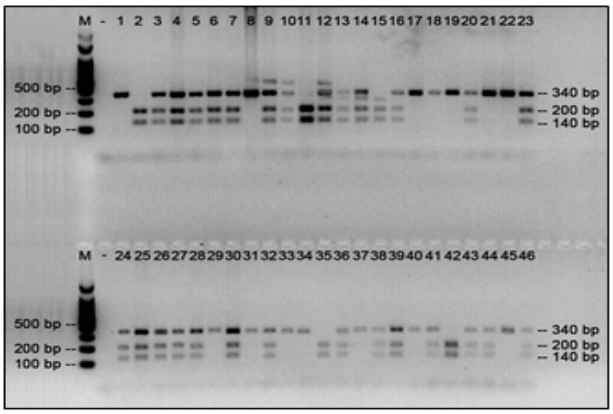

Figure 2. Gel electrophoresis showing PCR-RFLP product of CYP1A1 gene (3801TC) from controls on 32 well-2% agarose gel: Marker (lane1), blanco control (lane 2), samples 1-23 (lane 3-25 line 1), samples 24-46 (lane 3-25 line 2).

Analysis results showed that there were 26 (49.1%) patients with heterozygote T/C gene polymorphism, 16 (53.3%) patients with wild type T/T gene polymorphism, and only 4 (44.4%) patients with homozygote C/C gene polymorphism suffering from breast carcinoma. This study didn't find any significant relationship between CYP1A1 gene polymorphism (3801T/C) and breast carcinoma (p=0.877) (table 1), but if this relationship was assessed based on the association of CYP1A1 gene polymorphism (3801T/C) and clinicopathological characteristics, it showed that smoking patients with heterozygote T/C genotype were at risk 2.5 times higher to suffer from invasive breast carcinoma (table 2).

IV. DISCUSSION

The etiology of breast cancer involves various genetic, environmental, and behavior risk factors in each individuals. Genetic mutation with high susceptibility especially BRCA1 and BRCA2 gene is only found in trivial cases so it's suspected that there are other low penetrating genes can cause breast cancer.

The finding of this study was in accordance with most researches, including meta-analysis by Yao et al, Chen et al, Sergentanis et al, Khalili, and Garcia-martinez.[11-15] However, contrary to findings of several researches such as Shen et al, Shin et al, and Balmukhanov et al, we didn't find that heterozygote T/C genotype was associated with increased breast carcinoma risk.[16-18] Boyapati et al demonstrated that homozygote C/C genotype had lower risk for developing breast carcinoma, especially women with low body mass index and long endogenous estrogen exposure.[9] Controversial results was also discovered by Okobia et al stating that heterozygote T/C genotype can decrease risk of breast carcinoma about 21%.[19] Meanwhile, Naif et al said that homozygote C/C variant had higher risk for breast carcinoma, especially in post-menopausal women.[20] When comparing our results to those of older studies, we assumed that CYP1A1 gene polymorphism (3801T/C) wasn't a predisposing factor to invasive breast carcinoma.

Controversy between these studies could only attributable to ethnic diversity, giving difference in genetic and social factors. Different ethnic subjects had diverse culture and lifestyle contributing to diversity of genetics, susceptibility to malignancy, and exposure doses to procarcinogen in each populations. Specific genotype that could give protection/ susceptibility to any cancer usually showed protection/ promotion effect in subjects exposed normal dose procarcinogens.[20] Moreover, different study location caused different lifestyle, race, total samples and research sampling techniques.

Even though this study generally didn't find any relationship between CYP1A1 gene polymorphism (3801T/C) and invasive breast carcinoma, but after more specific analysis based on CYP1A1 gene polymorphism (3801T/C) was done, this study revealed that smoking women with heterozygote T/C genotype had greater risk about 2.5 times of suffering invasive breast carcinoma (P=0.004, OR=2.551[95%CI 1.304-4.989]) (table 2). This study was in line with Ambrosone et al stating that patients with CYP1A1 gene polymorphism (3801T/C) smoking more than 29 packs per year had higher risk.[21] Nakachi et al and Ishibe et al, concluded that smoking women with homozygote C/C genotype were at greater risk than other genotypes.[22,23]

Smoking is a potential risk factor in developing breast carcinoma. Cigarette smoke consists of thousands chemical metabolites such as PAH, aromatic amine, and N-nitrosamine, most of them are carcinogens to breast. Cell proliferation during pre-puberty until pregnancy can reduce DNA repair mechanism to correct damage before cell division, and breast tissue is more susceptible to carcinogen exposure during this period.[24] Palmer et al stated that smoking at young age could increase breast cancer risk.[25] Ishibe et al gave an impression that harmful effect of cigarette smoke exposure could occur during early maturation of breast tissue.[23]

Verde et al demonstrated that carcinogen from cigarette smoke will pass through alveolar membrane and then with lipoprotein will be transported to breast. In vitro study found that cigarette smoke will induce epithelial transition to mesenchymal, producing more aggressive fenotype.[26] Several researches had shown that smoke ingredients was found in breast milk, and nipple aspiration from smokers, suggesting direct involvement of cigarette smoke in breast tissue injury.[27,28] Perera et al in his study discovered that DNA adducts with cigarette smoke exposure was found in 4 of 7 smoking women with breast cancer but not in 8 non-smokers with breast cancer.[29] Another research also reported about the finding of benzo(a)pyrene-like adduct in breast tissue of breast cancer patients but not in control groups.[30]

Activation through AhR influencing cell signaling pathways reflected its role in tumor development. Interaction between AhR and CYP1A1 can cause changes in steroid levels modulating bioactivation of therapeutic and xenobiotic agents and increasing cancer risk in smoking women. Therefore, this gene polymorphism may decrease the ability of detoxifying carcinogen components of smoke cigarette causing a person more susceptible to breast cancer.[31]

There were several limitation to this study. First, lack of information about smoking, such as age began to be exposed to cigarettes, duration of smoking or smoke cigarette exposure, amount of cigarettes per year and etc. Second, there was no information about duration of hormonal contraceptive use, age at menarche, body mass index, age at first pregnancy, and parity. Third, the composition of contraception whether only consist of estrogen or combination of estrogen and progesterone was also not recorded in this study. Moreover, further studies are needed to evaluate other risk factors related to smoke exposure, such as previous occupation, inhabitation, history of eating burnt foods, all of which play important role in PAH metabolism.

V. CONCLUSION

This study found that CYP1A1 gene polmorphism (3801T/C) wasn't a predisposing factor to invasive breast carcinoma. However, smoking women with heterozygote T/C genotype had greater risk about 2.5 times of suffering invasive breast carcinoma.

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Table 1. Analysis of the association between CYP1A1 gene polymorphism (3801T/C) and invasive breast carcinoma

	Group				Total	P value
	Control	%	Case	%		
Polimorfisme gen						
• *Wild type T/T*	14	46,7	16	53,3	30	
• *Heterozygote T/C*	27	50,9	26	49,1	53	0,877
• *Homozygot C/C*	5	55,6	4	44,4	9	
Total	46	50,0	46	50,0	92	

Tabel 2. Association of hormonal contraception, smoking, and menstrual status and invasive breast carcinoma in each wild-type, heterozygote, and homozygote groups.

Variabel	Group				Total	OR (95% CI)	P
	Control	%	Case	%			
Wild-type T/T							
Hormonal contraception							
• No	6	35,3	11	64,7	17	0,574	0,290
• Yes	8	61,5	5	38,5	13	(95%CI:0,265-1,244)	
Smoking							
• No	6	42,9	8	57,1	14	0,857	
• Yes	8	50	8	50	16	(95%CI:0,394-1,867)	0,980
Menstrual status							
• Pre menopause	12	60	8	40	20	3,000	
• Pasca-menopause	2	20	8	80	10	(95%CI:0,826-10,901)	0,093
Heterozygote T/C							
Hormonal contraception							
• No	16	59,3	11	40,7	27	1,401	0,337
• Yes	11	42,3	15	57,7	26	(95%CI:0,801-2,421)	
Smoking							
• No	20	71,4	8	28,6	28	2,551	0,004*
• Yes	7	28,0	18	72	25	(95%CI:1,304-4,989)	
Menstrual status							
• Pre menopause	23	57,5	17	42,5	40	1,869	
• Pasca-menopause	4	30,8	9	69,2	13	(95%CI:0,793-4,407)	0,175
Homozygote C/C							
Hormonal contraception							
• No	4	50	4	50	8	0,5	1,000
• Yes	4	100	0	-	1	(95%CI:0,250-1,000)	
Smoking							
• No	4	1	1	20	5	3,2	0,206
• Yes	1	3	3	80	4	(95%CI:0,554-18,471)	
Menstrual status							
• Pre menopause	5	83,3	1	16,7	6	0,167	0,097
• Pasca-menopause	0	-	3	100	3	(95%CI:0,028-0,997)	