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Identification of CYP1A1 (3801T/C) Gene Polymorphism in Invasive Breast Carcinoma

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Abstract : Breast cancer is still the biggest health program in the world. In Indonesia, until 2016 was the most malignancy found in 10 years period. Cytochrome P450 (CYP1A1) is involved in the metabolism of both environmental carcinogens and oestrogen. Its polymorphism may contribute to individual susceptibility to breast cancer. The aim of this study is to identify the frequency and distribution of CYP1A1 (3801T/C) gene polymorphism in invasive breast carcinoma. This cross-sectional descriptive study was conducted in Department of Anatomical Pathology, University of Sumatera Utara and H. Adam Malik Hospital Medan with 46 samples of invasive breast carcinoma. CYP1A1 gene polymorphism (3801T/C) was analyzed using PCR-RFLP method followed by gel electrophoresis. CYP1A1 (3801T/C) polymorphism in invasive breast cancer were 52,2% heterozygot T/C, 39,1%, wild-types T/T and 8,7% homozygot C/C. In both invasive breast carcinoma non-specific and specific type, heterozygote T/C genotype was more often found (54,3% and 72,7%) respectively. Therefore, individual who has polymorphism CYP1A1 (3801T/C) heterozygote T/C genotype has a tendency to suffer invasive breast carcinoma.
Keywords : Polymorphisme, gene CYP1A1, Invasive Breast Carcinoma.

Introduction

Breast cancer remains a serious health issue worldwide. Invasive breast carcinoma is the most common type of cancer in women. Breast cancer accounts for 23% of all cancer cases and 16% cancer deaths in women wordwide[1].In Indonesia, based on the data collected from Dharmais Cancer Hospital, breast cancer ranked first of all types of cancer handled in that hospital during the last 10 years (2007 – 2016). Meanwhile, the number of cases in North Sumatera was 2,682[2].

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Epidemiologic studies of breast cancer show that there are multifactorial interactions and polygenic involments. The combination of genetic and environmental factors are involved in the development of breast cancer. Estrogen and environmental carcinogen are considered to be the major breast cancer factors[3]. Cytochrome P450 (CYP450s) enzymes are responsible for most phase I reactions. Cytochrome P450 1A1 (CYP1A1) is an enzyme of the cytochrome P450 superfamily which playing important roles xenobiotic and endogenous metabolism, and found mainly in extrahepatic tissues, especially in the epithelial cells, including breast epithelial[4]. CYP1A1 catalyses the metabolism of polycyclic aromatic hydrocarbons (PAHs) and contributes in the formations of reactive metabolites that are capable to damage the DNA. If this process was not intervened, it would initiate carcinogenesis[5].

CYP1A1 also involved in breast carcinoma through a estrogen-related mechanism. Estrogen initiates and promotes the process of breast cancer. One of the risk factor of breast cancer is long exposure of either endogenous or exogenous estrogen. Estradiol was metabolised through two pathways to produce inactive 2-hydroxyestrone and active 16-hydroxyestrone. 2-hydroxyestrone has a weak binding capacity to estrogen receptor. 16-hydroxyestrone was found increased in breast cancer and associated with tumorigenesis[6].

The association between the polymorphisms and breast cancer is still controversial. This study aims to evaluate the association between cytochrome CYP1A1 (3801T/C) gene polymorphisms and invasive breast cancer.

Experimental

There were 46 peripheral blood specimens with the suspicion of invasive breast carcinoma in oncological surgery clinic at the Haji Adam Malik Medan General Hospital. The samples were collected between March until Mei 2018 after the approval from health research ethics committee of the Medical Faculty of Universitas Sumatera Utara and H. Adam Malik General Hospital.

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) were determined using a PCR-RFLP-based assay method[4.] PCR amplificatioan of a 340-base DNA fragment containing an MspI restriction site was performed, using the primers 5'- CAGTGAAGAGGTGTAGCCGCT-3' (*Forward*) dan 5'-TAGGAGTCTTGTCTCATGCCT-3' (*Reverse*). PCR mix solution (by Promega) was prepared, containing 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 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 MspI (Bench Top 100bp DNA Ladder, Promega Corporation, Madison, USA), then incubated at 37^{o}c for 16 hours. The restricted products were analyzed by electrophoresis in 2% agarose gel containing ethidium bromide. 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 pair [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).

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 menses caused by pregnancy or breastfeeding. According to WHO classification of breast cancer invasive breast carcinoma no special type and its variant determined as non-specific type and the other as special type.

Results and Discussion

There were 46 patients included in this study The mean±SD age was 49.54 ± 8.09 years, ranging from 33 – 68 years. Each group of ≥ 50 year-old and < 50 year-old had a total of 23 patients. Twenty four (52.2%) patients were having menopausal transition,and 22 (47.8%) patients were in the postmenopausal period. On microscopic examination showed that majority (35 samples / 76.1%) of the samples showed non-specific

histologic type of breast carcinoma, while 11 (23.9%) samples had specific histologic type of breast carcinoma. (Table 1).

Table 1. Selected characteristic of study samples

Variable	Frequency	Percentage (%)
Age (yrs)		
mean±SD	49,54±8,09	
median	49,5	
range	33-68	
Menopausal status		
Premenopausal		52,2
Postmenopausal		47,8
Histologycal type		
Non-specific type	35	76,1
Specific type	11	23,9
Total	46	100

Figure 1 and table 2 shown CYP1A1(3801T/C) gene polymorphisms, there were 24 (52.5%) patients with heterozygousT/C genotype, 18 (39.1%) patients with wild typeT/T genotype, and 4 (8.7%) patients with homozygous C/C genotype. The T allele (57) was more found than C allele (35).

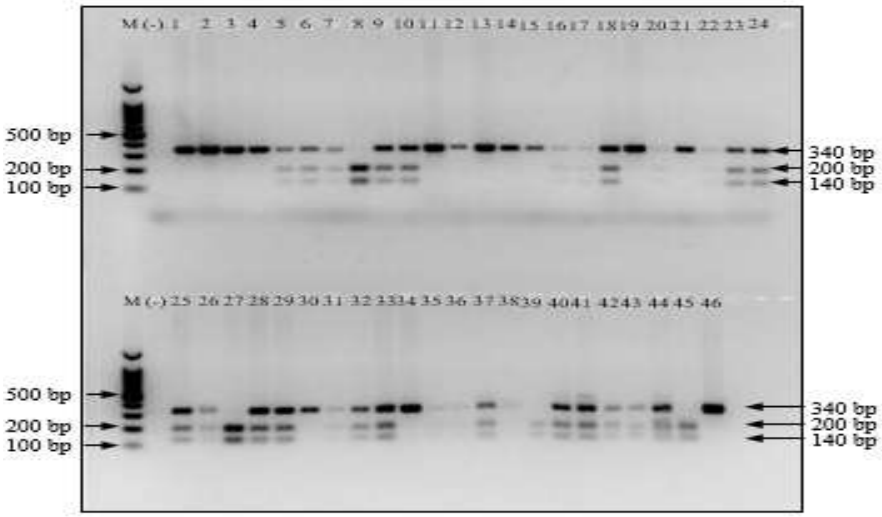

Figure 1. Electrophoresis results of PCR-RFLP generating fragments of polymorphism gen CYP1A1 (3801T/C) on a 2% agarose gel stained 60 well: Marker 100bp (line 1), negative control (line 2), samples (upper row: samples 1-24, lower row: line 2: samples 25-46)

Table 2. Frequency distribution polymorphism of CYP1A1 (3801T/C) gene and allele

Polimorfisme Gen *CYP1A1 (3801T/C)*	n	(%)	Allele T		Allele C	
Wild type T/T	15	32,6	30		-	
Heterozygote T/C	27	58,7	27		27	
Homozygot C/C	4	8,7	-		8	
Total	46	100	57		35	

Among 35 non-specific type breast carcinoma samples, the polymorphism CYP1A1 (3801T/C) gene were 19 (54.3%) samples with heterozygousT/C genotype, 12 (34.4%) samples with wild type T/T genotype, and 4 (11.4%) samples with homozygousC/C genotype. Meanwhile, among 11 specific type breast cancer samples, there were 8 (72,7%) samples with heterozygote T/Cgenotype, and 3 (27,3%) samples with wild typeT/T genotype. (table 3).

Table 3.Frequency distribution polymorphism of CYP1A1 (3801T/C) gene Related to histologic type of invasive breast carcinoma

Diagnosis	Polimorfisme Gen *CYP1A1 (3801T/C)*						Total
	Wild type	%	Heterozygote	%	Homozygote	%	
Non-specific type	12	34,3	19	54,3	4	11,4	35
Specific type	3	27,3	8	72,7	0	0	11
Total	15	32,6	27	58,7	4	8,7	46

Discussion

CYP1A1 gene located at 15q22-q24, comprise seven exon and six introns spanning 5,810 base pairs. It is an important Phase I enzyme that is induced by, and acts upon many of the potent mammary carcinogens and expressed in breast tissue. in human, CYP1A1 is under regulatory control of the aryl hydrocarbon receptor, a transcription factor that regulates gene expression. It is considered as one of the gene candidates related to breast cancer[7].

Mean of patients' age was 49.54 years, ranging from 33 – 68 years. Each group of ≥ 50 year-old and < 50 year old had a total of 23 patients. This finding was along with the report by Huang, et al finding (1998, Taiwan), but differed from those studies done by Taioli, et al (1995, New York), Boyapati, et al (2005, China), Sangrajrang (2009, Thailand), and Wang (2013, Guangzhou). Taioli et al reported that breast cancer occured mainly in older women, with a mean of 54.9 years[4,5,8,9,10].

Majority of the patients were in menopausal transition, only 47.8% were in the postmenopausal period. This finding was similar to the studies conducted by Boyapati, et al (2005, China), Shin, et al (2007, Korea), Sangrajrang (2009, Thailand). The Collaborative group on hormonal factors in breast cancer (2012) stated that 45–54 years-old women have 40% higher risk of suffering from breast cancer compared to postmenopausal women[5,6,9].

Based on the evaluation of CYP1A1(3801T/C) gene polymorphisms, majority of the patients had heterozygous T/C genotype, followed by wild type T/T genotype, and homozygousC/C genotype. This researches were in accordance with Wang, et al (2013, Guangzhou), Shen, et al (2006, Shanghai) Boyapati et al (2005, China), They concluded that heterozygous T/C genotype was the most common type, and homozygous C/C was the least common[5,10,11].Study of Shen, et al (2006, Shanghai) and Shin, et al (2007, Korea) showed that heterozygous T/C has a higher risk to develop breast cancer than wildtype T/T and homozygous C/C[6,11].In contrary, Okobia, et al. (2005, Nigeria) summarised that T/C heterozygous genotype reduced the risk of breast cancer as much as 21%[12]. However, Huang, et al. (1999, Taiwan) and Naif H et al. (2018, Iraq) argued their findings and concluded that those with homozygousC/C genotype was more likely to develop breast cancer, especially in postmenopausal women[4,13].Boyapati, et al (2005, China) reported that homozygousC/C genotype has a lower risk to have breast cancer, notably those with lower body mass index and longer endogenous estrogen exposure[5].The differences among those studies' results probably caused by the differences numbers of samples, races, genetic background, lifestyles, and exposure to risk factors of breast cancer.

To this day, the studies about association between histopathologic types and CYP1A1 (3801T/C) gene polmorphisms were still limited. This study found in both invasive breast carcinoma non-specific and specific type, heterozygote T/C variant was more often found (54,3% and 72,7%) respectively.

Our finding differed from Wang, et al, who summarised that specific type breast cancer was likely to have heterozygous T/Tgenotype and non-specific type breast cancer was likely to have wild type genotype. Nonetheless, statistical analysis showed that there was no significant association between histopathologic type and CYP1A1 (3801T/C) gene polmorphisms in breast cancer[4].

Based on the gene alleles showed that T allele (60) was more common than C allele (30). This was similar to the results obtained by Wang, et al and Shin, et al[6,11]. Furthermore, Shin, et al also concluded that T allel had 1.76 higher risk to have breast cancer (95%, CI = 1.19 – 2.61)[6]. However, Taoliet, al (1995) and Kiyohara (1996, Japan) disagreed, explaining that C allel increased the risk of breast cancer[8]. On the other hand, Miyoshi, et al (2003, Japan) reported that C allele reduced that risk of breast cancer, and individuals with T allele were susceptible to suffer from invasive breast carcinoma[14].The variation of allele possibly caused by the diversity of races and ethnic groups.

Conclusion

Our finding showed that the most patient invasive breast carcinoma has polymorphism CYP1A1(3801T/C) heterozygote T/Cgenotype. Based on alleles we found that T allele was the most often found than C allele. Therefore, individual who has polymorphism CYP1A1 (3801T/C) heterozygote T/C variant has a tendency to suffer invasive breast carcinoma. The discrepancy between the earlier studies polymorphism and our result may be due to several factors including difference in ethnicity, diet, study subject number and perhaps, most importantly ascribed to geographic variation and difference in study population and other environmental exposures.

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