

ORIGINAL ARTICLE

CAT-21 A/T GENE POLYMORPHISMS ASSOCIATED WITH BREAST CANCER SUSCEPTIBILITY

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Abstract:

Background: Oxidative stress induced cancer cell formation. Gene polymorphism plays roles in carcinogen metabolism, antioxidant and DNA repairing pathway was susceptibility to oxidative stress. This study aim to determine the association between CAT-21 A/T polymorphism with breast cancer susceptibility. **Methods:** Case control study was conducted on 65 breast cancer patient and 65 healthy control group. The whole blood samples were isolated from 65 breast cancer patients in Haji Adam Malik General Hospital Medan and 65 healthy control group. The CAT-21A/T polymorphism was analyzed by PCR-RFLP procedure. PCR-RFLP product was electrophoresed and visualized in agarose 4%. **Results:** The AA CAT-21 genotype were lower in breast cancer (BC) than healthy control (HC) group (31/47.7% vs 40/61.5%), in the contrary AT+TT genotype was greater in BC than HC group (34/52.3% vs 25/38.5%) with ($p=0.159$, $OR=1.755$, $CI=0.874-3.525$). A allele CAT-21 were found lower in BC than HC group (89/68.5% vs 105/80.8%) then T allele were greater in BC than HC group (41/31.5% vs 25/19.2%) with ($p=0.033$, $OR=1.935$; $CI=1.022-3.428$). **Conclusions:** There was significant difference in allele distribution of CAT-21 A/T between case and control group but no in genotype distribution. In this population study showed that allele of CAT -21 A/T polymorphism could represent as a risk factor to breast cancer.

Keywords: breast cancer, CAT-21 A/T, polymorphism.

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Introduction:

Breast cancer is the most common type of cancer in women. In 2016, there was estimated 245.299 new cases of female breast cancer and 41.487 women died of female breast cancer in the United States.¹ According to Indonesian Ministry of Health data in 2013, breast cancer in Indonesia has the second highest prevalence after cervical cancer.² Various research evidenced about cancer, it caused by a combination of various factors such as age, genetic mutation, age of menopause, first menstruation at young age, family heredity of breast cancer, alcohol consumption, radiation, obesity, hormone therapy and oxidative stress conditions in cells.³⁻⁵

Oxidative stress is an imbalance between free radicals and antioxidants that is triggered by reduced antioxidants and excess free radical production. There were three antioxidant groups, namely enzymatic antioxidants, chain-breaking antioxidants and transition metal antioxidants. The enzymatic antioxidant groups are superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and catalase (CAT).^{6,7}

The CAT is a common antioxidant that found in all oxygen-exposed organisms. Previous studies showed that rs7943316 (CAT-21 A/T) polymorphism in the promoter region can alter the binding affinity of transcription factors of CAT. This process leads to a

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decrease in the catalytic activity of CAT. The decrease activity of CAT can increase oxidative stress that caused damage to certain genes. Oxidative stress can trigger cancer development in the cells.^{8,9} The study by other researcher showed changes of expression of CAT "1518/"1226 in cancer cells. These gene changes support cell enhancement by inducing genetic instability and activating oncogenes.¹⁰ Study about association of CAT-21A/T polymorphism and breast cancer has never been done. Based on description above, we are interested in analyzing the association of CAT-21A/T polymorphism with breast cancer susceptibility in the Haji Adam Malik General Hospital Medan, Indonesia.

Methods:

Study subjects

This research was case control study design. We calculated the number of subjects based on the prevalence of breast cancer was 15-25%. Based on the equation, we got 65 breast cancer patients for case and 65 healthy women for control. Breast cancer patients that diagnosed by oncologist were recruited at Haji Adam Malik General Hospital, and healthy control group were recruited at Medical Faculty Universitas Sumatera Utara and gym in Medan. The inclusion criteria for case group were patients who had the age between 16-68 years old, had normal liver and kidney examination, had normal complete blood count (CBC) before underwent chemotherapy and approved to sign informed consent. The inclusion criteria for the control group were healthy women who had age between 16-68 years old, had normal liver and kidney examination. Exclusion criteria in healthy control group were family history of breast cancer.

Ethics

This study was done in March to November 2019 after ethical approval (E.C. No.8/ KEPKFK USU-RSUP HAM/ 2019) from ethical committee of Medical Faculty Universitas Sumatera Utara.

Blood Sample and DNA extraction

DNA was isolated from peripheral leukocyte. The 3 ml blood was put into EDTA tube and was centrifuged in 3000 rpm for 15 minutes, and then we collect 300 µl buffy coats in 1.5 ml eppendorf tube. From the incubation and centrifugation process of buffy coat, the form of sediment existed in the bottom of tube. We added 300 µl Nuclei Lysis Solution, 100 µl Protein precipitation 50 µl DNA Rehydration Solution to get the DNA (Promega®, USA).

PCR- RFLP and Detection of CAT-21A/T gene CAT-21A/T polymorphism analyzed by using conventional Polymerase Chain Reaction (PCR)-Restriction Fragment

Length Polymorphism (RFLP) in Laboratory of Medical Faculty of Universitas Sumatera Utara, Medan, Indonesia. The primers are Forward: 5'-AAT CAG AAG GCA GTC CTC CC-3' and Reverse: 5' TCG GGG AGC ACA GAG TGT AC-3'. The PCR cocktail mix volume is 23 µL + 2 µL isolat DNA. PCR cocktail mix consisted of 12.5 µL master mix, 1 µL each forward and reverse primer, and 8.5 µL nuclease free water. PCR was carried out with a primary denaturation step at 95°C for 4 minutes, with 30 cycles of denaturation at 94°C for 1 minute, primer annealing at 61°C for 40 seconds, elongation at 72°C for 1 minute, and a final elongation at 72°C for 5 minutes.¹¹

PCR product (250 bp) was digested by restriction enzyme Hinf1 for 2 hours at 37°C. RFLP product was visualized using agarose 4%, with wild type AA at 177 bp and 73 bp; heterozygote AT at 250 bp, 177 bp, 73 bp and mutant homozygote TT at 250 bp due to loss of restriction site for Hinf1 enzyme.

Statistical analysis

The association between CAT-21 A/T polymorphism with breast cancer was assessed by Chi Square with odds ratio and CI 95% using SPSS version 25.0 for windows program (SPSS, Chicago, IL, USA).

Results:

Histological presentations of breast cancer patients

65 histological data including Grade and Stage of breast cancer were retrieved from patients' hospital record. The Histological parameters of breast cancer group can be seen at Table I.

Table-I
Histological parameters of breast cancer

Characteristics	Grade	Case (Ca Mammae) n (%)
Invasive Duct Ca	I	18 (27.69)
	II	29 (44.61)
	III	2 (3.07)
Invasive Lobular Ca	II	4 (6.15)
Infiltrating Duct Ca	II	11 (16.92)
Infiltrating Duct Ca	III	1 (1.53)
Stage		
IIA		6 (9.23)
IIB		6 (9.23)
IIIA		2 (3.07)
IIIB		36 (55.38)
IV		15 (23.07)

Ditribution of CAT-21A/T genes among the study population

Fig.-1. is a representative gel showing the separation of CAT-21A/T gene amplicons from study subjects on agarose gel. PCR product of catalase gene electrophoresed in the form of DNA band of 250 bp.

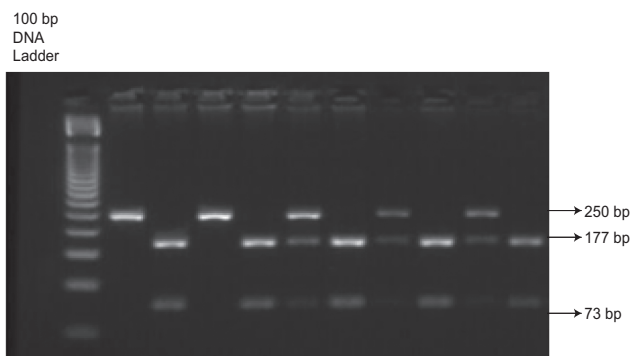


Fig- 1. The separation of CAT-21A/T gene amplicons in 4% agarose gel electrophoresis, RFLP products showed (AA genotype: 177 bp and 73 bp); (AT genotype: 250 bp, 177 bp, and 73 bp) and (TT genotype: 250bp)

In this study population, genotype CAT-21 had increasing the risk of breast cancer, although only clinically significant, not statistically ($p=0.159$, $OR=1.755$, $CI=0.874-3.525$). The A allele of CAT-21 was lower in breast cancer than healthy control group (68.5% vs 80.8%) whereas T allele were found greater in breast cancer than healthy control group (31.5% vs 19.2%). There was significant difference in allele distribution between case and control group ($p=0.033$). Comparison of allele proportion between breast cancer patients and healthy control group was showed in $OR = 1.94$ ($CI=1.022-3.428$). Genotype and allele distribution of CAT-21A/T in breast cancer and healthy control group showed at Table II.

There were no statistical differences in genotype distribution of CAT-21 gene polymorphism with breast cancer staging with $p = 0.937$ ($p>0.05$). The association between CAT-21A/T gene polymorphism and breast cancer stage showed in Table III.

Table II
Genotype and allele distribution in breast cancer and healthy control group.

CAT-21 A/T	Control n (%)	Breast Cancer n (%)	P Value	Odds Ratio	ConfidenceInterval (95% CI)
AA	40 (61.5)	31 (47.7)	-	1	(reference)
AT+TT	25 (38.5)	34 (52.3)	0.159	1.755	0.874 – 3.525
Total	65 (100)	65 (100)			
A	105 (80.8)	89 (68.5)	-	1	(reference)
T	25 (19.2)	41 (31.5)	0.033	1.935	1.022 – 3.428
Total	130 (100)	130 (100)			

Table-III
Genotype distribution of CAT-21A/T based on breast cancer stage

Genotype	Breast Cancer Stage			P Value
	IIA/IIIB	IIIA/IIIB	IV	
	n(%)	n(%)	n(%)	
AA	7 (58.3)	19 (50.0)	5 (33.3)	0.937
AT+TT	5 (41.7)	19 (50.0)	10 (66.7)	
Total	12 (100)	38 (100)	15 (100)	

Discussion:

Breast cancer is a disease that leads cells in breast to proliferate, change, and spread out of control. The proliferating cells will eventually form a lump or mass called tumor. Breast cancer can originate from breast tissue either from the gland for milk production or the ducts that connecting lobules to nipple. Early diagnosis of breast cancer provides a great opportunity for recovery and successful treatment. The gold standard for breast cancer is by histological examination. In this study, the histological grading of breast cancer was obtained and the most results were invasive duct carcinoma (grade II). These results are in line with previous studies that the invasive ductal grading was the highest. According to the American Cancer Society,

8 out of 10 invasive breast cancers were invasive ductal carcinoma type. Invasive ductal carcinoma type starts from cells in the lactiferous ducts of breast glands that come through the ductal walls and develop into breast fat tissue.¹²⁻¹⁴

In this study, the most common stage of breast cancer was stage IIIB with 36 cases (55.38%). The same results were found in study by Partini et al., (2018)¹⁵. The other study by Rondonuwu et al., (2016) found the most breast cancer is stage IV with 96 cases (63.6%).¹⁵ The discovery of many patients in advanced stage is due to low public awareness in checking themselves routinely or doing breast self-examination (BSE). Different data were obtained in Wang et al., study, which showed the highest percentage of staging was at the localized stage (stages I, and II).¹⁶ This may be due to the high awareness of women in that population through early detection of breast cancer such as mammography test, so that the prevalence can be suppressed.^{17,18}

Early detection of breast cancer can be done when someone knows the factors related to the risk of suffering from breast cancer. The risk factors are very complex such as increasing age, family history, body mass index, carcinogenic, hormonal exposure such as estrogen use, increased oxidative stress and etc.^{5,6,19} Oxidative stress is related to an imbalance between the production of reactive oxygen species (ROS) and antioxidant levels. The human body has antioxidant systems such as CAT to neutralize ROS. As an antioxidant, the function of CAT is releasing H₂O₂ into oxygen and water, H₂O₂ is important in cell defense against oxidative damage. The catalase gene encodes a single protein of 526 amino acids located on chromosome 11p13; the catalase gene length is 34 kb consisting of 12 introns and 13 exons. Lower of CAT gene expression in cancer cells is still an unanswered question, but the previous research reported the polymorphism of rs7943316 (CAT-21A/T) changed the catalytic activity of catalase enzymes. The reduced catalytic activity of CAT can increase the susceptibility of oxidative stress that ultimately results in damage to certain genes that induced cancer.^{20, 21}

We found that the CAT-21 A/T genotype had a risk of breast cancer 1.75 fold, whereas the CAT-21 A/T allele was at risk of developing breast cancer 1.94 fold. Recent studies have focused on the relationship of CAT polymorphism with various types of cancer but many results are inconsistent. Glorieux and Calderon (2017) study showed the expression of CAT altered in cancer cells, most likely supporting cell proliferation by

inducing genetic instability and oncogen activation.^{22,23} Regulation of catalase expression mainly controlled at the transcriptional level although other mechanisms may also be involved. In addition to transcription factors such as Sp1 and NF-Y, JunB and RAR α transcription factors are important regulators of breast cancer cells by recruiting proteins involved in chromatin transcription and remodeling complexes. Therefore, CAT can be the target of future therapy in the context of cancer by using a pro-oxidant approach.²⁴⁻²⁶

Conclusions:

This current study also assessed the association CAT-21A/T polymorphism with the stage of breast cancer, but there was no association. Many different opinions occur between the associations between CAT-21A/T gene polymorphism with breast cancer and therefore it is still necessary to do further research with a larger population by analyzing the association of CAT-21A/T polymorphism and the level of CAT expression and its risk factor of breast cancer. In this population study showed that CAT - 21 A/T polymorphism could represent as a risk factor to breast cancer.

Conflict of Interest:

The authors stated that there is no conflict of interest in this study

Funding:

No specific funding was received for this study.

Ethical consideration:

The study was conducted after approval from the ethical review committee. The confidentiality and anonymity of the study participants were maintained.

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