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C-1589T and G-1665A Polymorphisms of matrix Metalloprotein-9 Gene promoter increased level of matrix metalloproteinase-9 enzyme as a risk factor metastatic of breast cancer in balinese tribe

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ABSTRACT

Background: Metastatic breast cancer is a cancer which grows and develops in other tissues or organs with the nature and type of cancer similar to its origin. The prevalence of metastatic breast cancer is quite high increase in the overall incidence rate of approximately 1.5% per year. Cancer metastatic is one of the factors increasing mortality and morbidity in patients with a low cure rate (30%). Evidence suggests that breast cancer is affected by genetics and non-genetics (epigenetic). Gene promoter polymorphisms of MMP-9 is one of the genetic factors that play a role in breast cancer metastatic. This research was conducted with the aim of whether the polymorphism C-1589T and G-1665A on MMP-9 gene promoter and high levels are a risk factor for breast cancer metastatic.

Methods: This research has been done with cross sectional and case control study. Sixty six patient of breast cancer devided in two groups, thirty three with metastatic used as a samples and thirty three without metastatic used as a controls. PCR and sequencing techniques were used to presence of polymorphism and ELISA techniques used to determined levels of MMP-9 enzyme.

Results: The research found C-1589T polymorphism (genotype-CT) and G-1665A (genotype-GA) and also with both genotype-CT and genotipe-GA (genotype-CT/GA). Genotypes were found to be associated with the occurrence of breast cancer by 51%. C-1589T polymorphism (genotype-CT) and G-1665A polymorphism (genotype-GA) increased levels of MMP-9 but were not risk factor for breast cancer metastatic (OR= 1.61; 95% CI= 0.41-6.34; *p*= 0.367) and (OR= 1.86; 95%CI= 0.62-5.61; *p*= 0.204). While polymorphism with both genotype (genotype-CT/GA) increasing levels of MMP-9 and indicated risk factor for breast cancer metastatic (OR= 8.62; CI95%= 0.99-74.57: *p*=0.027).

Conclusions: Polymorphism with genotype-CT, genotype-GA and genotype-CT/GA found about 51% on breast cancer Balinese Tribe. Enzyme levels found to be higher in cases than in controls but not significantly different. Polymorphism with genotype-GA and genotype-CT increase levels of MMP-9 enzyme but not as risk factor for metastatic cancer while genotype-CT/GA increases levels of the enzyme MMP-9 and as a risk factor for breast cancer metastatic in Balinese Tribe.

Keywords: MMP-9 gene, polymorphism, levels of MMP-9, breast cancer, metastatic.
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INTRODUCTION

Breast cancer (carcinoma mammae) is a cancer that occurs in breast tissue, is the most common cancer in female.[1] Breast cancer is a disease with the highest mortality rate in the world and number two cause of death after cardiovasculer disease.[2,3] Incidence of breast cancer continues to rise and more common in grade or advanced stage and metastatic conditions. Increasing the number of incidents also occurred in Indonesia, especially in Bali and more than 70% of breast cancer patients came to Sanglah General Hospital be on advanced stage and afer metastatic condition.[4,5]

Metastatic cancer is a process of migration of cancer cells into the surrounding tissue.[6,7] Cancer metastatic is a major cause of increased mortality and morbidity in patients with breast cancer. The prevalence of metastatic breast cancer is quite high about 1.5% per year and is one of the factors increasing mortality with a low cure rate of approximately 30%.[8,9]

Development and metastatic of breast cancer was multifactorial process due to an accumulation of changes in both genetic and non-genetic (epigenetic).[9,10] Involvement of a gene in pathogenesis of breast cancer has been widely linked. This group of genes are metalo-proteinases (MMPs), especially MMP-9 gene.[9,10] Polymorphisms in the MMP-9 gene promoter can affect gene transcription and is associated with the expression or activity of enzymes and is very useful for assessing the progression and help predict metastatic and prognosis of breast cancer patients at this ethnic.[11,12,13,14]

MATERIALS AND METHODS

Cross sectional and case-control design used in this study. Seventy samples were used in the cross-sectional and sixty samples were used in the case-control (thirty three cases and thirty three controls).[15,16] PCR and sequencing techniques were used to determine the polymorphism and ELISA was used to measure levels of MMP-9 enzyme.[17] Sampling and histopathology analysis were done in Anatomical Pathology at Sanglah General Hospital, Denpasar Bali. DNA isolation, amplification, purification and ELISA were done at Biochemistry Departement Faculty of Medicine Udayana University in Denpasar and Sequencing was done at the Laboratory of Genetica Science, Jakarta.

Table 1 Characteristics of Cross Sectional Research Subjects

Variables	Total Samples (70)	Percentage (%)
Age (Years)		
35-40	14	20
41-55	48	69
55-65	8	11
Height (Cm)		
150-160	61	87
161-170	9	13
Weight (Kg)		
36-55	26	37
56-65	38	54
66-80	6	9
Gender		
Woman	70	100
Marriage Status		
Married	61	87
Not Married	9	13
Level MMP-9 (ng/mL)		
<50	14	20
>50	56	80
Grading Histologi		
I	2	3
11	23	33
III	45	64
Stage of Cancers		
IIA	4	6
11B	2	3
III A	3	4
IIIB	40	57
IIIC	3	4
IV	18	26
*Livovciscul*cir *Invission* (LVI)		
+	33	47
-	37	53

RESULT

Seventy subject were used in this study. All of this subject are female and there were 35 to 65 years old. Polymorphism with genotype-CT, genotype-GA and genotype-CT/GA found about 51% on breast

Table 2 Polymorphisms of MMP-9 Promoter Gene Distribution in Subject

Polymorphisms	Amount	Percentage (%)
C-1589T	10	14
G-1665A	18	26
C-1589T dan G-1665A	8	11
With polymorphism	36	**51**
Without polymorphism	34	**49**
Total Subjects	70	100

Table 3 Haplotypes of MMP-9 Gene Promoter

Polymorphism	Genotipe	Haplotipe	f (n = 70)	%
C-1589T	CC	CC/CC	60	86
	CT	CC/CT	10	14
	AleIC		0,91	
	AlelT		0,10	
G-1665A	GG	GG/GG	52	74
	GA	GG/GA	18	26
	AlelG		1	
	Alel A		0,14	
C-1589T dan G-1665A	CT dan GA	CT/GA	52	11

f = frequency

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Table 4 Distribution Polymorphisms of MMP-9 Gene Promoter in Cases and controls

Para Meter	Amount	Polymorphism			With Poymorphism	Without Poymorphism	Total
		C-1589T	G-1665A	CTdanGA			
Control	33	4	7	1	12	21	
Cases	33	6	11	7	24	9	
Amount		10	18	8			
Total					36	30	**66**

Table 5 Characteristics Case Control Research Subjects

Variable	Cancer Metastasis n = 33	Cancer Without Metastasis n = 33	P
Age (years)	46.45 ± 6,17	46.78 ± 7.64	0.082
Height (cm)	158 ± 4.47	157.58 ± 3.78	0.262
Weight (kg)	57.55 ± 10,01	57.88 ± 6.86	0.076
MMP-9 Level (ng/mL)	52.27 ± 2,83	51.88 ± 2.33	0.248
Marriage Status			
No Marriage	26	31	0.129
Marriage	7	2	

a. 2 cells (50%) have expected less than 5 minimum expected count is 4.00
b. Computed only for a 2 x 2 table
Difference analysis of variables between the cases and controls for age, height, weight, levels of MMP- 9 and mariage status but no significantly different (p> 0.05).

Tabel 6 Relationship C-1589T and G-1665A Polymorphisms with Levels of MMP-9 and Metastatic

Variable	Group		OR	95%CI		P
	Metastasis	Non Metastasis		Lower	Upper	
C-1589T	6 (21,21)	4 (18.18	1.61	0.410	6.337	0.367
G-1665A	11 (33.33)	7 (11.11)	1.86	0.615	5.605	0.204
C-189T and G-1665A	7 (21,21)	1 (3.03)	8.62	0.995	74,574	0.027
MMP-9 Level						
High	18	11	2.40	0.87	6.50	0.068
Low	15	22				

cancer Balinese Tribe. Found different haplotypes in this subject. This haplotypes are; haplotype CC/CC, CC/CT,GG/GG, GG/GA and haplotype CT/GA. Polymorphisms were found in this study were in Hardy-Weinberg Equilibrium (HWE).

Polymorphism with genotype-CT, genotype-GA and genotype-CT/GA found about 55% on breast cancer Balinese Tribe

Polymorphism with genotype-CT/GA increases MMP-9 enzyme levels are highest and are risk factors for breast cancer metastatic compared with genotype-CT and genotype-GA (OR = 8.615; 95% CI 0.99 - 74.57: p = 0.027).

Statistical analysis using Chi-square test with a confidence interval of 95% (p <0.05, significant p-value in bold.

DISCUSSIONS

Metastatic cancer is one of the factors increasing mortality and morbidity in patients with breast cancer. Cure rates of metastatic of breast cancer approximately within 30%.[18,19] Found of early-stage cancer is a very important factor both of handling, prevention of metastatic and patient treatment costs.

Founded subjects diagnosed with breast cancer in this study were aged between 35-65 years, but the greatest number of aged 41-50 years (52%). Age affects a person's risk of breast cancer. It is believed to be related with hormonal status because length to exposure of estrogen and progesterone hormones affect the breast tissue proliferation and other risk exposures that require a long time able to induce this cancer.[20,21.]

More samples are diagnosed on advanced stage (grade II and III) which is about 90% and most already metastatic to limfenody. This indicates there is delay in diagnosis. Delayed diagnosis can be caused by many factors, among others; ignorance about how to determine of cancer early, lazy to done for early detection of breast cancer and minim of infrastructure for screening to early detection of cancer.[7] Size of cancer was diagnosed are variety from T2 - T4. T4 sizes was found most (86%). The size of the cancer is also associated with late diagnosis, because the longer of the cancer is diagnosed, it will provide an opportunity for the cancer cells to grow or expand, although the size of the cancer/tumor is not significant for this tumor malignancy.[5,7,22]

C-1589T polymorphism (genotype-CT), G-1665A polymorphism (genotype-GA) and polymorphism with two genotypes (genotypes-CT/GA) were found to be 51%. This gives mean that the polymorphism contributes 51% in the occurrence of breast cancer and of this metastatic cancer.

Levels of the MMP-9 enzyme was found to average higher in cases than in controls, but not significantly different (p= 0.248). No meaningful results mean levels of the MMP-9 enzyme, this does not mean there is no relationship between levels of the MMP-9 enzyme with metastatic breast cancer, but this study no doing analysis levels of Tissue Inhibitors of Metalloproteinase-1 (TIMP-1). TIMP-1 is a glycosylated protein and is mainly involved in the regulation of proMMP-9, capable of binding or slowing the activity of MMP-9 enzyme.[23,24]

Degradation ability of MMP-9 in the human body depends on the balance between the amount active of enzyme and TIMP-1 because the activity of MMP -9 fully can be inhibited by inhibitors TIMP-1.[25,23] In general cell complexes would secrete MMP-9/TIMP-1, although MMP-9 is activated but TIMP-1 can bind covalently proMMP-9. No meaningful levels of MMP-9 enzyme between cases with controls this suspected inhibition activity of MMP-9 enzyme by TIMP-1 as a natural inhibitor, this requires further research to this prove.

MMP-9 enzyme serves to maintain the elasticity of cells on ECM degradation processes and on several physiological functions such as; embryonic development, reproduction, tissue remodeling and leukocyte migration. Conditions polymorphism makes the enzyme more active, thus increasing ECM degradation process and provide ease of migration or metastatic of cells for another tissue.[9,10] Increased activity of MMP-9 enzyme would trigger synthesis of the Vascular Endothelial Growth Factor/Receptor (VEGF/VEGFR), which is proangiogenesis important factor in the process of angiogenesis. Angiogenesis is also synthesized when experiencing ischemic tissue tumor because of a large tumor size or tumor intra pressure increases. VEGF/VEGFR is needed for the growth of cancer cells in the new place.[19,24] Progressive growth of cancer cells depend on angiogenesis. The ratio of the growth of new blood and cancer cells, also used as a prognostic factor, the ability of metastatic and indicator for aggressivity of cancer cell.[26,27,28] Another factor is the influence on the process of protein synthesis because although the expression and activity increased but still there are other activities after the translation process in the synthesis protein.

C-1589T polymorphism (genotype-CT) was found to be higher in cases than in controls and increase the risk of cancer metastatic 60%. This polymorphism increase levels of MMP-9 enzyme with an odds ratio 1.61. G-1665A polymorphism (genotype-GA) was found to be higher in cases than in controls and increase the risk of cancer metastatic 61%. This polymorphism increase levels of MMP-9 enzyme with an odds ratio 1.86. Polymorphism with genotype-CT/GA found to be higher in cases than in controls and increase the risk of cancer metastatic 86%. This polymorphism increase levels of MMP-9 enzyme and as a risk factor of cancer metastatic with an odds ratio 8.62.

Difference of risk metastatic found in this study are related with type and number of alleles were found. Allele T (-1589T) and allele A (-1665A) has been higher for transcription activity compared with allele C and allele G, thereby increasing the translation and synthesis of proteins or levels of MMP-9 enzyme.[28] Results of polymorphism found in this study can be used to predict person's susceptibility to the occurrence or metastatic breast cancer, especially in Balinese Tribe.

Polymorphisms in a gene associated with the disease are not fully because of about 1% occur in the population, but this study has shown that polymorphisms were found to be related in the process metastatic of breast cancer. Increase number of polymorphisms were found in a gene specifically in the promoter region of a gene provide greater effect on metastatic. This is evidenced because found the highest risk of metastatic in polymorphism with two variants (genotype-CT/GA) compared with only one variant.

Enzyme levels were also found with the average highest levels of the polymorphism with two variants (genotype-CT/GA). Increased levels of the enzyme could be due to the increased activity of the enzyme. This leads to increased tissue degradation process or become faster and thus the process of invasion or metastatic of cancer cell more easily and quickly.[12]

Early detection of metastases is very important to be able to overcome this complication, although some studies and clinical trials for metastatic breast cancer has not been entirely successful, the identification of genes that are vulnerable may help in designing future therapies.

The discovery of genetic markers associated with the incidence of metastatic as polymorphism C-1589T and G-1665A can help identify patients who have a high risk for the occurrence of metastases early so that it can be overcome. Given the levels of the enzyme MMP-9 were high and genotype CT/GA is a risk factor for metastatic, checks the levels of the enzyme MMP-9 and genotypes in patients diagnosed with cancer will be able to detect the risk of metastatic from the beginning so that metastatic can be anticipated.

Various studies of the MMP-9 gene polymorphism and its relation to the occurrence of metastatic breast cancer remains an interesting topic to

be studied and researched. This is done because of genetic factors in particular C-1589T and G-1665A polymorphism on MMP-9 gene promoter has been shown to be associated with the occurrence of breast cancer metastatic. Thus the role of genetic factors in reducing the risk of metastatic, especially those that proved to be a risk factor can be early detected and well anticipated.

Three variants of polymorphisms were found in this study, polymorphism with genotype-CT/GA or the haplotype CT/GA increases MMP-9 enzyme levels are highest and are risk factors for breast cancer metastatic compared with genotype-CT and genotype-GA (OR = 8.615; 95% CI 0.99 to 74.57: p = 0.027). Results of this study prove that the polymorphism analysis is very important to be done, especially in the promoter region of genes as associated with gene expression to transcription and translation in the process of protein synthesis and also to the activity of the enzyme.[19,12]

CONCLUSIONS

Polymorphism with genotype-CT, genotype-GA and genotype-CT/GA found 51% in breast cancer patients Balinese Tribe. Levels of MMP-9 enzyme found to be higher in cases than in controls but not significantly different.

Polymorphism genotype-CT and genotype-GA increase levels of MMP-9 enzyme but is not as risk factor for cancer metastatic while polymorphism with genotype CT/GA increase levels of MMP-9 enzyme and as a risk factor for breast cancer metastatic Balinese Tribe.

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