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ASSOCIATION OF MMP-9 GENE POLYMORPHISM (RS3918242) WITH BREAST CANCER STAGE (CARCINOMA MAMMAE)

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ABSTRACT

Background: Breast cancer ranks as the world's second most common cause of mortality associated with cancer and remains highly prevalent in Indonesia. Genetic factors were believed to influence its progression, one of which is the Matrix Metalloproteinase-9 (MMP-9) rs3918242 polymorphism. This gene had an important function in breaking down the extracellular matrix and was involved in angiogenesis, invasion, and metastatic processes. Previous studies reported associations between polymorphic genotypes and increased breast cancer susceptibility, although other research found no significant link, leaving the contribution of this polymorphism uncertain.
Objective: This study aimed to assess whether the MMP-9 (rs3918242) polymorphism is associated with breast cancer stage.
Methods: A descriptive-analytical study with a cross-sectional design was conducted involving 22 breast cancer patients. DNA was isolated from biological samples, and MMP-9 polymorphism detection was performed using PCR, electrophoresis, and sequencing. Data were analyzed with the Chi-Square test, Odds Ratio (OR), and a 95% Confidence Interval (CI).
Result: Most patients were aged 51- 60 years (36.4%) with a mean age at diagnosis of 54.55 years, and 72.7% were diagnosed at an advanced stage. Comparison of CT and TT genotypes with the CC genotype showed no significant association with stage ($p = 0.624$). The OR of 2.2 (95% CI: 0.323-14.975) indicated a possible increase in risk, but the result lacked statistical significance.
Conclusions: The MMP-9 (rs3918242) polymorphism showed no significant association with breast cancer stage, though a slight trend toward increased risk was noted. Larger sample studies are needed to confirm its role.
Keywords: Breast Cancer; MMP-9 Polymorphism (rs3918242); Stages of Breast Cancer

INTRODUCTION

Breast cancer remained a major worldwide health concern and added significantly to the overall cancer burden due to its high incidence and mortality rates. Based on GLOBOCAN statistics, cancer-related deaths reached 4.4 million cases in 2020, and breast cancer ranked as the second leading cause of cancer mortality globally.[1] In Indonesia, breast cancer represented 16.2% of newly diagnosed cancer cases, amounting to 66,271 out of 408,661 new cancer cases reported in 2022.[2]

One of the major challenges in managing breast cancer, including in the Bali region, was the tendency for patients to face delays in receiving a diagnosis and beginning appropriate therapy. The majority of patients are only diagnosed when the disease has reached an advanced stage.[3,4] Given the high rate of disease progression at the time of initial diagnosis, this condition suggests that many patients are already in advanced stages when first detected. Identify genetic markers that predict tumor aggressiveness and progression, not just susceptibility, but to improve prognostic stratification and optimize therapy strategies. The progression of breast cancer from a localized tumor (in situ)

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to invasive carcinoma and metastasis essentially depends on the ability of malignant cells to penetrate the basal membrane and spread through the surrounding extracellular matrix.[5]

Matrix Metalloproteinase-9 (MMP-9) also termed Gelatinase B functions as a protease that breaks down key elements of the extracellular matrix, particularly Type IV collagen. MMP-9 is encoded by a gene located on chromosome 20q13.12.[6] Elevated MMP-9 expression was linked to accelerated tumor growth, the stimulation of angiogenesis through the release of growth factors like VEGF and bFGF from the extracellular matrix, and enhanced invasion and metastatic spread. Increased MMP-9 activity allows tumor cells to undergo an epithelial-to-mesenchymal transition (EMT) and enter the vascular or lymphatic system which directly contributes to the development of higher clinical stages.[7]

Genetic variation, particularly A Single Nucleotide Polymorphism (SNP) within the MMP-9 gene, was studied for its potential influence on enzyme activity and cancer risk. The SNP of concern in this study, rs3918242, was defined by a nucleotide change from Cytosine (C) to Thymine (T) at the -1562 base pair position, located in the promoter region of the gene. This promoter-region polymorphism was important because it did not alter protein sequences directly, but rather influenced the regulation of gene expression processes. The presence of the C allele is thought to provide a binding site for nuclear proteins which acts as a transcription repressor thus keeping MMP-9 activity regular and relatively low in normal tissues. Instead, the transition to the T allele is hypothesized to disrupt or eliminate these repressor binding sites. The loss of suppression allows transcriptional activity to increase which leads to higher production of the MMP-9 enzyme thus allowing greater ECM degradation.[5]

MMP-9 (rs3918242) had a direct relationship with a greater ability for tumor cells to invade and metastasize, which clinically appeared as diagnosis at a more advanced stage. Even though the biological mechanism was well supported, the clinical relevance of rs3918242 remained debated. Findings from the study by Yan et al reported that the MMP-9 (rs3918242) polymorphism was strongly associated with heightened vulnerability to breast cancer in several groups. Among Asian populations, individuals with the TT genotype showed a much greater susceptibility to breast cancer compared to those with the CC genotype (OR=1.777; p<0.001), and the CT genotype also demonstrated increased risk compared to CC (OR=1.631; p=0.003). [8]

Considering the ongoing debate in international research and the lack of studies examining the effect of MMP-9 (rs3918242) in relation to clinical staging in Indonesia, this study was designed to evaluate whether rs3918242 polymorphism was associated with breast cancer stage. This research is conducted to investigate the distribution of the Matrix Metalloproteinase-9 (rs3918242) genetic polymorphism and to assess its association with breast cancer staging, focusing on differences in disease severity among the observed genotypes.

OBJECT AND METHOD

This study uses a quantitative method that is classified as descriptive analytical with a cross-sectional design. This study utilizes clinical data and genomic DNA samples from a total of 22 breast cancer patients who meet the inclusion criteria, namely having undegraded genomic DNA with a volume of > 2 µl. Clinical information including age, age of diagnosis, age of menarche, and parity was collected retrospectively from stored medical records. For the purposes of bivariate analysis, clinical stages are grouped into two category variables, which were categorized as early stages (0, I, IIA, IIB) and advanced stages (IIIA, IIIB, IIIC, IV).

Genotyping for MMP-9 polymorphism (rs3918242) begins with the process of amplifying DNA samples with Polymerase Chain Reaction. The reaction mixture amounted to 35 µl containing 13.3 µl nuclease-free water, 1.4 µl forward primer (5'-TCCAGCCCCAATTATCACAC-3'), 1.4 µl reverse primer (5'-TGGTCAACGTGAAACCC -3'), 17.5 µl GoTaq® Green Master Mix, and 1.4 µl DNA template. The thermal cycle is carried out for 40 cycles. The protocol began with a pre-denaturation step at 95°C for 5 minutes, followed by cycles of denaturation at 95°C for 1 minute, annealing at 54.4°C for 1 minute, and extension at 72°C for 1 minute, ending with a final 5 minute extension at 72°C.

The amplified product was examined using electrophoresis on a 1% agarose gel stained with ethidium bromide. The amplification of the target MMP-9 fragment corresponding to the locus rs3918242 was confirmed through a clear single band of approximately 385 bp observed under UV transillumination. After confirming the amplification results, the PCR products that have been selected and processed are sent for sequencing to PT Genetika Science. Genotype determination (CC, CT, TT) was performed by analyzing the sequencing electroforogram using Chromas software that identifies a specific base at the -1562 locus of the MMP-9 gene. This research has received ethical legitimacy with the number 0057/UN14.2.2.VII.14/LT/2025.

RESULT

Following the established inclusion and exclusion criteria, the researchers used a total of 22 samples for the study. The results of the amplification were then analyzed by the electrophoresis method as shown in **Figure 1**

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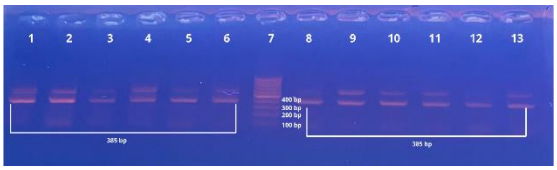

Figure 1. Electrophoresis results using 1% agarose gel

The amplified PCR product has a size of 385 bp according to the target MMP-9 gene fragment at the locus rs3918242. The sequencing analysis of the 22 samples demonstrated a genotypic variation in the matrix metalloproteinase-9 (MMP-9) gene (rs3918242). The most common genotype was CC, which was observed in 14 samples. This was followed by the CT genotype (5

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samples) and the TT genotype (3 samples). The results of the sequencing can be shown in **Figure 2.**

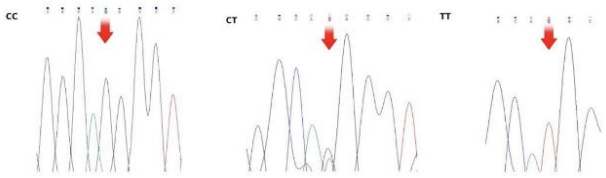

Figure 1. Sequencing results in the form of electropharograms

Univariate analysis showed that the average age of breast cancer patients was 56.73 ± 12.37 years, while the mean age at diagnosis in the sample was 54.55 ± 11.71 years. The average age at menarche recorded among the participants was 13.09 ± 1.26 years. When observed by age category, most of the participants were between 51 and 60 years old, representing 36.4%. Regarding the age at diagnosis, the majority fell within the 41–60 year range, accounting for 54.6%. The distribution of menarche age showed that 59.1% of patients began menstruation at 13 years or older. In terms of parity status, most women in this study were classified as multiparous (90.9%). In relation to breast cancer progression, the participants included in this study were 72.7% in the advanced stage.

The relationship between the MMP-9 (rs3918242) polymorphism and breast cancer stage was analyzed using the Chi-Square test, comparing polymorphic genotypes with the most frequent genotype found in the population, which helped evaluate the potential risk of progression to more severe stages of breast cancer. **Table 1** presents the findings from the Chi-Square analysis.

Table 1. Results of the Chi-Square analysis assessing the relationship between the MMP-9 gene variant (rs3918242) and breast cancer stage.

MMP-9 Gene Polymorphism (rs3918242)	Stages of Breast Cancer		OR	*p*	95%CI
	Early Stage	Advanced Stage			
CT+TT	3	5	2.2	0.624	0.323 – 14.975
CC	3	11			

Significant value p <0.05

In the analysis, it was found that the CT and TT genotypes, which are polymorphic variants of the matrix metalloproteinase-9 gene (rs3918242) compared to the CC genotype as the reference variant, was not significantly related to breast cancer stage. This is indicated by the value p = 0.624 (p>0.05). According to the Odds Ratio (OR) findings, a value of 2.2 was obtained with a 95% confidence interval (0.323 - 14,975) which showed that the CT and TT genotypes had a 2.2 times greater chance of being in the advanced stage than the CC genotype. Although there is a tendency that polymorphisms are potentially associated with advanced breast cancer, the available evidence is not strong enough to confirm a meaningful difference. The wide range of intervals also indicates a high level of uncertainty in the estimates, these results cannot be directly generalized to a broad population which may be influenced by a limited number of samples or variations in the data.

If further analysis is carried out using the codominant model (TT vs CC), the results as shown in **Table 2 are obtained.** This analysis was intended to identify potential differences in the distribution of breast cancer stages between individuals who have the TT genotype and the CC genotype on the MMP-9 gene polymorphism (rs3918242).

Table 2. Results of Chi-Square test analysis of the association between MMP-9 gene variation and breast cancer stage with the TT vs CC codominant model

MMP-9 Gene Polymorphism (rs3918242)	Stages of Breast Cancer		*p*
	Early Stage	Advanced Stage	
TT	0	3	1.000
CC	3	11	

Significant value p <0.05

The test results showed a value of p = 1,000, indicating that there was no significant relationship regarding the polymorphic variation of the MMP-9 gene (rs3918242) with the stage of breast cancer in the TT vs CC codominant model. If further analyzed using the codominant model (TT vs CT), the results as presented in **Table 3 are obtained.** The test results showed a value of p = 0.429 which means that there was not a significant association between TT and CT genotype variations and the stage of breast cancer.

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Table 3. Results of Chi-Square test analysis of the association between MMP-9 gene variation and breast cancer stage using the TT vs CT codominant model.

MMP-9 Gene Polymorphism (rs3918242)	Stages of Breast Cancer		*p*
	Early Stage	Advanced Stage	
TT	0	3	0.429
CT	2	2	

Significant value p <0.05

DISCUSSION

The polymorphism of the MMP-9 gene (rs3918242) was located in its promoter segment, which had a functional role in controlling transcriptional activity. Substitution of bases from C to T at the -1562 position can reduce transcriptional repressor factor binding.[9] In the C allele, repressor factors can suppress promoter activity so that gene expression is more controlled. Conversely, in the T allele, there is a decrease in repressor binding which results in increased transcriptional activity while increasing the production of the MMP-9 enzyme. This finding indicated that breast cancer patients carrying CT and TT genotypes exhibited elevated expression of MMP-9, which subsequently hastened the destruction of the basement membrane as well as the extracellular matrix. This process will allow tumor cells to more easily invade the surrounding tissues, spread to the lymph nodes, and support the process of angiogenesis which plays a role in advanced development and a worse prognosis.[10]

Yan et al. reported a significant association between MMP-9 gene polymorphism (rs3918242) and with increased the likelihood of developing breast cancer among various populations. In Asian populations, the probability of breast cancer was significantly increased in individuals with the TT genotype compared to CC (OR=1,777; p<0.001) and CT compared to CC (OR=1,631; p=0.003). Meanwhile, in the Iranian population, there was an increased risk of TT genotype against CC (OR=7,466; p=0.001) and CT against CC (OR=2,310; p<0.001).[8] This opens up opportunities for the development of genetic biomarkers that have the potential to be used in breast cancer risk early detection and stratification strategies.[11] However, a number of other studies have not shown a significant association between MMP-9 gene polymorphism (rs3918242) on predisposition at the genetic level or clinical severity of patients.

In this study, the analysis of the relationship between the polymorphism of the matrix metalloproteinase-9 (MMP-9) gene rs3918242 in relation to the clinical staging of breast cancer showed that the CT and TT genotypes as a group of polymorphic variants, when compared with the CC genotype as the reference variant failed to show a measurable statistical relationship regarding the clinical staging of breast cancer (p=0.624; OR=2.2; 95% CI: 0.323-14.975). These findings are consistent with previous investigations that also found not significant association between the polymorphism of within the MMP-9 (rs3918242) gene and its and the stage or risk of breast cancer.

A study conducted in China reported that no significant correlation was identified between MMP-9 polymorphism (rs3918242) and increased susceptibility to breast cancer. The results showed that the comparison of the T to C alleles gave an OR of 0.761 (95% CI: 0.523-1.107; p=0.153), while TT and CT to CC obtained an OR of 0.715 (95% CI: 0.430 - 1.189; p=0.196).[8] Research performed by Felizi et al in Brazil showed that MMP-9 polymorphisms did not show a meaningful association with the severity or stage of breast cancer. In the early-stage group (IA-IIB), the majority of patients had the CC genotype (81%) while the polymorphic genotype (CT + TT) was only found about 19%. This distribution is relatively comparable to the advanced stage group (IIB-IV), where the CC genotype remains dominant up to 89%, while the proportion of CT and TT genotypes drops to 11% (p=0.4075).[12]

A study recently performed in Taiwan involving 1,232 breast cancer cases and an equal number of controls indicated that the TT genotype was linked to an increased risk, but the association was not statistically significant (p= 0.1869; OR = 1.43; 95% CI = 0.88 - 2.36). The analysis indicated that people carrying the TT genotype showed a 1.41 fold increase of developing breast cancer when compared with the combined CC and CT genotypes, although the association was not statistically significant (95% CI: 0.86 - 2.30; p=0.2155). In addition, individuals with the CT or TT genotype showed a 1.12 times increased risk of breast cancer relative to those with the CC genotype, but remained statistically meaningless (95% CI: 0.94 -1.34; p=0.2189). The TT genotype actually showed a significant association with an increased risk of triple negative breast cancer (p=0.0072; OR=2.49; 95%CI=1.32 – 4.72).[13]

In this study, it was concluded that the MMP-9 gene polymorphism (rs3918242) was not act as the only determinant of breast cancer stage. The progression of breast cancer was multifactorial and could be affected by hormonal, genetic, inflammatory, and lifestyle factors. Hormonally, women who experience prolonged estrogen exposure such as early menarche, delayed menopause, and parity status or have tumors whose estrogen/progesterone hormone receptor expression is positive show greater potential for breast epithelial cell proliferation and more aggressive cancer cell adaptation.[14] On the genetic side, germline mutations such as BRCA1/BRCA2 or genetic alterations occurring in tumor-inhibiting genes, for example TP53 result in genome instability, loss of cycle control mechanisms, and failure to carry out the apoptosis process. Advanced molecular processes such as epithelial-to-mesenchymal transition (EMT) contribute to cancer cell invasion capabilities. This whole mechanism strengthens the capacity of cancer cells to perform intravation, survive in circulation, and eventually metastasize to distant organs, a condition that generally reflects a more severe clinical stage of cancer and a higher rate of disease progressivity.[15]

One of the factors most commonly showed a relationship with a higher risk of breast cancer is obesity, especially in postmenopausal women. After menopause, the ovaries are no

longer the main source of estrogen, and fat tissue becomes a major contributor through the process of aromatizing androgens into estrogen. This excess fat tissue increases circulating estrogen levels, which can further trigger the proliferation of breast epithelial cells, especially in types of breast cancer that have positive hormone receptors.[16] In addition to obesity, excessive alcohol consumption also contributes to an elevated the risk of developing breast cancer. Alcohol consumption also led to higher estrogen levels in the blood and resulted in DNA injury through the formation of carcinogenic acetaldehyde metabolites, as well as produce oxidative stress that accelerates genetic mutations in breast cells. Radiation can cause direct or indirect DNA damage through the formation of free radicals, increasing the risk of genetic mutations underlying the development of breast cancer.[17] Thus, when a patient is diagnosed with advanced breast cancer, the condition is not only due to the size of the tumor or the number of lymph nodes involved, but also to the accumulation of hormonal disorders, genetic predispositions, and lifestyles that do not support long-term health.

CONCLUSIONS AND SUGGESTIONS

Based on this study's findings, the CT and TT genotypes of the MMP-9 polymorphism (rs3918242) compared to the CC genotype were not significantly associated with breast cancer stage (p = 0.624; OR = 2.2; 95% CI: 0.323 -14.975). Some suggestions that can be given through this study are the need for further research with a larger sample size; analysis should consider other clinicopathological characteristics, such as hormone receptor status, HER2 expression, tumor size, lymph node involvement, and histopathological degree so that the relationship between genetic polymorphisms can be understood more comprehensively; Further research is expected to integrate genetic analysis with environmental and lifestyle factors such as diet, carcinogen exposure, and hormonal status to evaluate the interaction between genes and the environment in the development of breast cancer.

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