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THE ASSOCIATION BETWEEN MATRIX METALLOPROTEINASE-1 (MMP-1) GENE POLYMORPHISM -1607 1G/2G (RS1799750) AND BREAST CANCER METASTASIS

I Komang Chandra Yogananda[1], Ni Putu Tesi Maratni[2], I Wayan Surudarma[2], Putu Anda Tusta Adiputra[3], I Gede Putu Supadmanaba[2*]
Department of Biochemistry
[1]Bachelor of Medicine Program, Faculty of Medicine, Udayana University
[2]Department of Biochemistry, Faculty of Medicine, Udayana University
[3]Department of Surgery, Faculty of Medicine, Udayana University

***Corresponding Author**
I Gede Putu Supadmanaba
Department of Biochemistry, Faculty of Medicine, Udayana University
E-mail: supadmanaba@unud.ac.id
E-mail: yogananda.2202511091@student.unud.ac.id

ABSTRACT

Background: Breast cancer is the most common malignancy among Indonesian women and is frequently diagnosed at advanced stages with high metastatic risk. The MMP-1 -1607 1G/2G (rs1799750) promoter polymorphism increases transcriptional activity and may enhance metastatic potential, yet evidence in Indonesian populations is limited.
Methods: A cross-sectional observational study was conducted using archived venous blood samples from 24 breast cancer patients at the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University. Genotyping was performed using genomic DNA extraction, PCR, and Sanger sequencing. Associations between genotypes and metastatic status were analyzed using Chi-Square and Fisher's Exact Tests, with Odds Ratios (OR) calculated under a dominant 2G allele model.
Results: Six patients (25%) had metastasis and 18 (75%) were non-metastatic. Genotype frequencies were 1G/1G 75%, 1G/2G 20.8%, and 2G/2G 4.2%. A significant association was observed (p=0.017). Under the dominant 2G model, the association remained significant (p=0.006; Fisher p=0.018) with OR=16.0 (95% CI: 1.694–151.110).
Conclusions: The MMP-1 rs1799750 polymorphism, under a dominant 2G allele model, is significantly associated with increased breast cancer metastasis risk.
Keywords : Breast Cancer, Metastasis, MMP-1, Polymorphism, rs1799750, 2G Allele.

INTRODUCTION

Breast cancer is a condition characterized by abnormal and uncontrolled cell growth in the ductal and lobular tissues of the breast.[1,2] It is one of the most common diseases affecting women worldwide. Millions of new breast cancer cases are diagnosed annually, with more than two million new cases and hundreds of thousands of deaths reported each year. Breast cancer is also the second leading cause of cancer-related mortality globally. Its prevalence varies across countries, with higher incidence rates observed in developed nations such as the United States, Canada, and several European countries.

In Indonesia, breast cancer represents a serious public health problem. Tens of thousands of new cases are diagnosed annually, with incidence rates continuing to increase each year.[1] Breast cancer is also one of the leading causes of cancer-related death in Indonesia. These data indicate that breast cancer requires serious attention within the national health system.

Breast cancer is classified into several stages based on tumor size and the extent of cancer spread to surrounding tissues or distant organs. Staging is determined using the TNM system, which assesses the primary tumor size, lymph node involvement, and distant metastasis. Early-stage breast cancer is characterized by relatively small tumors without lymph node or distant organ involvement and is generally associated with a favorable prognosis. In contrast, advanced-stage breast cancer involves regional lymph nodes or distant organs such as bone, liver, lungs, or other tissues, resulting in lower survival rates and increased mortality risk. Metastasis is a critical factor influencing prognosis and treatment outcomes in breast cancer.

Several challenges remain in breast cancer management in Indonesia, including low public awareness regarding early detection and appropriate treatment. Limited awareness often

leads to delayed medical consultation, resulting in diagnosis at advanced stages. Addressing this issue requires strategies that identify biological components capable of predicting breast cancer stage and metastatic risk.[2,5]

Genetic factors play an important role in breast cancer development. A proportion of breast cancer cases are associated with inherited genetic risk factors. While mutations in specific genes such as BRCA1 and BRCA2 are well established, other genetic variations may also influence breast cancer risk. One such variation is gene polymorphism, which can alter gene function and contribute to disease susceptibility, including breast cancer.

Genetic polymorphism analysis is essential for identifying associations between genetic variations and breast cancer risk. Gene polymorphisms represent variations in DNA sequences within populations and may influence cancer development, disease stage, and metastatic potential. Among genes implicated in breast cancer metastasis is the matrix metalloproteinase (MMP) gene family.

Matrix metalloproteinases are a family of metal-dependent proteolytic enzymes involved in cell migration, embryogenesis, tissue remodeling, and wound healing. Their most significant function is the degradation of the basement membrane and extracellular matrix components such as elastin, collagen, gelatin, growth factor–binding proteins, tyrosine kinase receptors, and cell adhesion molecules. These processes contribute to cancer initiation, invasion, and metastasis.

Matrix metalloproteinase-1 (MMP-1), also known as interstitial collagenase and located on chromosome 11q, is an important member of the MMP family and is expressed in various normal cells. Previous studies have shown that MMP-1 facilitates tumor growth and lymph node metastasis, with high expression levels observed in many neoplastic tissues and low expression in normal physiological tissues. MMP-1 expression can be influenced by single nucleotide polymorphisms located at the -1607 bp position within its promoter region.[3,6]

Single nucleotide polymorphisms are the most common form of genetic variation underlying differences in disease susceptibility among individuals. The MMP-1 (-1607) 1G/2G (rs1799750) polymorphism involves the insertion of a guanine nucleotide at position 1607 in the MMP-1 promoter. This polymorphism enhances MMP-1 transcriptional activity by creating a binding site for transcription factors of the Ets family. Individuals carrying the 2G/2G genotype exhibit higher MMP-1 expression levels. Elevated MMP-1 expression plays a crucial role in degrading type I and III collagen, which are key components in cancer metastasis, thereby indicating a poorer prognosis in breast cancer patients.[4,8]

In Indonesia, studies specifically evaluating the role of the MMP-1 (-1607) 1G/2G (rs1799750) polymorphism in breast cancer metastasis risk are still lacking.[9,10,11] Furthermore, available literature addressing this issue remains limited. Therefore, further investigation and comprehensive analysis are needed to elucidate the relationship between the MMP-1 (-1607) 1G/2G (rs1799750) polymorphism and breast cancer metastasis.

METHODS

This study is an analytical study using a cross-sectional design aimed at determining the association between Matrix Metalloproteinase-1 (MMP-1) gene polymorphism -1607 1G/2G (rs1799750) and metastasis status in breast cancer patients at RSUP Prof. dr. I.G.N.G. Ngoerah. The target population of this study consisted of all breast cancer patients treated at RSUP Prof. dr. I.G.N.G. Ngoerah. The accessible population included patients who had been diagnosed with breast cancer at RSUP Prof. dr. I.G.N.G. Ngoerah and had undergone venous blood sampling for DNA extraction. The DNA samples were stored as Biobanked Biological Materials at the Department of Biochemistry, Faculty of Medicine, Universitas Udayana.

The inclusion criteria were breast cancer patients with complete medical record data, including age, sex, and metastasis status, as well as patients diagnosed with breast cancer at RSUP Prof. dr. I.G.N.G. Ngoerah whose venous blood samples had been collected and stored as biobanked biological materials at the Department of Biochemistry, Faculty of Medicine, Universitas Udayana. The exclusion criteria included biobanked samples that did not meet usability standards, such as samples that were dry, foul-smelling, had changes in color or consistency, or failed to produce visible DNA bands during electrophoresis. In addition, genomic DNA samples with volumes less than 2 µL and PCR products that could not undergo sequencing were excluded from the study.

The sample size was calculated using the formula for analytical cross-sectional studies, expressed as $n = (Z\alpha^2 \times p \times (1-p)) / d^2$. Using a Zα value of 1.96, a breast cancer prevalence of 4.93%, and a precision of 10%, the minimum required sample size was 18 samples. In this formula, n represents the sample size, Zα is the standard normal distribution value, p is the prevalence, q is equal to 1−p, and d is the accepted clinical precision.

The study variables consisted of independent and dependent variables. The independent variable was the MMP-1 gene polymorphism -1607 1G/2G (rs1799750), which was identified based on the presence of the 1G and 2G alleles in individual samples. The dependent variable was the metastasis status in breast cancer patients. The MMP-1 rs1799750 polymorphism represents a genetic variation at nucleotide position -1607 of the MMP-1 gene, characterized by the presence of either the 1G or 2G allele, contributing to genetic diversity within the population. This variable was measured on a nominal scale. Breast cancer metastasis status was determined based on the TNM staging system recorded in patients' medical records, specifically M0 indicating no metastasis and M1 indicating the presence of metastasis, and was also measured on a nominal scale.

The research materials consisted of biobanked biological materials stored at the Department of Biochemistry, Faculty of Medicine, Universitas Udayana. The research instruments included data collection sheets used to record study variables obtained from previously collected medical records; therefore, no additional medical record retrieval was required. All data were stored in secured computer systems to maintain patient confidentiality.

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Laboratory instruments included equipment and consumables for DNA isolation, polymerase chain reaction (PCR), and electrophoresis procedures. Equipment used comprised microcentrifuge tubes, centrifuges, DNA isolation kits, micropipettes, thermocyclers, PCR tubes, microwaves, Erlenmeyer flasks, and gel documentation systems. Consumables included absolute alcohol, nuclease-free water, forward and reverse primers for MMP-1 rs1799750, DNA templates, GoTaq® Green Master Mix, agarose gel, TBE buffer, ethidium bromide, and micropipette tips.

The study was conducted at the Integrated Biomedical Laboratory and the Department of Biochemistry, Faculty of Medicine, Universitas Udayana, over a period of approximately six months. The research workflow included sample selection, DNA isolation, DNA amplification using PCR, visualization of PCR products through electrophoresis, sequencing analysis, and statistical data analysis.

Data collection was performed using consecutive sampling until the required sample size was achieved. The samples consisted of DNA extracted from venous blood of breast cancer patients stored as biobanked biological materials at the Department of Biochemistry, Faculty of Medicine, Universitas Udayana. Medical record data and patient identities had been preserved from previous studies; therefore, no additional medical record collection was required. Additional relevant patient data were recorded using data collection sheets. The DNA samples underwent PCR amplification to identify the MMP-1 polymorphism (-1607 1G/2G, rs1799750), after which the PCR products were sent to Genetica Science for sequencing to determine allele and genotype variations. The resulting data were analyzed to assess the association between the polymorphism and clinicopathological characteristics, particularly metastasis status and breast cancer subtype, using SPSS version 26.0.

PCR amplification was performed by preparing a reaction mixture with a total volume of 35 µL, followed by amplification using a thermocycler with predefined temperature settings. PCR products were visualized using 1% agarose gel electrophoresis and observed under ultraviolet light. PCR products that met quality criteria were subsequently subjected to sequencing analysis.

Data processing and statistical analysis were conducted using SPSS software version 26.0. Univariate analysis was used to describe the distribution of subject characteristics, while bivariate analysis was performed using the chi-square test or Fisher's exact test when chi-square assumptions were not met. This analysis aimed to determine the association between alleles and genotypes of the MMP-1 (-1607 1G/2G, rs1799750) polymorphism and breast cancer metastasis status. Risk estimation was assessed using odds ratios (ORs) with 95% confidence intervals (CIs). A p-value of less than 0.05 was considered statistically significant.

RESULTS

The number of samples that met the inclusion and exclusion criteria in this study was 24 breast cancer patients. The median current age was 56.5 years (range 39–86 years), while the median age at breast cancer diagnosis was 53.5 years (range 38–84 years). The majority of study subjects were aged ≥50 years, both at the current age (62.5%) and at the time of diagnosis (54.2%). Based on parity, most subjects were multiparous with parity ≥3 (41.7%), followed by parity 1–2 (33.3%) and nulliparous (25.0%).

Based on the results of MMP-1 -1607 1G/2G (rs1799750) gene polymorphism analysis in 24 study samples, the genotype distribution was as follows: the homozygous 1G/1G genotype was the most frequently observed, found in 18 subjects (75.0%), followed by the heterozygous 1G/2G genotype in 5 subjects (20.8%), and the homozygous 2G/2G genotype was observed in only 1 subject (4.2%). Thus, the overall frequency of the 2G allele (both heterozygous and homozygous 2G) was 25.0%, while the 1G allele accounted for 75.0%. The complete characteristics of the study subjects are presented in Table 1.

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Table 1. Genotype and Allele Distribution of MMP-1 -1607 1G/2G (rs1799750)

Characteristic	Category	n	%
Total subjects		24	100%
Current age (years)	Median (min–max)	56.5 (39–86)	
	<50 years	9	37.5%
	≥50 years	15	62.5%
Age at diagnosis (years)	Median (min–max)	53.5 (38–84)	
	<50 years	11	45.8%
	≥50 years	13	54.2%
Parity	0 (nulliparous)	6	25.0%
	1–2	8	33.3%
	≥3	10	41.7%
MMP-1 rs1799750 polymorphism	1G/1G (homozygous 1G)	18	75.0%
	1G/2G (heterozygous)	5	20.8%
	2G/2G (homozygous 2G)	1	4.2%
Metastasis	No	18	75.0%
	Yes	6	25.0%

The association between the MMP-1 -1607 1G/2G (rs1799750) gene polymorphism and metastatic status in breast cancer patients was analyzed using the chi-square test. The analysis demonstrated a statistically significant association between MMP-1 rs1799750 polymorphic genotypes and the occurrence of breast cancer metastasis. More detailed chi-square analysis results are presented in Table 2.

Table 2. Association between MMP-1 Genotype (Dominant 2G Model) and Metastasis Status

MMP-1 rs1799750 Polymorphism	Non-Metastatic n (%)	Metastatic n (%)	Total n (%)	p-value*
2G/2G	0 (0.0%)	1 (16.7%)	1 (4.2%)	
1G/2G	2 (11.1%)	3 (50.0%)	5 (20.8%)	0.017
1G/1G	16 (88.9%)	2 (33.3%)	18 (75.0%)	
Total	18 (100%)	6 (100%)	24 (100%)	

Based on the chi-square test results for the 3×2 table (three genotypes versus metastatic status), a p-value of 0.017 ($p < 0.05$) was obtained, indicating a significant association between the MMP-1 rs1799750 gene polymorphism and breast cancer metastasis. However, because 83.3% of the cells had an expected count of less than 5 (minimum expected count = 0.25), a further analysis was performed using a dominant genetic model of the 2G allele (genotypes 2G/2G + 1G/2G as the 2G allele carrier group) compared with the 1G/1G genotype as the reference, as presented in Table 3

.

Table 3. Results of Analysis Using the Dominant 2G Model (1G/1G vs 2G/2G + 1G/2G)

Dominant 2G Model	Total n (%)	p-value**	Odds Ratio (95% CI)
1G/1G	18 (75.0%)		1 (reference)
2G/2G + 1G/2G	6 (25.0%)	0.006	16.0 (1.69–151.11)
Total	24 (100%)		

**Pearson Chi-Square test (df = 1); Fisher's Exact Test p = 0.018

The analysis using the dominant 2G model demonstrated a stronger and statistically significant association, with a p-value of 0.006 (Pearson Chi-Square test, df = 1). Because several cells had expected counts <5 (75%), the more appropriate p-value was derived from Fisher's Exact Test, yielding $p = 0.018$ ($p < 0.05$). The Odds Ratio (OR) of 16.0 (95% CI: 1.694–151.110) indicates that breast cancer patients carrying the 2G allele (1G/2G or 2G/2G genotypes) had a 16-fold higher risk of developing metastasis compared with patients carrying the homozygous 1G/1G genotype.

Data distribution showed that among the six patients with metastasis, four (66.7%) were carriers of the 2G allele, while only two (33.3%) had the 1G/1G genotype. Conversely, in the non-metastatic group (n = 18), the majority (88.9%) had the 1G/1G genotype, and only 11.1% were 2G allele carriers. This pattern supports the hypothesis that the 2G allele in the MMP-1

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gene promoter acts as a risk factor for breast cancer metastasis. Although the Odds Ratio is relatively high and the wide confidence interval reflects the small sample size (n = 24), the findings remain statistically significant.

DISCUSSION

This study involved 24 breast cancer patients who met the inclusion criteria. The analyzed sample characteristics included current age, age at diagnosis, parity, MMP-1 rs1799750 gene polymorphism, and metastatic status. The median current age was 56.5 years (range 39–86 years), with 62.5% of patients aged ≥50 years. The median age at diagnosis was 53.5 years (range 38–84 years), and 54.2% were diagnosed at ≥50 years of age. These findings are consistent with broader epidemiological patterns showing that breast cancer incidence peaks in the fifth and sixth decades of life. Increasing age is a major risk factor due to the accumulation of somatic mutations, decreased immune system efficiency, impaired DNA repair mechanisms, and prolonged cumulative estrogen exposure.[3,4,5]

Based on parity, 41.7% of subjects had ≥3 children, 33.3% had 1–2 children, and 25.0% were nulliparous. High parity and lactation are known to have protective effects by reducing the lifetime number of ovulatory cycles, thereby lowering endogenous estrogen exposure and promoting differentiation of mammary ductal cells. The relatively high proportion of nulliparous women in this study aligns with an increased risk of breast cancer. Metastasis was identified in 25% of subjects, reflecting the aggressive nature of the disease within the study sample.[6,7]

Overall, the characteristics of this study sample reflect the clinical profile of breast cancer patients in Indonesia, with classical risk factors such as a relatively high proportion of nulliparity. This background is relevant for interpreting the main finding of the study, namely the significant association between the 2G allele of the MMP-1 rs1799750 polymorphism and an increased risk of breast cancer metastasis.[8,9]

MMP-1 is a major interstitial collagenase that degrades type I, II, and III collagen in the extracellular matrix and plays a crucial role in tissue remodeling, tumor invasion, angiogenesis, and metastasis. The functional -1607 1G/2G polymorphism in the promoter region of the MMP-1 gene results in the insertion of an additional guanine (2G allele), creating a binding site for the Ets-1 transcription factor and markedly increasing transcriptional activity compared with the 1G allele. The 2G allele has consistently been associated with higher MMP-1 expression and increased malignancy risk in breast cancer.[10,11]

In this study, the distribution of MMP-1 rs1799750 genotypes was 75% for 1G/1G, 20.8% for 1G/2G, and 4.2% for 2G/2G. The frequency of the 2G allele was consistent with that reported in Asian populations. Initial analysis using the chi-square test showed a significant association between genotype and metastatic status. Because most cells had expected counts below five, further analysis was performed using a dominant 2G genetic model, grouping 2G/2G and 1G/2G genotypes as 2G allele carriers and comparing them with the 1G/1G genotype. This analysis demonstrated a stronger and statistically significant association, with an Odds Ratio of 16.0, indicating that patients carrying the 2G allele had a sixteen-fold higher risk of developing metastasis compared with those with the 1G/1G genotype.[12,13]

Although the confidence interval was wide due to the small sample size and low frequency of the 2G allele, the association remained statistically significant. Biologically, the 2G allele is thought to promote excessive degradation of stromal collagen, facilitating local invasion, intravasation, and extravasation of tumor cells.[14,15,16] Increased MMP-1 activity is also associated with enhanced epithelial–mesenchymal transition, enabling tumor cells to migrate from the primary tumor into circulation and form metastases.[17,18,19] High MMP-1 expression has been linked to higher histopathological grade, positive lymph node status, and poorer prognosis in breast cancer.[20,21,22]

The main limitations of this study are the small sample size and the absence of serum MMP-1 or protein expression measurements. Nevertheless, these findings contribute important insight into the genetic risk of breast cancer metastasis in Indonesia. The MMP-1 rs1799750 polymorphism, particularly under the dominant 2G allele model, has potential as a simple and cost-effective genetic biomarker for metastasis risk stratification in breast cancer patients, especially in settings with limited access to advanced molecular testing.

CONCLUSION

In this Indonesian breast cancer cohort, the MMP-1 -1607 1G/2G (rs1799750) polymorphism, particularly the dominant 2G allele model, is significantly associated with increased metastatic risk. These findings highlight its potential as a genetic biomarker for prognostic stratification and underscore the need for expanded genomic studies in underrepresented populations to inform personalized management strategies. Further studies with larger sample sizes are needed to confirm the association between the MMP-1 rs1799750 gene polymorphism and the risk of breast cancer metastasis.

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