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Correlation of MMP-9 (Matrix Metalloproteinase 9) and SMA (Smooth Muscle Actin) expression with Basal Cell Carcinoma's risk of recurrence

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Abstract

Basal Cell Carcinoma (BCC) is the most common cancer in many countries, with its incidence steadily rising. Data from the Indonesian Pathologists Association show that among 1,530 skin cancer cases, BCC was the most frequent (39.93%), followed by Squamous Cell Carcinoma (23%) and Malignant Melanoma (7.9%). According to the 2018 WHO classification, BCC is divided into Low-Risk and High-Risk groups based on the risk of recurrence. BCC invades surrounding tissues through extracellular matrix degradation and enhanced cell motility, driven by the expression of MMP-9 (Matrix Metalloproteinase 9) and SMA (Smooth Muscle Actin). This study aims to evaluate the expression of MMP-9 and SMA in BCC using immunohistochemistry and analyze their correlation with the risk of recurrence. A total of 40 paraffin blocks from BCC patients diagnosed between 2019 and 2021 at Dr. Saiful Anwar General Hospital in Malang, Indonesia, were sampled. The blocks were sectioned, stained with MMP-9 and SMA antibodies, and examined for positive expression indicated by brown staining in the cytoplasm of tumor cell and peritumoral stroma. Spearman correlation showed r = 0.811 for MMP-9 and r = 0.857 for SMA ($p < 0.05$, CI: 95%), indicating a significant relationship between these markers and the risk of recurrence. Higher MMP-9 and SMA expression corresponded to a higher risk of BCC recurrence, suggesting their potential as prognostic biomarkers. However, further research is needed to assess recurrence risk more accurately and determine the most appropriate therapy.

Keywords: Basal Cell Carcinoma; MMP-9; SMA; Risk of Recurrence

1. Introduction

Basal Cell Carcinoma (BCC) is the most common cancer in several countries, with its incidence steadily rising [1]. BCC and Squamous Cell Carcinoma (SCC) are the two most common subtypes of Non-Melanoma Skin Cancer (NMSC), and although they share some similarities, they exhibit distinct characteristics [1]. According to 2020 data from the Global Cancer Observatory, the incidence of NMSC ranks fifth after lung, breast, colorectal, and prostate cancers [2]. However, NMSC poses its own challenges, including post-treatment recurrence and the potential to cause disability [3].

Although BCC has an exceptionally low mortality rate, it significantly contributes to morbidity, particularly because it often occurs in visible areas such as the face, head, and neck. This makes BCC not only a health issue but also a source of psychological and social burden. BCC is a slow-growing disease, invasive to surrounding soft tissues, but it has a high recurrence rate [3]. The rising incidence of BCC emphasizes the importance of public health awareness and the need for preventive measures [1].

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According to data from the Indonesian Pathologists Association Cancer Registration Agency, among 1,530 cases of skin cancer, the most common was BCC, accounting for 39.93%, followed by SCC (23%), Malignant Melanoma (7.9%), and other skin cancers [4].

Based on the 2018 World Health Organization (WHO) histopathological classification, BCC is divided into several subtypes based on the risk of recurrence, as outlined in Table 1:

Table 1 Basal Cell Carcinoma Recurrence Rate Classification adapted from Elder DE 2018 (5)

High-Risk Basal Cell Carcinoma	Low-Risk Basal Cell Carcinoma
Nodular	Sclerosing / Morpheaform
Superficial	Infiltrating
Pigmented	Sarcomatoid
Infundibulocystic	Micronodular
Fibroepithelial	Basosquamous

The incidence of carcinoma, particularly BCC, can be caused by genetic mutations that lead to uncontrolled cell proliferation. These mutations, especially patched homolog 1 (PTCH-1) and smoothened receptor (SMO) gene mutations, are acquired through prolonged exposure to Ultraviolet B (UVB) radiation and other carcinogens, which affect the Sonic Hedgehog pathway [6]. BCC is characterized by cell proliferation and invasion into the surrounding stroma and soft tissue. This invasiveness depends on several processes, including the degradation of the basement membrane and interstitial connective tissue, alterations in tumor cell attachment to the Extracellular Matrix (ECM), and enhanced cell motility to infiltrate the surrounding stroma. The expression of Matrix Metalloproteinases (MMPs), particularly MMP-2 and MMP-9, plays a critical role in these processes, along with the expression of Smooth Muscle Actin (SMA), which influences cell motility [6–8].

Previous studies have indicated that MMP-9 is differentially expressed in the tumor and peritumoral stroma between infiltrative and nodular types of BCC [8]. SMA is also considered to show stronger expression in more aggressive forms of BCC, which are associated with a higher recurrence rate [9].

Understanding the correlation between the expression of MMP-9 and SMA with each risk group of Basal Cell Carcinoma (BCC) is crucial as it can serve as a predictive factor for the aggressiveness and recurrence rate of BCC, even in small biopsy samples. This knowledge enables the implementation of appropriate therapy for patients and helps prevent recurrence.

This study aims to evaluate the expression of MMP-9 and SMA in BCC using immunohistochemistry and analyze their correlation with the risk of recurrence, categorized into low-risk and high-risk groups.

2. Material and methods

The study adopted an analytical observational design and used total sampling as the sample collection method. The sample consisted of 40 paraffin blocks from patients diagnosed with Basal Cell Carcinoma between 2019 and 2021, along with medical record data from histopathological results. However, clinical data could not be analyzed due to the inconsistent and incomplete nature of the medical records found in the sample. The research was conducted from January to June 2023 at the Anatomical Pathology Department of Dr. Saiful Anwar General Hospital, Malang, Indonesia.

2.1. Histopathological Examination

The paraffin blocks were all prepared by applying a standard tissue processing procedures issued by Indonesian Association of Anatomical Pathologists. The fixation used 10% formalin for a minimum of 4-6 hours. The Paraffin blocks that met the criteria were cut using a microtome, soaked in a tissue floating bath, then continued with deparaffinized for 2 hours in a microwave oven. Processing was then continued with a routine staining using hematoxylin-eosin [10]. These processes were applied to all chosen paraffin block samples by the technician in our Anatomic Pathology Laboratory.

2.2. Immunohistochemistry Procedure

The immunohistochemistry examination began with thinly cut tissue placed on a *poly-L-lysine-coated slide*, followed by deparaffinization using xylol and alcohol solutions in decreasing concentrations. The tissue was then soaked in a peroxide block solution for 25 minutes, followed by Diva Decloaker solution, and placed in a decloaking chamber at 90°C for 45 minutes. The process continued with soaking in PBS (Phosphate Buffered Saline) solution for 5 minutes, followed by incubation with the primary antibody for 60 minutes and the application of polymer. The next step involved applying DAB (Diaminobenzidine) chromogen and performing counterstaining with hematoxylin for 2 minutes, followed by lithium carbonate. The process was completed by clearing the tissue in xylol solution and performing mounting [11]. The antibodies used were MMP-9 LOT #H0620 (Santa Cruz Biotechnology) and SMA LOT 010423 (Biocare Medical).

2.3. Evaluation of MMP-9 Expression

The expression of the antigen was measured using a light microscope, counting neoplastic tumoral and peritumoral cells with a brown stain on the cytoplasmic, as many as 1,000 cells from 5 large fields of view (x40) in the largest area. Each large field of view counted 200 cells-stained brown granules contained in the cytoplasm [12].

MMP-9 expression scores were obtained from the multiplication of grade (percentage of positivity) and intensity (degree of positivity of tumor cells), which is brown granules contained in the cytoplasm of tumor cells [12], shown in Table 2. The MMP-9 score was calculated as a numeric ordinal scale of 0-12 [12].

Table 2 MMP 9 Expression representation adapted from Liu 2019 [12]

Grade	0	No positivity in tumor
	1	Positivity 0-25% in tumor
	2	Positivity 26-50% in tumor
	3	Positivity 51-75% in tumor
	4	Positivity 76-100% in tumor
Intensities	1	Weak
	2	Moderate
	3	Strong
Skor (Grade x Intensities)	<8	Low Expression
	≥8	High Expression

2.4. Evaluation of SMA Expression

The expression of antigen was measured using a light microscope, counting neoplastic tumoral and peritumoral cells with a brown stain in the cytoplasm, as many as 1,000 cells from 5 large fields of view (x40) in the largest area. Each large field of view counted 200 cells stained brown [13].

The SMA expression score was calculated by applying criteria in Table 3. SMA appeared as a brown color in the cytoplasm of tumor cells and peritumoral stromal cells. The SMA expression scores were presented with a scale of 0-100% [13].

Table 3 SMA Expression representation adapted from Law 2003 [13]

Score 0	No positivity expressed
Score +1	1-25% positivity
Score +2	26-50% positivity
Score +3	51-75% positivity
Score +4	76-100% positivity

2.4.1. Statistical Analysis

MMP-9 expression scoring, before being grouped into low and high expression, was carried out first on a scale of 0-12 which is the result of the multiplication of grade and intensity based on criteria [12]. Statistical analysis was performed to compare MMP-9 scores (with ratio data scale) in high and low risk BCC groups, after a previous normality test. As suggested in the literature, normality test was performed using the Shapiro-Wilk test because the number of samples was less than 50 [14]. The Shapiro-Wilk test showed that all of the group have p value < 0.05, which meant that the data was not normally distributed. In this regard, we continued by assessing the differences of MMP-9 scores in 2 groups of Basal Cell Carcinoma's risk of recurrence by performing the non- parametric test i.e., Mann Whitney test. The correlation between MMP-9 expression that had been grouped into low and high expression with BCC high and low-risk of recurrency groups was then analyzed with chi square (X^2) and determining the correlation coefficient with Spearman test.

SMA expression, before being grouped based on scoring, the percentage of SMA positivity in the BCC group was tested for normality (to determine whether the data distribution was normal or not) using Shapiro-Wilk test because the number of samples <50 [14]. It was found that the data distribution was not normal, we then assessed the differences of SMA percentage in 2 groups of BCC's risk of recurrence by performing the non- parametric test i.e., Mann Whitney test. The correlation between SMA expression that had been grouped into score 1+, 2+, 3+, and 4+ with BCC high and low-risk of recurrency groups was then analyzed with chi square (X^2) and determining the correlation coefficient with Spearman test.

3. Results and discussion

The demographic characteristics of the 40 samples with Basal Cell Carcinoma diagnosis are featured in Table 4.

Table 4 Research Sample Characteristics

Characteristic	**Variables**	**Frequency (N=40)**	**Percentages (%)**
Gender	Male	19	47.5
	Female	**21**	**52.5**
Age (Years Old)	31-40	2	5
	41-50	6	15
	51-60	7	17.5
	61-70	**13**	**32.5**
	71-80	11	27.5
	81-90	1	2.5
	BCC type		
Low-Risk Group	Nodular	**21**	**52.5**
	Superficial	2	5
High-Risk Group	Infiltrative	7	17.5
	Basosquamous	7	17.5
	Nodular-Infiltrative	1	2.5
	Morpheaform	1	2.5
	Micronodular	1	2.5

The sample was then divided into two groups based on the risk of recurrence, namely the high-risk group and the low-risk group, according to the 2018 WHO Classification. The calculation and percentage of each is presented in the Figure 1.

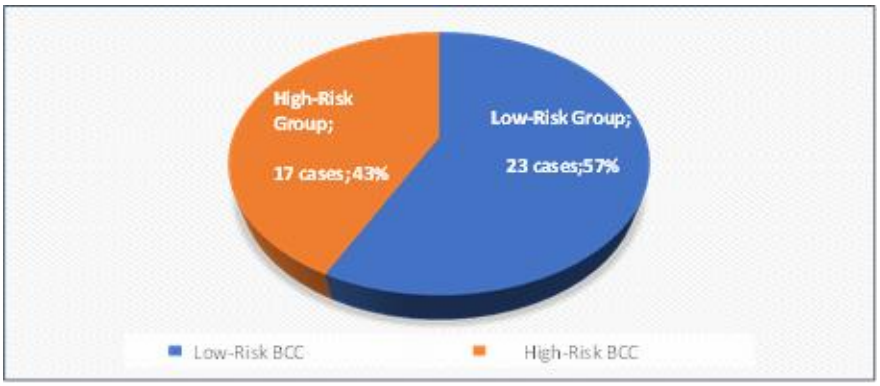

Figure 1 Number of cases in each group. There are 17 cases of High-Risk BCC and 23 cases Low-Risk BCC

The histopathologic features of Basal Cell Carcinoma of the low and high-risk groups appear as shown in Figure 2.

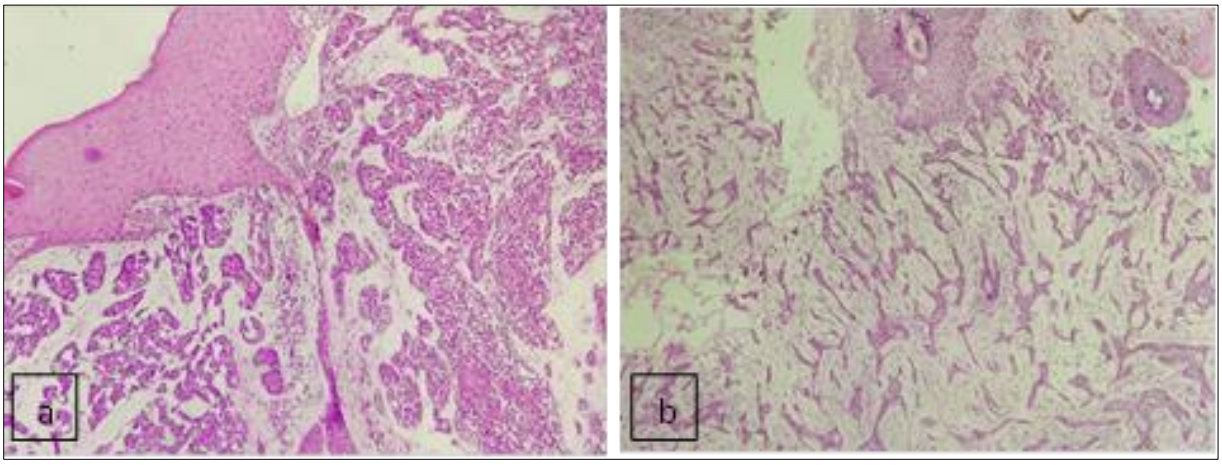

Figure 2 Histopathologic features of Basal Cell Carcinoma with Hematoxylin Eosin staining at 100x magnification (a) Nodular Subtype Basal Cell Carcinoma, belongs to the Low-Risk group. (b) Infiltrative Subtype Basal Cell Carcinoma, belongs to the High-Risk group

The immunohistochemical feature of the expression in MMP-9 and SMA staining is presented in Figure 3.

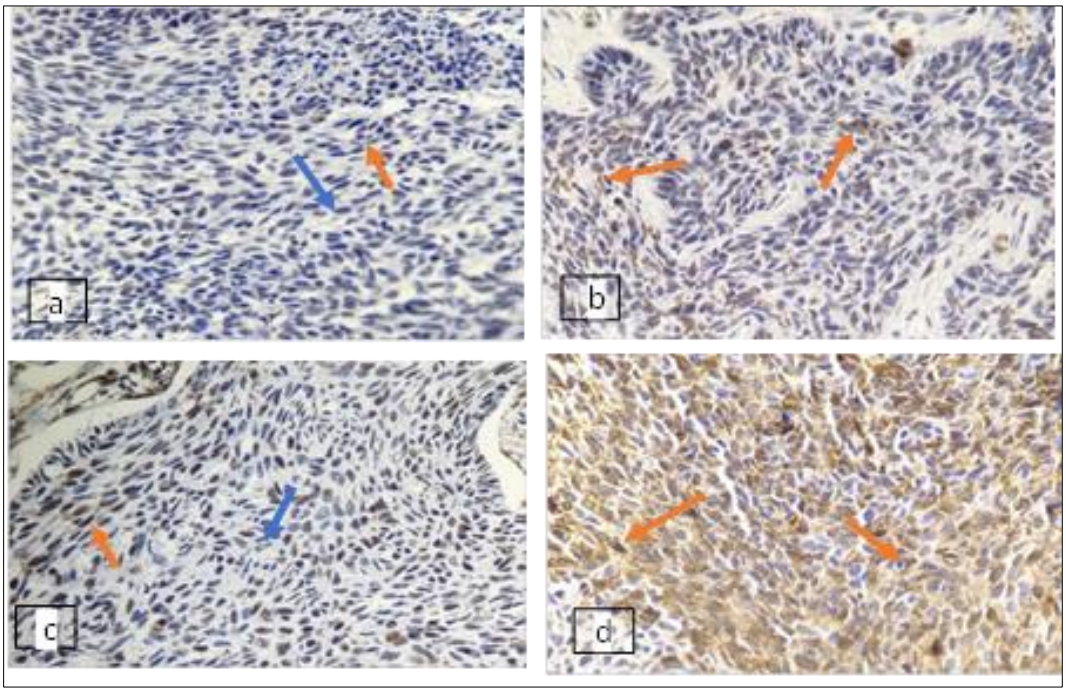

Figure 3 Immunohistochemical Staining Feature in Strong Magnificent (400x) (a) MMP-9 feature in Nodular Basal Cell Carcinoma (Low-Risk) with weak staining and low positivity. (b) MMP-9 feature in Infiltrative Basal Cell Carcinoma (High-Risk) with moderate staining and high positivity (c) SMA feature in Nodular Basal Cell Carcinoma (Low-Risk) with low percentage of positivity (d) SMA feature in Basosquamous Cell Carcinoma (High-Risk) with high percentage of positivity

The distribution of the number of BCC cases from low and high-risk groups that have been assessed for positivity of MMP-9 (Low / High) and SMA expression scores are shown in the Table 5.

Table 5 Number of case and expression in both antibodies SMA and MMP-9

Antigen Expression		**Case Frequency (n=40)**	
		Low Risk BCC	**High Risk BCC**
SMA	+1	15	0
	+2	8	0
	+3	0	10
	+4	0	7
MMP-9	Low	21	0
	High	2	17

The comparative analysis between MMP-9 score and SMA score is featured in Table 6. It is shown in Table 6 that SMA expression in low-risk group is significantly different to ($p<0.05$, CI 95%) the expression in high-risk group. The table is also featuring that MMP-9 expression in low-risk and high-risk group is significantly different ($p < 0.05$, CI 95%).

Table 6 Comparative Contingency Table between SMA and MMP-9 Expression in Low-Risk and High-Risk type of BCC

	Risk of Recurrence	N	Mean ± SD	Median	Test Statistics	p-value
SMA percentage (0-100%)	Low	23	20.5261 ± 10.606	18.2 (11.4 – 29.00)	5.349	0.000
	High	17	65.5882 ± 14.431	59.6 (52 – 81.25)		
MMP-9 Score (0-12)	Low	23	2.1739 ± 2.2085	2 (1 – 2)	5.062	0.000
	High	17	8.8235 ± 0.3929	9 (9 – 9)		

Table 7 Cross Table SMA and MMP-9 Expression in BCC Low Risk and High-Risk

Variables	Score	Number of BCC cases		p-value	Correlation Coefficient
		Low-Risk	High-Risk		
SMA expression	+1	15 (65.20%)	0 (0.00%)	0,000	0.857
	+2	8 (34.80%)	0 (0.00%)		
	+3	0 (0.00%)	10 (58.80%)		
	+4	0 (0.00%)	7 (41.20%)		
MMP-9 expression	Low	21 (91.30%)	0 (0.00%)	0,000	0.811
	High	2 (8.70%)	17 (100.00%)		

Notes: CI: Confident Interval 95%

From Table 7, we found a significant correlation between MMP-9 expression and the risk of recurrence of BCC ($r = 0.811$; $p < 0.05$, CI: 95%). The results for SMA expression were similar, showing a statistically significant correlation with the risk of recurrence of BCC ($r = 0.857$; $p < 0.05$, CI: 95%). This indicates that higher MMP-9 and SMA expression corresponded to a higher risk of BCC recurrence.

Our study also showed that the incidence of BCC was more prevalent in female patients than in males, with a percentage ratio of 52.5% (female) to 47.5% (male). This prevalence is consistent with nationwide data indicating that BCC is more common in females than in males [12]. Considering the risk factors for BCC, our findings support the argument that women, especially in Asian countries, are more prone to accumulate higher-intensity UV exposure than men, even with similar occupational risks in open-air environments, such as gardening, cooking in open kitchens, or selling in traditional markets. This is likely because women's skin is generally thinner than men's, providing less protection against environmental stress, including UV exposure [15]. However, this finding requires further exploration, as the data in our study do not fully cover all clinical and demographic information.

The occurrence of BCC in this study tended to occur in elderly patients, with 25 out of 40 cases (80%) being in individuals above the age of 60. This finding is consistent with extensive epidemiological research indicating that the risk of BCC is higher in those over 60 [16, 17]. Theoretically, this increased risk is due to the reduced ability to repair DNA (deoxyribonucleic acid) damage in elderly patients [18]. The rising incidence in older age may also result from the accumulation of somatic mutations due to the development of neoplasms, as well as a decline in immune function associated with aging [19].

The most common type of BCC identified in this study was the nodular type. This is consistent with previous studies conducted in the country; for example, data from the National Cancer Registry in 2016 showed that 35.4% of cases were of the nodular type [12], and in Makassar, from 2017 to 2019, 53.1% of BCC cases were also classified as nodular [20].

Statistical tests revealed a significant correlation between MMP-9 expression with the risk level of Basal Cell Carcinoma recurrence, which is categorized into high and low risk of recurrence, with a p-value of 0.000 (<0.05) and a correlation coefficient ($r = 0.811$). This indicates that higher expression of MMP-9 is associated with a greater risk of BCC risk of recurrence. This finding is consistent with previous research by Gozdzialska, which states that MMP-9 mRNA levels in nodular type BCC are significantly lower than those in infiltrative BCC [8]. In this context, nodular type BCC is classified as a low recurrence risk group, while infiltrative type BCC is classified as a high recurrence risk.

Matrix Metalloproteinases (MMPs) are synthesized by the tumor cells themselves, leading to increased proteolysis and degradation of type IV collagen, which is mainly produced by the basement membrane. This results in tumor growth and migration of cancer cells into blood vessels and throughout the body [8]. Matrix Metalloproteinases are also mentioned in the literature to be associated with nuclear palisading. This nuclear palisading pattern is reduced in BCC that has a high recurrence rate (high risk). The change in the nuclear palisading picture is related to structural changes in the tumor, causing the histopathological feature to be poorly differentiated, resulting in a poor prognosis [21].

There was a positive relationship between SMA expression and the recurrence rate of BCC, with a p-value of 0.000 (<0.05) and a correlation coefficient (r) of 0.857. This indicates that higher expression of SMA increases the probability of a greater risk of BCC recurrence. This finding is consistent with previous research. SMA plays a role in cancer cell motility, making it closely related to the aggressiveness of BCC. The higher the aggressiveness of BCC, typically found in high-risk subtypes, the greater the SMA expression observed [22].

SMA is also strongly expressed in myofibroblast cells surrounding the tumor islands, which increases the motility of carcinoma cells, allowing BCC invasion to penetrate deeper. A study conducted by Christian et al. found positive expression of SMA in high-risk types of BCC, including micronodular, morpheaform, and infiltrative types [7].

The limitations of this study include the inability to establish a strong cause-and-effect correlation due to the cross-sectional study design and the limited clinicopathological information. Another limitation is the small number of samples, despite employing a total sampling method.

4. Conclusion

Different expression of MMP-9 and SMA exists between high-risk and low-risk groups which are possibly related to degree of aggressiveness of BCC histological subtypes and lead to its recurrence risk level. The results of this study may serve as an initial endeavor for further research in investigating the potential benefit of SMA and MMP-9 markers to assess BCC progression and thus prognosis. This study did not provide strong cause-and-effect correlation due to the nature of the cross-sectional study design and the limitations of clinicopathological information. Therefore, more careful cohort study with a larger sample size is warranted to be conducted.

Compliance with ethical standards

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Disclosure of conflict of interest

Authors declare no conflict of interest in constructing this manuscript.

Statement of ethical approval

Ethical approval was obtained from Health Research Ethics Commission General Hospital Dr. Saiful Anwar Malang number 400/021/K.3/102.7/2023

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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