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Relationship between MMP-11 Expression in Invasive Ductal Breast Carcinoma with its Clinicopathologic Parameters

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Abstract

Background: Breast cancer is one of the most common cancers in the world, particularly in Iran. There are many genomic and molecular factors that cause the occurrence of breast cancer. Many markers are associated with tumor invasiveness. Matrix metalloproteinase includes a family of zinc-dependent endopeptidases that degrade various components of the extracellular matrix and basement membrane. Matrix metalloproteinase expressions increase in thyroid, colorectal, head and neck squamous cell carcinoma, lung, and ovarian cancers. It is correlated with tumor angiogenesis, invasion, and metastasis. Matrix metalloproteinase 11 is a member of the stromelysin subclass of the matrix metalloproteinase family. This enzyme is secreted to become a potentially active form against other matrix metalloproteinases. Contradictory results exist regarding the correlation between matrix metalloproteinase 11 expression and clinicopathologic parameters in breast cancer.

Methods: This case-control study examined 80 invasive ductal carcinoma of the breast and 80 adjacent nonneoplastic breast tissue paraffin blocks to identify the relationship between matrix metalloproteinase 11 expression and clinicopathologic parameters such as age, tumor size, microscopic grade, perineural and vascular invasion, lymph node involvement, and stage by immunohistochemistry analyses

Results: Among the 80 patients, 86.3% showed matrix metalloproteinase 11 expression in tumor cells and 17.5% had matrix metalloproteinase 11 expression in adjacent normal breast tissue. This expression correlated with stage, grade, lymph node metastasis, and perineural and vascular invasion (P<0.001), but not with age and tumor size (P>0.05).

Conclusion: Matrix metalloproteinase 11 expression is increased in breast cancer and may be used as a predictive factor for tumor invasiveness.

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Introduction

Breast cancer is the most common malignant tumor and leading cause of cancer deaths in women. The incidence is high in North America and Northern Europe, intermediate in Southern European and Latin American countries, and low in most Asian and African countries. A rapid increase in recent years with increased affluence of some of these countries is noted.[1] Breast carcinoma is the most common cancer in Iranian women.[2]

Matrix metalloproteinase (MMP) includes a family of zinc-dependent endopeptidases that degrade various components of the extracellular matrix (ECM) and basement membrane resembling collagen, laminin, actin, proteoglycan and glycosaminoglycan.[3-5] Matrix metalloproteinases play an important role in many physiological and pathological processes which include embryonic development, inflammation, wound healing, angiogenesis, immunity, tumor invasion, and metastasis.[6,7] The MMPs are divided into distinct subclasses: collagenases (MMP-1, MMP-8, MMP-13, and MMP-18), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10, and MMP-11), matrilysins (MMP-7, MMP-26), and other MMPs. Matrix metalloproteinase expressions are increased in cancers of the thyroid, colorectal, head and neck squamous cell carcinoma, lung, and ovaries. It is correlated with tumor angiogenesis, invasion, and metastasis.[8,9]

Matrix metalloproteinase 11 (MMP-11) is overexpressed in more than 90% of invasive breast carcinomas.[10] The MMPs are inhibited by specific endogenous tissue inhibitors, known as tissue inhibitors of MMPs (TIMPs) that are used in the treatment of cancers such as leukemia, lymphoma, testicular cancer, lung, gastrointestinal, and oropharyngeal cancer.[9]

MMP11 has secreted in form of activated enzyme in spite of other MMPS.[11-13] In addition, the activated form of MMP-11 is unable to hydrolyze ECM molecules and its action is limited to enzymes such as Beta-casein, Alpha2-macroglobulin and serine proteinase inhibitors.[14]

Researchers reported contradictory results about the correlation between MMP-11 expression and clinicopathologic parameters in breast cancer.

We have investigated the expression of MMP-11 in breast cancer and its correlation with clinicopathologic parameters due to the increasing prevalence of breast cancer in different communities, such as Iran, lack of appropriate treatment response, and contradictions that exist in numerous studies in this field. Perhaps the findings from this study can be a step towards better recognition of breast cancer behavior and impact intervention and follow-up of these patients.

Materials and Methods

Sampling

This analytic case-control study aimed to investigate the correlation between abnormal expression of the MMP-11 marker with clinicopathologic parameters in breast cancer. We examined paraffin blocks from the Pathology Archives at Imam Khomeini Hospital in Sari, Iran during 2010-2015. The case group consisted of paraffin blocks of breast cancer tissue from patients who underwent mastectomy without a history of chemotherapy or radiotherapy before surgery. The control group consisted of paraffin blocks of adjacent normal looking tissue.

The sample size in this study was compared with previous studies and calculated using the formula:

$$n_1 = n_2 = \frac{2\times(z_{1-\frac{\alpha}{2}} + z_{1-\beta})^2 \times p \times (1-p)}{\varsigma^2} = 79\,;\ 80,\quad p = 0.79\quad \alpha = 0.05\quad \beta = 0.1, \varsigma = 0.2$$

$$80,\quad sample\quad in\,each\quad group$$

The case group consisted of tissue from 80 patients with breast cancer and the control group consisted of the same number. The clinicopathologic parameters evaluated included age, tumor size, histological grade, lymph node metastasis, perineural and vascular invasion, and stage. We divided patients into two age groups (under 50 and above 50 years). Tumor size was divided into three groups (<2 cm, 2 to 5 cm, and >5 cm). Clinicopathologic parameters was retrieved from pathological reports.

Immunohistochemistry procedure

All paraffin blocks were removed from the archive and we prepared several slides with hematoxylin-eosin staining of the tumor and adjacent normal looking tissue.

For immunohistochemistry, sections of the selected blocks were placed on specific slides and incubated at 60ºC for one hour. In order to clean the paraffin, xylene solution, absolute ethanol, and ethanol 96° were used in three steps (each solution 2 times for 5 min), after which the slides were washed with running water. After drying, the slides were transferred to 1% hydrogen peroxide which is mixture with methanol (to eliminate the internal peroxidase), therefore the slides were followed to the target solution after 10 minutes. The slides were heated in an autoclave with 100 ºC for 13 min. The slides were removed and allowed to cool and were washed with running water and wash buffer in a moist chamber. Therefore the slides were incubated at envision for 60 min that used a diagnostic kit for MMP-11 antibody diluted 1:100 (Abcam). Next, we washed the slides twice with wash buffer. DAB solution was poured on the glass slides; if the color of slides were changed to brown after 1-2 min, they were placed again in wash buffer for 2 min. Afterward, the washed slides with distilled water were stained with Mayer's hematoxylin, rinsed in distilled water, and fixed in xylene. Finally, the slides were mounted with Entellan (a kind of glue). Normal tonsil tissue and prostate carcinoma were used for negative and positive controls of the marker, respectively.

Table 1. Clinicopathologic findings in patients with breast cancer.

		Number	Percent
Age (years)			
	≤ 50	41	51.2
	> 50	39	42.8
Tumor size (cm)			
	< 2	22	27.5
	2-5	38	47.5
	> 5	20	25
Histologic grade			
	1	20	25
	2	50	62.5
	3	10	12.5
Perineural invasion			
	YES	32	40
	NO	48	60
Vascular invasion			
	YES	38	47.5
	NO	42	52.5
Lymph node metastases			
	YES	48	60
	NO	32	40
Stage			
	1	11	13.8
	2	28	35
	3	41	51.2

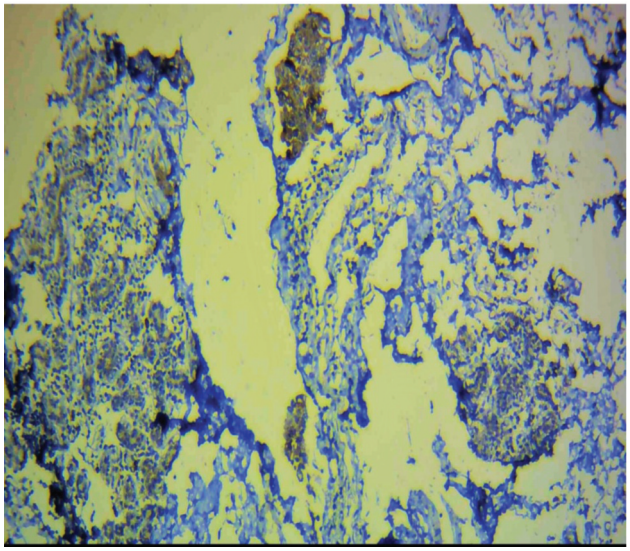

Figure 1. Positive staining in tumor cells with MMP-11 marker. No stain was observed in the normal appearing tissue located adjacent to the tumor (IHC; 100×).

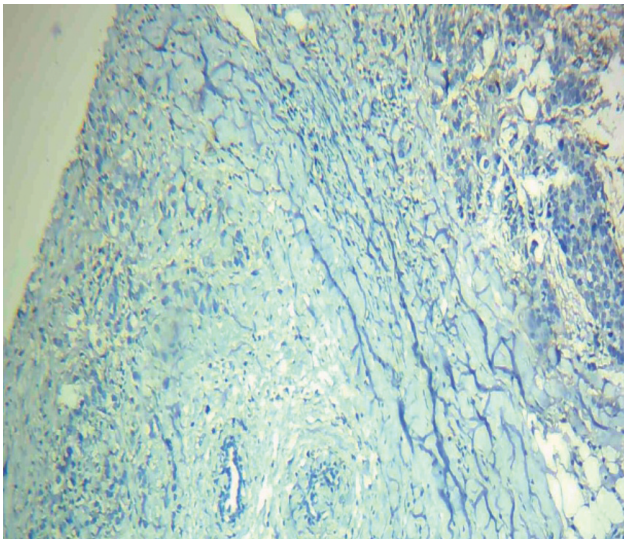

Figure 2. Negative staining in the tumor cells with MMP-11 marker according to immunohistochemistry staining (IHC; 100×).

Table 2. Comparison of the expression of matrix metalloproteinase 11 (MMP-11) staining in case and control groups.

Staining	Negative		Positive	
Group	Number	Percent	Number	Percent
Case	11	13.7	69	86.3
Control	66	82.5	14	17.5

The slides were studied and reported by two expert pathologists who had no information about the clinicopathologic data of the patients, MMP-11 expression, and staining intensity. To improve diagnostic accuracy, we examined multiple microscopic fields at low (100×) and high (400×) power.

Scoring

For this study, positive MMP-11 immunostaining was defined as only cytoplasmic without nuclear staining and graded according to both the intensity and percentage of positively-stained tumor or stromal fibroblast-like cells. We scored MMP-11 staining intensity on a scale of 0 to 3 (0: negative; 1: weak; 2: moderate; 3: strong). The percentage of MMP-11 positive cells was also scored into one of four categories: 1 (0–25%), 2 (26%–50%), 3 (51%–75%), or 4 (76%–100%). The level of MMP-11 staining was analyzed as an immunoreactive score (IRS) calculated by multiplying the scores of the staining intensity and the percentage of positive cells. We divided MMP-11 expression into negative (IRS ≤1) and positive groups (IRS >1).[15]

Statistical analysis

The results were analyzed by statistical software SPSS (IBM SPSS Statistics 23). Chi-square, Fisher's exact and McNemar's tests were used to analyze the correlation between expression of MMP-11 in breast cancer and clinicopathologic parameters. A *P*-value <0.05 was considered statistically significant.

Results

We evaluated tissue from 80 patients. The clinicopathologic parameters of the patients are summarized in table 1.

After immunohistochemical staining, we compared both MMP-11 expression and staining intensity in both groups (Table 2).

According to the definition given in "Materials and Methods", the case group had 86.3% (n=69) of samples with positive staining and 13.7% (n=11) negative samples. In the control group,

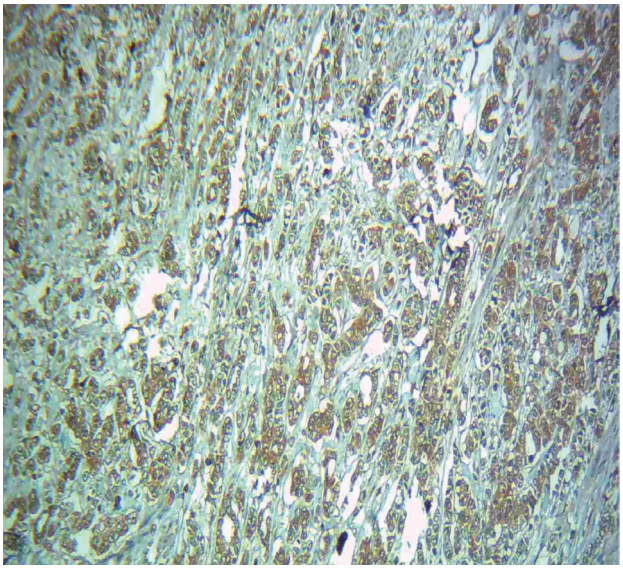

Figure 3. Mild staining in the tumor cells with MMP-11 marker according to immunohistochemistry staining (IHC; 100×).

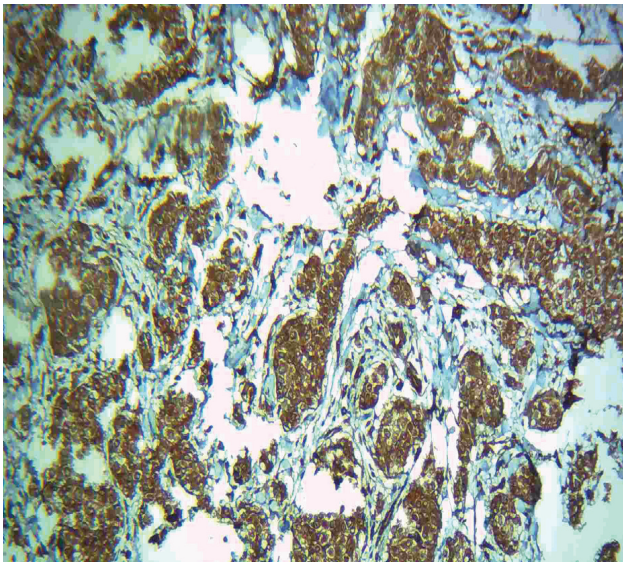

Figure 4. Moderate staining in the tumor cells with MMP-11 marker according to immunohistochemistry staining (IHC; 100×).

Table 3. Correlation between matrix metalloproteinase 11 (MMP-11) expression and clinicopathologic parameters in breast cancer.

Clinicopathologic parameters		Expression of MMP-11				*P*-value
		Positive		Negative		
		Number	Percent	Number	Percent	
Age (years)						
	≤50	29	36.2	12	15	0.814
	>50	29	36.2	10	12.6	
Tumor size (cm)						
	<2	11	13.7	11	13.7	0.29
	2-5	28	35	10	12.5	
	>5	19	23.7	1	1.4	
Grade						
	1	5	6.2	15	18.7	0.001
	2	43	53.7	7	9	
	3	10	12.4	0	0	
Perineural invasion						
	Yes	29	36.2	3	3.7	0.024
	No	29	36.2	19	23.9	
Vascular invasion						
	Yes	38	47.5	0	0	0.001
	No	20	25	22	27.5	
Lymph node metastases						
	Yes	47	57.4	1	1.4	0.001
	No	11	13.7	22	27.5	
Stage						
	1	4	5	6	7.5	0.005
	2	15	18.9	13	16.3	
	3	39	48.8	2	2.5	

17.5% (n=14) showed positive staining and 82.5% (n=66) had negative staining (Figures 1-5).

We used the McNemar test to analyze and compare expressions of MMP-11 in the two groups. The results showed a significant difference between the groups ($P<0.001$).

A comparison of MMP-11 expression with clinicopathologic features in patients with breast cancer and statistical data analysis (Table 3) showed a significant correlation between MMP-11 expression in tumor cells with histological grade, lymph node metastasis, perineural and vascular invasion, and stage ($P<0.05$). There was no significant correlation between MMP-11 expression in tumor cells to age and tumor size ($P>0.2$).

Discussion

In the current study, 86.3% of cases of breast cancer expressed MMP-11 according to immunohistochemistry analysis. MMP-11 expressed in 79.1% of breast cancer tissue according to a study by Kyuen et al. in south Korea,[15] whereas Chenard et al. reported 78.3% in France[16] and Cheng et al. reported 61.1% in Taiwan.[17]

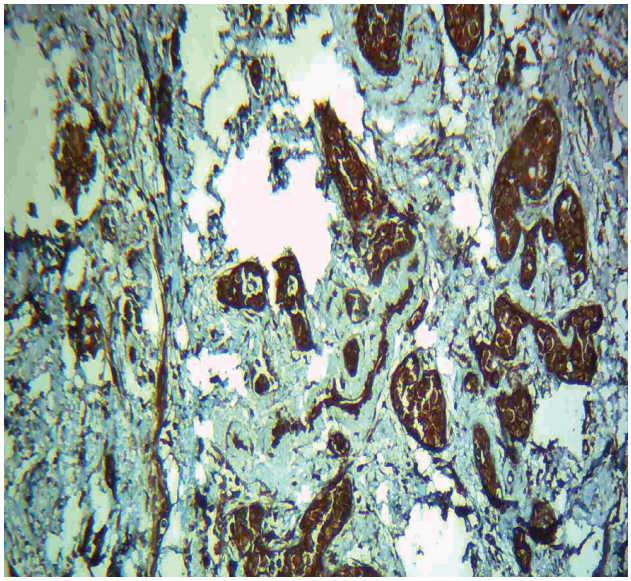

Figure 5. Intense staining in the tumor cells with MMP-11 marker according to immunohistochemistry staining (IHC; 100×).

In our study, we observed a significant correlation between MMP-11 expression and tumor grade, stage, lymph node involvement, as well as perineural and vascular invasion. These parameters showed invasiveness of the tumor but expression of this marker did not correlate with age and tumor size. Hähnel et al., in a study in Australia, reported that expression of MMP-11 was not associated with the age, tumor size vascular invasion, and lymph node involvement.[18]

Kwon et al. reported a significantly higher frequency of MMP-11 overexpression in breast tumor tissues of invasive ductal carcinoma patients, whose clinical features included advanced stage and lymph node involvement.[19] Kwon et al., in the US, observed a correlation between invasion and lymph node involvement.[19]

In the present study, we found a significant correlation between expression of MMP-11 and tumor grade and lymph node involvement, however there was no correlation with tumor size. Chenard et al. reported that MMP-11 expression in breast cancer did not correlate with tumor size, grade, or lymph node involvement.[16]

Kyuen et al. reported that expression of MMP-11 in the tumor was not correlated with histologic grade, stage, tumor size, lymph node involvement, and perineural and vascular invasion.[15]

Unlike the present study, Roy and Walsh reported that expression of MMP-11 in tumors was not correlated with histologic grade, tumor size, and lymph node involvement.[20]

Conclusion

Our study has shown MMP-11 expression in 86.3% of breast cancer cases. Since expression of MMP-11 is associated with more aggressive breast tumors, this marker can be used as a prognostic factor.

In order to achieve more conclusive results we recommend evaluating the correlation between serum level of MMP-11 and clinicopathologic parameters in future studies. Also, we recommend studying the effect of TIMPs in the treatment of breast cancer.

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Conflict of interest

No conflict of interest is declared.

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