MMP9 EXPRESSION IN THE CYTOPLASM OF SURROUNDING STROMAL CELLS OF BREAST CARCINOMA IN RELATION TO PATHOLOGICAL STAGE, GRADE AND RECEPTOR STATUS

Pathology

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ABSTRACT

INTRODUCTION: Breast cancer is the most common malignancy and the second leading cause of cancer-related death after lung cancer among women in the United States and Europe. Due to major advances in screening and early diagnostic procedures, most breast cancer patients are diagnosed at an early stage. However, 6% to 10% of patients still present with metastatic breast cancer at the time of diagnosis; for those patients, relapses tend to occur earlier and survival rates are shortened. **AIMS AND OBJECTIVES:** To study MMP 9, Estrogen receptor, Progesterone receptor and Her-2-Neu expression in breast carcinoma by immune histochemistry. Compare MMP 9 expression with result of Estrogen receptor expression, Progesterone receptor expression and Her-2-Neu expression in breast carcinoma. To study MMP 9 expression in relation to staging and grading of breast cancer. **MATERIALS AND METHOD:** This cross-sectional study was conducted on a group of patients admitted in Department of General Surgery, R G Kar Medical College & Hospital, Kolkata from January 2018 - Jun 2019. Approval from an institutional review board was obtained at the initiation of the study. All patients were provided with signed informed written consent. Cross sectional, descriptive and observational. **RESULTS AND PUBLICATION:** From the above table it is evident that only 2 of the patients with stage II tumor showed MMP9 positivity while 8 were negative, but in case of patients with stage III disease 20 out of 22 showed MMP9 positivity with only 2 of them showing a negative MMP9 pattern. From the above table it is evident that only 1 out of 6 cases with grade 1 breast cancer was positive for MMP9, while 11 out of 16 cases of grade 2 disease showed MMP9 positivity and all cases with grade 3 was found to be MMP9 positive. The above table clearly shows that out of 12 ER positive breast cancer cases only one third i.e. 4 were MMP9 positive. However, out of 20 ER negative cases 90% i.e. 18 were found to be MMP9 positive.

KEYWORDS

MMP9 Expression, Breast Carcinoma, Pathological Stage, Grade And Receptor Status

INTRODUCTION

Breast cancer is the most common malignancy and the second leading cause of cancer-related death after lung cancer among women in the United States and Europe¹. Due to major advances in screening and early diagnostic procedures, most breast cancer patients are diagnosed at an early stage. However, 6% to 10% of patients still present with metastatic breast cancer at the time of diagnosis; for those patients, relapses tend to occur earlier and survival rates are shortened. Cancer metastasis is considered to develop in a step-wise fashion leading to the acquisition of new capabilities by tumor cells helping them to thrive and evade natural barriers. Cancer cells detach themselves from the primary tumor, migrate and invade surrounding tissues, enter the vasculature, circulate throughout the body and eventually reach secondary sites where they extravasate, and populate distant organs².

Degradation of the extracellular matrix (ECM) is thought to be a crucial step in the formation of tumor metastasis. Multiple proteolytic enzymes such as plasmin, cathepsins, and matrix metalloproteinases (MMPs) are known to degrade ECM³. Matrix metalloproteinase-9 (MMP-9) is a zinc-dependent peptidase that belongs to the gelatinase subfamily of MMPs. It is excreted as an inactive pro-enzyme that undergoes activation upon cleavage by different types of extracellular proteases. MMP-9 activity is thought to be regulated by different biochemical stimulators such as growth factors and cytokines whose expression appear to modulate intracellular signaling pathways⁴. MMP-9 has the ability to degrade denaturated collagens which have been first cleaved by various collagenases such as MMP-1, MMP-8 and MMP-13. In addition, MMP-9 degrades type IV collagen which is the main component of the basement membrane⁵. It exerts different roles in the dissemination process such as tumor invasion, tumor-induced angiogenesis, and immunomodulation of the tumor microenvironment. In addition, MMP-9 is instrumental in creating so-called premetastatic niches that foster colonization of distant organs⁶. Elevated tissue levels of MMP-9 are also associated with invasion, metastasis and poor prognosis in different types of cancer including

cervical, colorectal, ovarian and breast cancer. Furthermore elevated levels of MMP-9 in the serum and urine have also been found to be associated with metastasis and poor prognosis in a diversity of cancers.

Our goal was to assess the potential clinical usefulness of MMP-9 as a prognostic biomarker of breast cancer. To achieve that aim, we first studied MMP-9 mRNA expression using in silico analysis on available DNA microarray and RNA sequencing data of human breast cancer tissues and breast cancer cell lines. We next evaluated MMP-9 expression at the protein level using immunohistochemical analyses on tissue microarrays containing both normal and neoplastic breast tissues. Our data were next correlated with patients' outcome specifically looking at the incidence of metastases, relapse and overall survival. Our results indicate that MMP-9 is not only differentially expressed in different molecular breast cancer subtypes but also over expressed in triple-negative and HER2-positive breast cancers.

GENERAL OBJECTIVES

1. To study MMP 9, Estrogen receptor, Progesterone receptor and Her-2-Neu expression in breast carcinoma by immune histochemistry.
2. To compare MMP 9 expression with result of Estrogen receptor expression, Progesterone receptor expression and Her-2-Neu expression in breast carcinoma.
3. To study MMP 9 expression in relation to staging and grading of breast cancer.

SPECIFIC OBJECTIVES

To study MMP 9 expression level in relation to known prognostic factors in breast carcinoma; like tumour stage, tumour grade, ER status, PR status and Her-2-neu expression.

MATERIALS AND METHOD

Place and Period: This cross-sectional study was conducted on a group of patients admitted in Department of General Surgery, R G Kar

Medical College & Hospital, Kolkata from January 2018 - Jun 2019. Approval from an institutional review board was obtained at the initiation of the study. All patients were provided with signed informed written consent.

Type of Study: Cross sectional, descriptive and observational.

Inclusion criteria

- Epithelial breast tumour with known Estrogen, Progesterone, Her2neu receptor expression.
- Type of mastectomy whether modified or radical to be decided by the surgeon.

Exclusion criteria

- Benign breast lesion
- Mastectomy due to cosmetic reasons.
- History of trauma.
- History of any other stromal tumour.
- Any history of previous chemo or radiotherapy for other type of cancer.
- History of neo-adjuvant chemotherapy or radiotherapy in breast carcinoma tissue specimens.

Sample size: 32 cases of breast carcinoma mastectomy specimens were taken.

RESULTS AND ANALYSIS

From the above table it is evident that only 2 of the patients with stage II tumor showed MMP9 positivity while 8 were negative, but in case of patients with stage III disease 20 out of 22 showed MMP9 positivity with only 2 of them showing a negative MMP9 pattern. From the above table it is evident that only 1 out of 6 cases with grade 1 breast cancer was positive for MMP9, while 11 out of 16 cases of grade 2 disease showed MMP9 positivity and all cases with grade 3 was found to be MMP9 positive. The above table clearly shows that out of 12 ER positive breast cancer cases only one third i.e. 4 were MMP9 positive. However, out of 20 ER negative cases 90% i.e. 18 were found to be MMP9 positive.

The above table shows that among PR negative breast cancer cases only 2 were also MMP9 negative while 18 were positive, but 8 out of 12 were MMP9 negative among the PR positive breast cancer cases. From the above table it is evident that out of 21 her-2-neu positive breast cancer cases 19 were also positive for MMP9 with only 2 being negative. However, among the 11 her-2-neu negative cases only 3 were MMP9 positive and 8 were MMP9 negative.

DISCUSSION

32 cases of breast carcinoma obtained by mastectomy were sent to the Department of Pathology, R.G. Kar Medical College & Hospital, Kolkata- 700004 during the period from April 2018 to March 2019.

MMP9 is a relatively new biomarker. Though the role of membrane metalloproteinase is well known in the pathogenesis and spread of invasive breast carcinoma it is relatively less studied and consequently there are only limited numbers of studies available that correlates MMP9 positivity with tumor staging, grading and receptor status.

Our study showed that The highest number of cases (12 patients, 37.5 %) were in the age group 41-50 years. 28.125% patients were in the 51-50 years age group, 15.625 % were aged above 60 years and 18.75% were in the age group 31-40 years. However none were aged below 30 years. The mean age of the subjects was 50.59 years, (median 49 yrs) with ages ranging from 34-73 years.

Our study Evidently showed that about one third of the patients had grade 3 cancer with about half of the patients having grade 2 disease and only about 6 patients having grade 1 cancer.

Our study shows that more than two third of the patients were in stage 3 disease and none were in stage 1 disease. So, evidently our disease population was mostly middle aged with proportionally more number of patients presenting with relatively high grade disease in late stage.

Our study shows a multilinear graph between MMP9 and Staging. As the MMP9 became negative to positive the number of cases of Stage III cases also increased considerably compared to Stage II cases.

Our study shows a multilinear graph between MMP9 and histopathological grade. As the MMP9 became negative to positive

the number of cases of tumour grade 3 cases increased considerably compared to tumour grade 2 cases.

Our study shows a multilinear graph between MMP9 and Estrogen receptor expression. As the MMP9 became negative to positive the number of cases of Estrogen receptor negative cases increased than Estrogen receptor positive cases.

Our study shows a multilinear graph between MMP9 and Progesterone receptor expression. As the MMP9 became negative to positive the number of cases of Progesterone receptor negative cases increased than Progesterone receptor positive cases.

Our study shows a multilinear graph between MMP9 and Her2neu expression. As the MMP9 became negative to positive the number of cases of Her2neu positive cases increased than Her2neu negative cases.

A meta-analysis by Song et al. suggested that positive MMP-9 expression confers a higher risk of relapse and worse survival in patients with breast cancer⁷. Table 1,2,3 and 4 of our study also found a positive correlation between MMP9 positivity and higher stage and grade of breast carcinoma respectively; that confers a poorer prognosis. So our study is in line with the aforementioned meta analysis by Song et al.

A study by Egeblad M et al reported the association of elevated levels of MMP-9 with a higher grade, incidence of metastases and poor clinical outcome of invasive breast carcinoma. In accordance Table 3 and 4 of our study also showed that high expression of MMP-9 is specifically correlated with high-grade breast cancers. This study by Egeblad M et al showed that MMP9 positivity also correlated with both triple-negative and HER-2 positive breast cancers.⁸ This finding is in line with our finding at table 5 and 6, that showed a negative correlation of MMP9 with ER positivity. This finding is also in line with our finding at table 7 and 8, that showed a negative correlation of MMP9 with PR positivity. However table 9 and 10 showed a positive correlation of MMP9 with Her-2-neu positivity, which is also in line with the study by Egeblad M et al.

A study by Caldarella A et al showed that those cell lines with a basal-like phenotype and those that overexpressed HER2 reached the highest levels of MMP-9 expression.⁹ Table 9 and 10 of our study also showed a positive correlation between MMP9 and her-2-neu positive cases of breast cancer. So our study finding is in line with the finding of aforementioned study.

A study by Johanna et al reported that MMP-9 expression has prognostic value of overall survival and relapse-free survival in breast cancer patients and that positive stromal MMP-9 expression indicates poor survival¹⁰. Table 1,2 and table 3,4 of our study showed positive correlation of MMP9 positive status with higher stage and grade of breast cancer respectively. Both higher stage and grade are associated with poorer survival and overall bad prognosis of the breast cancer patients. So our study findings are in accordance with the aforementioned study.

MMP9 plays an important role in the malignancy and the growth of the tumour. and has been linked to cancer cell proliferation, tumor invasion, and epithelial-to-mesenchymal transformation.¹¹ Accordingly, Table 1,2,3 and 4 of our study showed that MMP9 positive status is associated with higher grade and stage of breast cancer cases, those are result of increased cancer cell proliferation, tumor invasion, and epithelial-to-mesenchymal transformation.

A very recent study by Thammineni KL et al¹² showed that the levels of MMP-9 were significantly increased in larger tumor size, this finding is in line with our finding in table 1 and 2 showing significant positive correlation between MMP9 positivity and higher stage of disease.

TABLE - Correlation between MMP9 expression and Histopathological Grade

MMP9 Histopathological Grade	Grade 1	Grade 2	Grade 3	Total
NEGATIVE	5	5	0	10
POSITIVE	1	11	10	22

TABLE - Correlation between MMP9 expression and Estrogen receptor expression

MMP9 Positivity	ER Positive	ER Negative	Total
NEGATIVE	8	2	10
POSITIVE	4	18	22

TABLE - Correlation between MMP9 expression and Her-2-neu receptor expression

MMP9 Positivity	Her 2 Neu Positive	Her 2 Neu Negative	Total
NEGATIVE	2	8	10
POSITIVE	19	3	22

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