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# Immunohistochemical Expression of MMP-3 and MMP-8 in Breast Carcinoma. A Clinicopathological Study

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## Abstract

**Background** Matrix metalloproteinases are enzymes that are involved in the digestion of the components of the extracellular matrix (ECM), cell surface receptors for soluble factors and junctional proteins and physiological processes such as tissue remodeling, but also in the stimulation of tumor growth, invasion, and metastasis.

**Objectives** To assess the immunohistochemical expression of MMP-3 and MMP-8 in breast carcinoma and to correlate this expression with clinicopathological parameters including patient's age, tumor size, grade, subtype, lymph node status and lymphovascular invasion.

**Method** Sixty-two tissue blocks of breast carcinoma specimens were collected from Al-Kadhimiya Teaching hospital and Teaching Laboratories of the Medical City Center. Three sections of 5µm thickness were taken from each block and stained with H&E and Immunohistochemically for MMP-3 and MMP-8.

**Results** MMP-3 and MMP-8 expression were statistically correlated with patient's age, tumor grade, tumor size, histological subtype, lymph node involvement, and lymphovascular permeation with the exception of MMP-8 and age. Strong expression for MMP-3 was noticed in invasive carcinoma, high grade tumors, large size tumors, cases associated with positive lymph nodes and lymphovascular permeation (positive correlation). Negative expression for MMP-8 was noticed in most of the cases associated with lymphovascular permeation and positive lymph node(s) involvement by the metastatic cells (inverse relationship). Also a negative expression for MMP-8 was noticed in most cases associated with high grade, large size tumors.

**Conclusions** Assessment of MMP-3 in breast carcinoma reflects the grade of tumors and can predict progression of insitu to invasive cancer, lymph node involvement and lymphovascular permeation so that it may be useful additional prognostic factor. Expression of MMP-8 correlates with a lower incidence of lymph node metastasis and lymphovascular permeation and can be utilized as a marker indicating a good prognosis to these patients.

**Keywords** Breast carcinoma, MMP-3, MMP-8.

## Introduction

Breast cancer is the most common cancer affecting women in the world today. It is the leading cause of cancer related death for women aged between 35 and 55 years worldwide. One in nine women will suffer from breast cancer during her life and in excess 130 thousand women die from breast cancer each year [(1)]. In Iraq, cancer of the breast is the commonest cancer in females, constituting 31% of all other malignancies in women [(2)]. Matrix Metalloproteinases (MMPs) family consists of

more than 26 endopeptidases that share homologous protein sequences, with conserved domain structures and specific domains related to substrate specificity and recognition of other proteins [3].

Considering the main action mechanisms, MMPs roles may be discussed in terms of tissue destruction, cancer invasion and metastasis, angiogenesis, apoptosis, escaping mechanisms, and antitumor defensive mechanism, and as a pivotal role in the pathogenesis of arthritis, atherosclerosis, pulmonary emphysema, and endometriosis. Tissue inhibitors of metalloproteinases (TIMPs) may act in the tissue environment to neutralize used proteinases thereby preventing excessive and unwanted degradation away from the sites of metalloproteinase production [4].

Stromelysins (MMP-3 or Stromelysin 1 and MMP-10 or Stromelysin 2) digest a wide array of substrates, including aggrecan, fibronectin, nidogen, laminin, type IV, IX and X collagens, tenascin, vitronectin and decorin [5]. Studies have shown that the expression of MMP-3in the mammary glands of transgenic mice causes the production of invasive carcinomas by stimulating epithelial mesenchymal transition (EMT), acting as a natural tumor promoter and enhancing cancer susceptibility in mammary glands of transgenic mice [6]. In humans EMT is associated with the most aggressive breast cancers. A particular molecule involved in cell-cell contact (E-cadherin) is known to be lost in EMT. Stromelysin-1 induces cleavage of E-cadherin, a process that may be the initial step in EMT and subsequent tumor formation [7].

Collagenases (MMP-1 or collagenase-1, MMP-8 or collagenase-2 and MMP-13 or collagenase-3) can digest major fibrillar collagens in their triple-helical domain at physiological pH [5]. Analysis of MMP-8 in breast cancer patients revealed that the expression of this metalloproteinase by breast tumors correlates with a lower incidence of lymph node metastasis and confers good prognosis to these patients. However, to date, no information is available about the molecular mechanisms underlying the putative role of MMP-8 in the regulation of the metastatic process [8].

The aim of the present study is to assess the immunohistochemical expression of MMP-3 and MMP-8 in breast carcinoma and to correlate this expression with clinicopathological parameters including patient's age, tumor size, grade, subtype, lymph node status and lymphovascular invasion.

## Methods

A retrospective study included the collection of 62 formalin fixed, paraffin embedded tissue blocks from archived material at Al-Kadhimiya Teaching Hospital and Teaching Laboratories of the Medical City Center covering for the period from January 2009 to November 2010. These blocks represent the mastectomy specimens of breast carcinoma cases. Clinicopathological parameters such as (age, size, grade histological subtype, lymph node involvement and lymphovascular permeation) were obtained from the available histopathological reports and patients' files. An ethical approval was obtained from the institution in which the study was carried out in order to enable us to record patients' clinical data from their files.

Three sections of 5µm thickness were taken from each block, the first was stained by hematoxylin and eosin stain (H&E) for revision of the histopathological diagnosis, the rest two sections were stained immunohistochemically using three steps- indirect streptavidin method for MMP-3 and MMP-8.

Technical negative controls were obtained by omitting the primary antibody for the two markers under identical test condition, respectively.

Immunohistochemical expression of MMP-3 in ductal epithelial cells of the breast is cytoplasmic (brown color) and is better evaluated by the

intensity of the staining as to classify the result to negative (0 score), weak positive (+ score) (Figure 1), and strong positive (++ score) (Figure 2) depending on an intensity in control cases of endometroid endometrial carcinoma as weak positive MMP-3 is expressed in Grade I endometroid endometrial carcinoma and strong positive MMP-3 expression is detected in Grade III endometrial carcinoma [9-11].

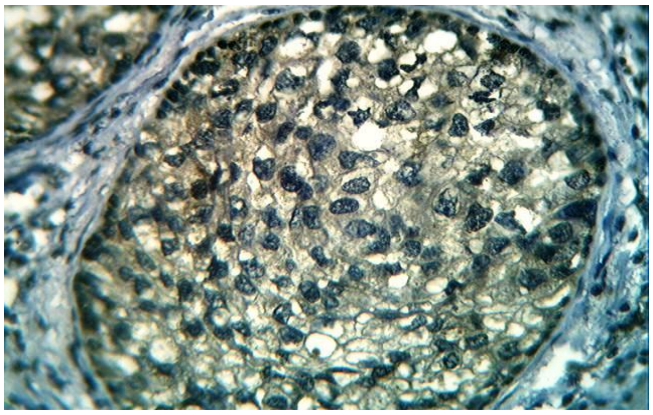

**Figure 1. Ductal carcinoma *insitu* showing weak cytoplasmic immunohistochemical expression of MMP-3 by the malignant cells (X40).**

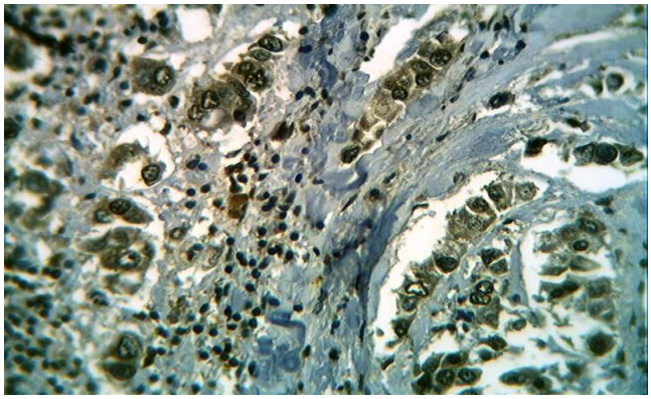

**Figure 2. Invasive ductal carcinoma(NOS), Grade II, showing strong cyoplasmic immunohistochemical expression of MMP-3 (X40).**

Immunohistochemical expression of MMP-8 in malignant ductal epithelial cells of the breast is cytoplasmic (brown color) and it is either negative (0 score) (Figure 3) or positive (+ score) (Figure 4) [12,13]. The positive control for MMP-8 is neutrophils in sections of acute supportive appendicitis according to the leaflet instructions. Statistical analysis was performed using SPSS V.17 (statistical package for social sciences) and Microsoft Excel 2007 programs. Data analysis was done using chi –square test and ANOVA. P-value is considered statistically significant when it is less than 0.05.

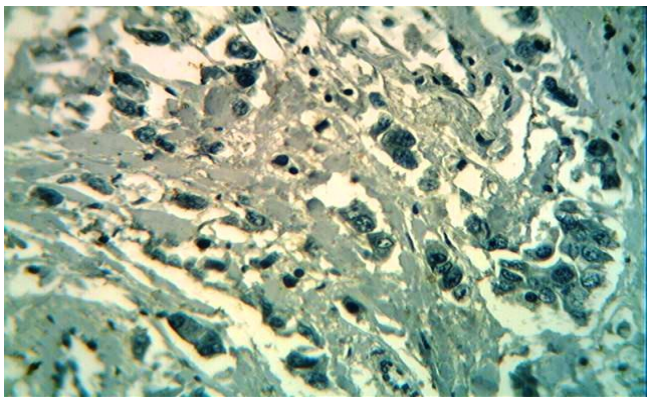

**Figure 3. Invasive ducal carcinoma (NOS), Grade II, with negative IHC expression of MMP-8 (X40).**

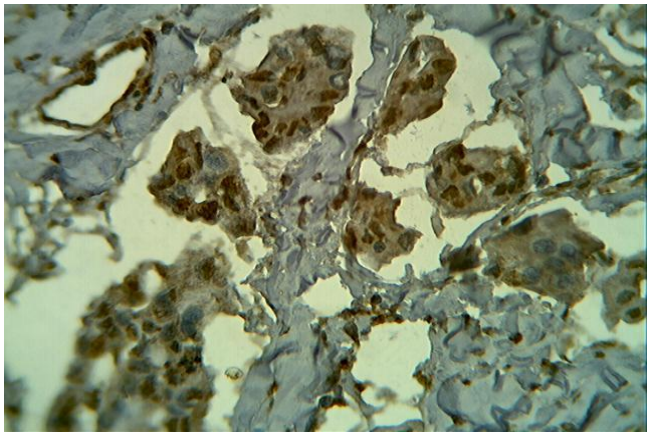

**Figure 4. Invasive ductal carcinoma (NOS), showing positive cytoplasmic IHC expression of MMP-8 by the malignant cells (X40).**

## Results

The age of patients ranges between 30-70 years with a mean of (48.55±1.3 year). Regarding grade of the tumor, 64.5% of cases were Grade II, 25.8% Grade III and 9.7 % were Grade I breast carcinoma. The majority of the studied cases (46 cases) (74.2%) were invasive ductal carcinoma

(IDC), not otherwise specified (NOS). Forty four cases (70.96%) were associated with lymph node involvement by metastatic tumor cells while 18 cases (29.03%) were negative for lymph node metastasis. Forty six cases (74.19%) were associated with lymphovascular permeation while 16 cases (25.8%) negative for lymphovascular permeation. There was a statistically significant correlation between the lymphovascular permeation and lymph node(s) involvement (P<0.001) with an Odd ratio of 23(95% CI). Thirty two cases (51.6%) were with T2 tumor size (2-5 cm), 18 cases (29%) were with T3 (>5 cm), and 12 cases (19.4%) were T1 (<2 cm).

In the present study the overall expression of MMP-3 in breast carcinoma cases was 90.31% (56 cases) while MMP-8 was positively expressed in 29.03% (18 cases).

There was a statistically significant correlation between MMP-3 expression and age of the patients, tumor grade, tumor size, histological subtype (Figures 1 and 2), lymph node involvement, and lymphovascular permeation. Strong expression for MMP-3 was noticed in invasive carcinoma (Figure 2), high grade tumors, large size tumors, cases associated with positive lymph nodes and lymphovascular permeation (positive correlation) (Tables 1-3).

There was a statistically significant correlation between MMP-8 expression and tumor size, grade, histological subtype, lymph node involvement, and lymphovascular permeation, while there was no statistically significant correlation between MMP-8 expression and age of the patients. Negative expression for MMP-8 was noticed in most of the cases associated with lymphovascular permeation and positive lymph node(s) involvement by the metastatic cells (inverse relationship). Also a negative expression for MMP-8 was noticed in most cases associated with high grade, large size tumors (inverse or negative correlation) (Tables 1-3).

**Table 1. Correlation of MMP3 and MMP8 immunohistochemical expression with age of patients, tumor size and grade**

| Marker Expression | Age range (Years) | P-value | Size <2 cm | Size 2-5 cm | Size >5 cm | P-value | Grade I | Grade II | Grade III | P-value |
|---|---|---|---|---|---|---|---|---|---|---|
| -ve MMP3 | 51.67±2.58 | | 2 | 4 | 0 | | 4 | 2 | 0 | |
| Weak MMP3 | 43.08±8.31 | 0.003 | 10 | 14 | 0 | <0.001 | 2 | 18 | 4 | <0.001 |
| Strong MMP3 | 52.06±10.78 | | 0 | 14 | 18 | | 0 | 20 | 12 | |
| -ve MMP8 | 50.05±9.87 | 0.072 | 4 | 26 | 14 | 0.006 | 2 | 28 | 14 | 0.044 |
| Positive MMP8 | 44.89±10.47 | | 8 | 6 | 4 | | 4 | 12 | 2 | |

**Table 2. Correlation of MMP3 and MMP8 immunohistochemical expression with tumor subtypes**

| Marker Expression | Comedo carcinoma | DCIS* | IDC+DCIS** | IDC(NOS)*** | P-value |
|---|---|---|---|---|---|
| Negative MMP3 | 0 | 2 | 0 | 4 | |
| Weak MMP3 | 2 | 2 | 2 | 18 | 0.016 |
| Strong MMP3 | 0 | 0 | 8 | 24 | |
| Negative MMP8 | 2 | 4 | 10 | 28 | 0.032 |
| Positive MMP8 | 0 | 0 | 0 | 18 | |

*ductal carcinoma insitu ** combined invasive ductal carcinoma and insitu carcinboma ***invasive ductal carcinoma not otherwise specified

**Table 3. Correlation of MMP-3 and MMP-8 immunohistochemical expression with Lymph node(s) involvement and Lymphovascular permeation**

| Marker Expression | | LN involvement | | P-value | LV permeation | | P-value |
|---|---|---|---|---|---|---|---|
| | | Negative | Positive | | Negative | Positive | |
| MMP3 | Negative | 4 | 2 | <0.001 | 4 | 2 | <0.001 |
| | Weak | 14 | 10 | | 12 | 12 | |
| | Strong | 0 | 32 | | 0 | 32 | |
| MMP8 | Negative | 6 | 38 | <0.001 | 6 | 38 | 0.001 |
| | Positive | 12 | 6 | | 10 | 8 | |

## Discussion

In the present study there was a statistically significant positive correlation between MMP-3 expression and the age of the patients with a P-value of 0.003. MMP-3 has been implicated in overall age-associated risk of cancer development.

senescent stromal fibroblasts secrete soluble and insoluble factors that can, at least in principle, disrupt the architecture and function of the surrounding tissue and stimulate (or inhibit) the proliferation of neighboring cells. These factors include inflammatory cytokines (e.g. IL1), epithelial growth factors (e.g. heregulin) and matrix metalloproteinases (e.g. MMP-3). Thus, senescent cells may create a tissue environment that synergizes with mutation accumulation to facilitate the progression of epithelial malignancies. Consistent with this idea, human and rodent cells with senescent characteristics accumulate in vivo with age and at sites of age related pathology, including hyperplastic and premalignant lesions. Moreover, senescent human fibroblasts can promote the proliferation and tumorigenic conversion of premalignant (non-tumorigenic, but bearing potentially oncogenic mutations), but not normal, epithelial cells in culture and in vivo [(14)].

In a study conducted by Nakopoulou et al reported no significant correlation of MMP-3 expression with age of patients with breast cancer, a finding which disagrees with this study due to difference in sample size, technique or population [(15)].

In the present study, the relation between the MMP-8 expression and patient's age was not statistically significant with a P- value of 0.072. This finding goes with a study done by Decock et al which recorded that the higher percentage of positive MMP-8 was expressed in pre-menopausal age; however the correlation of MMP-8 expression with age in breast cancer was not significant [(16)].

Regarding tumor grade, the current works revealed a statistically significant correlation between MMP-3 expression and the grade of breast carcinoma ($p< 0.001$). It is obvious that the expression of MMP-3 is more in higher grade tumors that all cases of Grade III were positive for MMP-3 expression. MMPs have also been shown to be involved in malignant transformation of the mammary gland. Overexpression of MMPs like MMP-3 (stromelysin-1) and MMP-7 (matrilysin-1) in the mammary gland of transgenic mice, results in premature differentiation and increased incidence of mammary tumor formation. A potential molecular basis for such an effect has been elucidated by the demonstration that some MMPs like MMP-3 and MMP-7 promote epithelial to mesenchymal transition (EMT), an early step in malignant transformation of epithelial cancers [(17)]. In humans EMT is associated with the most aggressive breast cancers [(7)]. However, studies by McGowan and

Duffy [18] and by Krippl et al [19] revealed no significant correlation of MMP-3 with tumor grade. This discordance could be attributed to environmental, racial and geographical differences, in addition to the difference in the sample size and antibodies used for detection of MMP-3 antigen.

In the current study there is a statistically significant correlation between MMP-8 expression and the grade of the carcinoma with P -value of 0.044, but the opposite to MMP-3, here we can notice that most of the higher grade cases (Grades II, III) were negative MMP-8, i.e., poorly differentiated breast carcinomas associated with negative MMP-8 expression while positive MMP-8 expression is detected mainly in low grade carcinomas. MMP-8 is not expressed in normal breast tissue while a low positive expression of MMP-3 is noticed in normal breast tissue. The unexpected finding that MMP-8 might play tumor-defying functions first derived from studies of cancer susceptibility in a murine model of MMP-8 deficiency. The absence of MMP-8 strongly increased the incidence of tumors in male $MMP^{-}/^{-}$ mice .Bone marrow transplantation studies provided additional evidence that neurtophil-derived MMP-8 is sufficient to restore the antitumor protection mediated by this metalloproteinase [20]. A study by Decock et al agrees with the present work [16]. Other studies revealed discordant results due to technical, statistical, racial or sample size differences [1,18].

The present study reported a statistically significant correlation between MMP-3 expression and the histological type of the tumor with P- value of 0.016. The expression of MMP-3 is more intense with invasive breast carcinoma and less with the in situ carcinomas. A particular molecule involved in cell-cell contact (E-cadherin) is known to be lost in EMT. Stromelysin-1 induces cleavage of E-cadherin, a process that may be the initial step in EMT and subsequent tumor formation [7]. Holliday et al [21] reported similar results with the current study; however, Nakopoulou et al disagrees with these findings [15].

This study showed a statistically significant correlation between MMP-8 expression and the histological type of breast carcinoma with P -value of 0.032 . The positive MMP-8 expression is found only in invasive carcinomas and is not detected in *in situ* tumors. Analysis of this negative regulation of cell invasiveness mediated by MMP-8 revealed that it is associated with an increased adhesion of cells expressing MMP-8 to different extracellular matrix components, such as type I collagen and laminin-1 and actin fiber reorganization, consistent with the increased adhesion of cells expressing MMP-8 [22,23].

There is a statistically significant correlation between the lymphovascular permeation and lymph node involvement with P- value of less than 0.001 and an Odd ratio (95% CI) of 23, which means that the patient with lymphovascular permeation is 23 times more risky to have a positive lymph node(s) than a patient without lymphovascular permeation. In the present study we found that there is a statistically significant correlation between MMP-3 expression and lymph node(s) involvement ($P < 0.001$) and with lymphovascular permeation ($P < 0.001$).

MMP-3 expression was more intense (strong) with positive lymphovascular permeation and positive lymph nodes. Lymphangiogenesis plays an important role in tumor biology; it is directly linked with the formation of lymphatic metastases. MMP-3 plays a role in activation of MMP-9 which is important in the modulation of the Vascular Endothelial Growth Factor (VEGF) bioavailability (the most potent inducer of tumor Lymphangiogenesis) and making sequestered VEGF bioavailable for its receptor VEGFR2, in turn, promotes dissemination of metastases into the lymph. So increase MMP-3 is linked with lymphatic invasion and lymph node metastases[24]. Krippl et al [19] reported also a

significant correlation between MMP-3 expression and lymph node involvement; however, the current study disagrees with a study done by McGowan and Duffy [18] due to similar reasons mentioned above.

In the present study there was a statistically significant negative correlation between MMP-8 expression and Lymph node(s) involvement (P <0.001) and also significant negative correlation with lymphovascular permeation (P = 0.001). MMP-8 expression was more with negative nodal metastases and negative lymphovascular permeation while the majority of positive nodal cases and positive lymphatic permeation were negative for MMP-8. MMP-8, like other MMP enzymes, is secreted as a proenzyme, which can subsequently be activated by a number of other enzymes including MMP-3 and serine proteases, which themselves can be inactivated by specific tissue inhibitors. The interplay between these potential activators of MMPs and their inhibitors plays a significant role in the function of these enzymes. Therefore, in addition to the differential expression of MMP-8 in tumor cells, activation of the procollagenase could be an important regulatory step in its inhibitory effect on metastasis, also MMP-8–expressing cells had an increased adhesion to type I collagen and laminin-1 so potentiates cell adhesion, and this might be a candidate mechanism by which this protease reduces cell invasion and metastasis, but (to date) the exact mechanism by which MMP-8 protect against lymph node metastasis is not clear [23,25]. Pennington *et al*. found that there was a significant correlation between MMP-8 expression and lymph node involvement and that the reduced expression of MMP-8 equating to greater nodal spread and suggested that the function of MMP-8 antagonizes metastasis of breast carcinomas[26] Decock et al revealed also a significant correlation of MMP-8 with nodal involvement and that MMP-8 is less expressed with node positive patients [16]. However, McGowan and Duffy [18] disagrees with this result.

When tumor size is taken into consideration, the present work found that there is a statistically significant correlation between MMP-3 expression and the tumor size (P value< 0.001) and that the staining is more intense with large tumors (T2 and T3), the strongest expression is in T3 tumors, and no strong expression in T1 carcinomas. Possible mechanisms by which MMP3 contributes to tumor cell growth include promotion of angiogenesis (which is necessary for a tumor to grow to a size greater than approximately 2mm in diameter, MMP-3 have been shown to breakdown endothelial-derived perlecan, releasing basic fibroblast growth factor (FGF), a potent endothelial mitogen, activation of stimulating growth factors or their receptors, and inactivation of inhibitory growth factors **[27]**. Other studies found no significant correlation of MMP-3 with tumor size [18,19].

In the present study there is a statistically significant correlation between MMP-8 expression and the tumor size (P = 0.006) and that MMP-8 expression in the majority of large tumors (T2 and T3) is negative (the opposite to MMP-3). The mechanism by which MMP-8 act as tumor defying agent is still unclear, but the possible explanation for increase expression of MMP-8 in small size tumor is that it develop it`s functions by targeting TNF (Tumor Necrosis Factor) which decreases tumor size by apoptosis, also this enzyme may target substrates distinct from collagens or other matrix components. The potential proteolytic processing activity of MMP8 on inflammatory mediators, which contribute to the host antitumor defense system, could help to explain this [23,25]. Studies by McGowan and Duffy [18], and Decock et al [16] recorded different results. This disagreement could be caused by difference in sample size, antibody used, methods of quantifying immunohistochemical staining(manual versus automated) and racial differences.

In conclusion, assessment of MMP-3 in breast carcinoma reflects the grade of tumors and can predict progression of *insitu* to invasive cancer, lymph node involvement and lymphovascular permeation so that it may be useful additional prognostic factor. Expression of MMP-8 correlates with a lower incidence of lymph node metastasis and lymphovascular permeation and can be utilized as a marker indicating a good prognosis to these patients.

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