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A STUDY OF MMP7 EXPRESSION IN TRIPLE NEGATIVE BREAST CANCER PATIENTS

Bhoomi Vaniya, Nupur Patel, Hemangini Vora

Immunohematology Lab, Cancer Biology Department,
The Gujarat Cancer & Research Institute, Ahmedabad
*Corresponding author: hemangini.vora@gcriindia.org

ABSTRACT:

MMP7 has a part in preventing apoptosis and promoting angiogenesis. According to both in vivo and in vitro evidence, MMP7 is mostly seen during tumour invasion and metastasis. Controlling MMP7 expression may be a method for treating this cancer because its overexpression encourages the occurrence, progression, and invasiveness of breast cancer. The aim is to study the expression of MMP7 in triple negative breast cancer patients. Formalin fixed paraffin embedded breast tumor tissues from 50 triple negative breast cancer patients were collected for this study. immunohistochemistry method was used to study MMP7 expression and correlated with clinical and pathological parameters as well as disease status. MMP7 positivity was observed in 48% triple negative breast cancer patients. No significant correlation of MMP7 expression with age, menopausal status, tumour size, histopathology, stage and lymph node status were observed. A significant higher incidence of MMP7 expression was noted in patients with high grade tumor (63%) as compared to patients with low grade tumor (00%). Further, a significant higher incidence of MMP7 was observed in patients with high BR score (65%) as compared to the patients with low BR score (00%). Also, a significant higher incidence was observed in patients with relapse (63%) than patients in remission (35%). A significant higher incidence of MMP7 expression was observed with respect to disease free survival in univariate survival analysis. In multivariate survival analysis, for disease free survival MMP7 entered at step 1 and lymph node status and MMP7 entered at step 2. The findings imply that MMP7 is important for tumour invasion and metastasis, and that elevated MMP7 expression is associated with tumour development and recurrence.

Keywords: MMP7, Triple negative breast cancer, Immunohistochemistry

INTRODUCTION:

Triple negative breast cancer means they test “negative” for the 3 receptors for estrogen, progesterone, and HER2. TNBC is typically linked to a younger diagnosis age, aggressive profile, and high frequencies of p53 gene mutations, along with strong p53 immunohistochemically found (1). Carcinogenesis in the breast is a complex, multi-stage process leading to excessive tissue proliferation that cannot be controlled by natural mechanisms due to predominance of cell proliferation over cell death. However, local neoplastic growth would not be possible without reconstruction of the extracellular matrix (ECM). Stromal degradation and looseness also allow migration of cancer cells and thus their dissemination, vascular invasion and formation of distant metastases (2). The reconstruction of the ECM involves a variety of digestive enzymes (3). However, a major role can be ascribed to metalloproteinases, which are intensively investigated in carcinogenic processes (2). MMP7 gene is also thought to be involved in cancer progression. MMP-7 has been detected in the majority of adenomas and carcinomas, including breast fibroadenomas and carcinomas (4), in addition to the digestion of stroma, MMP-7 also takes part in other processes involved in the carcinogenesis through breakdown of the cell surface protein (5). In this way, the enzyme can induce proliferation (6), and regulate apoptosis (7), angiogenesis and escape of cancer cells against the immune system (8). The secretion of matrix metalloproteinases (MMPs) is crucial in the metastasis of cancer cells; MMP-7 overexpression led to an increased incidence of breast hyperplasia, breast cancer, and accelerated tumor progression (9). IHC staining revealed significantly higher MMP-7 expression in surgical samples of breast cancer cells than in adjacent non-tumor tissues (10). Expression of MMP-7 is upregulated in most

cancers, making it a pro-tumor factor. This is clinically significant and should be evaluated further to serve as a diagnostic and prognostic biomarker for early tumors (11).

MATERIAL AND METHODS:

Patient characteristics:
This retrospective study was approved by institutional scientific and ethics committees, included 50 triple negative breast cancer patients treated at The Gujarat Cancer & Research Institute. Detailed clinicopathological history such as age, menopausal status, lymph node status, histopathological type, tumor size, stage, histopathology grade, metastasis, BR score and treatment offered were recorded from the case files maintained at the Medical Record Department of the Institute. Disease staging was done according to AJCC classification. Disease status was assessed by clinical examination, radiological investigations and biochemical investigations.

Immunohistochemical Localization:
Localization of marker MMP7 expression was analysed by immunohistochemistry method, which was performed on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). Primary antibody MMP7 was procured commercially from Epitomics. The primary and secondary antibodies were incubated as follows: MMP7 for 32 minutes at 37°C with dilution 1:80; and HRP multimer for 8 minutes.

Scoring:
Two individual observers scored the sections under microscope. Cytoplasmic staining pattern was noted for MMP7. For MMP7, scoring was done using the ASCO and CAP guidelines 2007 where immunoreactivity scored as 0 for negative (no cytoplasmic staining), 1+ (faint or incomplete cytoplasmic staining), 2+ (10-30% with moderate cytoplasmic staining) and 3+ (>30% tumor cells with complete cytoplasmic staining). For statistical analysis 1+, 2+ and 3+ were clubbed as positive and 0 considered as negative.

Statistical analysis:
Statistical analysis was carried out using SPSS statistical software version 26 (SPSS Inc. USA). Mean, Standard error (SE) of mean and median were calculated and Pearson's Chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between two parameters. Univariate survival analysis was carried out by Kaplan Meier and Log Rank statistics was used to assess the prognostic significance of disease-free survival (DFS) and overall survival (OS); Multivariate survival analysis was performed using Cox regression model with forward stepwise (likelihood ratio) method. P values ≤ 0.05 were considered to be statistically significant.

RESULT:

Patient characteristics with outcome:
This retrospective study included 50 patients, 66% had age ≤ 50 years, whereas 34% patients had >50 years. Further, 68% patients had postmenopausal status. In relation to pathological characteristics, more than 50% were of T2 tumour size, Positive lymph node status, IDC subtype, disease stage II, histological grade III (Table 1) and in this study cohort 50% patients had metastasis and 50% patients had no metastasis. (Table 1). The primary treatment offered to the patients was surgery followed by adjuvant chemotherapy and radiotherapy. The maximum follow-up period was 108 months.

MMP7 expression:
Cytoplasmic expression of MMP7 was noted in 48% of the cases of triple negative breast cancer (Figure 1). No significant correlation of MMP7expression was observed with age and menopausal status. MMP7 expression was significantly higher in patients with high grade of tumor ($p = 0.04$) (Table 1). further, a significant higher incidence of MMP7 was observed in patients with high BR score ($p = 0.05$) (Table 1). Similarly, significant higher incidence of MMP7 expression was noted in patients with in patients having relapse ($p = 0.04$) (Table 1).

MMP7 expression in relation to survival

According to Kaplan and Meier univariate survival analysis, with respect to DFS, a significant higher incidence of disease relapse was noted in MMP7 positive patients (63%, 15/24) than in MMP7 negative patients (39%, 10/26) (Table 2, Figure 2A). While with respect to OS, similar incidence of death was noted in MMP7 negative patients (08%, 02/26) and MMP7 positive patients (12%, 03/24) (Table 2, Figure 2B).

Multivariate survival analysis using Cox regression model with forward stepwise (likelihood ratio) method was carried out to evaluate the prognostic significance of clinical and pathological parameters such as age, menopausal status, tumour size, lymph node status, disease stage, histopathology, histological grade, BR score, treatment and MMP7. MMP7 expression entered at step 1 as significant factor (Wald statistic = 4.06, df = 1, Exp (B) = 2.55, p = 0.04) and lymph node and MMP7 expression entered at step 2 (Wald statistic = 3.80, df = 1, Exp (B) = 2.76, p = 0.05) and (Wald statistic = 5.36, df = 1, Exp (B) = 2.98, p = 0.02) respectively. (Table 3)

Table 1: Correlation of MMP7 expression with clinicopathological parameters and disease status

Parameters		**Incidence of MMP7 Expression**		**P**
	N (%)	**Negative N (%)**	**Positive N (%)**	
Age (years)	**50 (100)**	**26 (48)**	**24 (52)**	
≤ 50 years	33 (66)	15 (45)	18 (55)	0.19
> 50 years	17 (34)	11 (65)	06 (35)	
Menopausal Status	**50 (100)**	**26 (48)**	**24 (52)**	
Premenopausal	16 (32)	08 (50)	08 (50)	0.84
Postmenopausal	34 (68)	18 (53)	16 (47)	
Tumor Size	**50(100)**	**26(48)**	**24(52)**	
T1	04(08)	03(75)	01(25)	0.32
T2	36(72)	18(50)	18(50)	
T3	08(16)	05(63)	03(37)	
T4	02(04)	00(00)	02(100)	
Lymph node Status	**50(100)**	**26(48)**	**24(52)**	
Negative	17(34)	08(47)	09(53)	0.85
Positive	33(66)	18(55)	15(45)	
Histopathology	**50(100)**	**26(48)**	**24(52)**	
ILC	01(02)	01(100)	00(00)	0.16
IDC	48(96)	25(52)	23(48)	
IDC+DCIS	01(02)	00(00)	01(100)	
Grade	**50(100)**	**26(48)**	**24(52)**	
Grade I	02(04)	02(100)	00(00)	**0.04**
Grade II	21(42)	14(67)	07(33)	
Grade III	27(54)	10(37)	17(63)	
BR score	**45(100)**	**22(49%)**	**23(51%)**	
5 (Low)	02(04)	02(100)	00(00)	**0.05**
6-7 (Intermediate)	17(34)	11(65)	06(35)	
8-9 (High)	26(52)	09(35)	17(65)	
Stage	**50(100)**	**26(48)**	**24(52%)**	
Early (Stage IIA+ IIB)	29(58)	15(52)	14(48)	0.96
Advanced (Stage IIIA+ IIIC)	21(42)	11(52)	10(48)	
Disease status	**50(100)**	**26(48)**	**24(52)**	
Remission	25(50)	17(65)	09(35)	**0.04**
Relapse	25(50)	09(37)	15(63)	
Disease status	**50(100)**	**26(48)**	**24(52)**	
Alive	45(90)	24(53)	21(47)	0.58
Dead	05(10)	02(40)	03(60)	

Figure 1: Positive cytoplasmic MMP7 staining of breast tumour cells of cancer patients (40X)

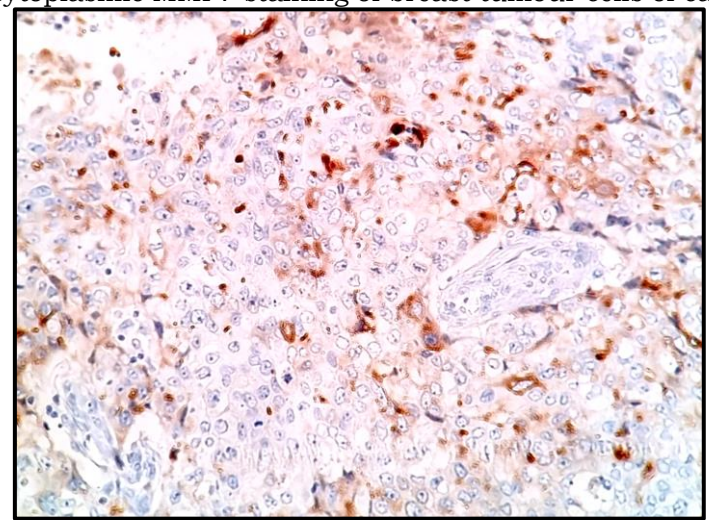


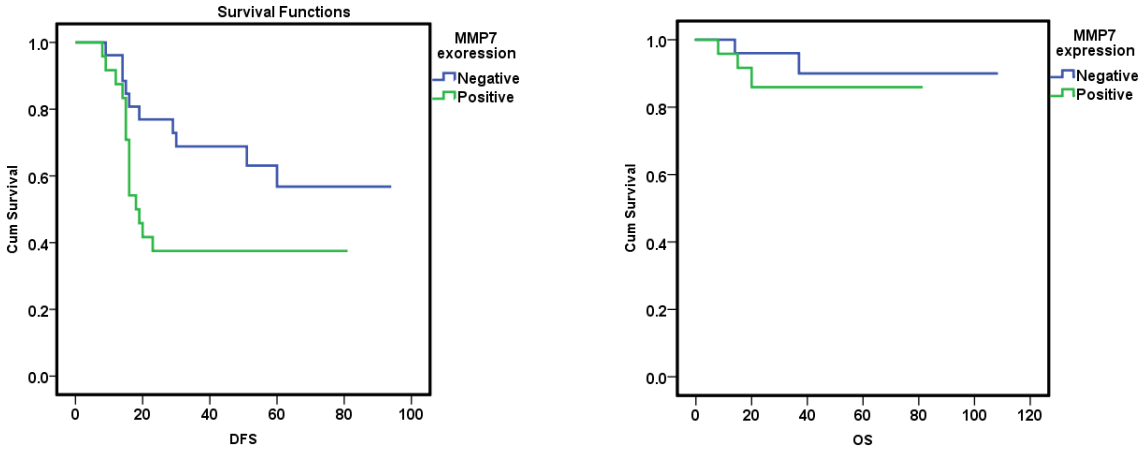
Table 2: Univariate analysis of MMP7 expression

MMP7 expression	**N**	**DFS in months Mean ± SE**	**Remission N (%)**	**Relapse N (%)**
Negative	26(48)	65.82 ± 7.01	16(61)	10(39)
Positive	24(52)	38.02 ± 6.60	09(37)	15(63)
Log rank = 4.21, df = 1, P = **0.04**				
MMP7 expression	**N**	**OS in months Mean ± SE**	**Alive N (%)**	**Dead N (%)**
Negative	26(48)	99.9 ± 5.41	24(92)	02(08)
Positive	24(52)	71.7 ± 5.02	21(88)	03(12)
Log rank = 0.50, df = 1, P = 0.48				

Table 3: Multivariate analysis of MMP7 expression

							95% CI for Exp (B)	
Patients	**Step**	**Variables**	**Wald statistics**	**Df**	**P**	**Exp(B)**	**Lower**	**Upper**
DFS	1	MMP7	4.06	1	0.04	2.55	1.02	6.36
	2	LN	3.80	1	0.05	2.76	0.99	7.65
		MMP7	5.36	1	0.02	2.98	1.18	7.53

Figure 2: (A) Kaplan - Meier survival analysis for disease free survival (DFS)
(B) Kaplan - Meier survival analysis for overall survival (OS)



(A) (B)

DISCUSSION:

In the present study, MMP7 expression was noted in 48% of triple negative breast cancer patients. While other studies found that expression of MMP7 was variable ranging from 23% to 61% of the cases (12) (13) (14). In this study MMP7 expression was correlated with clinical parameters such as age and menopausal status. No significant association was observed with age and menopausal status was observed, which is in concordance with study of Mylona E., et al. (15) and Fernandez-Garcia Belen, et al. (16) respectively.

Expression of MMP7 was also correlated with the various pathological parameters. Such as tumor size, lymph node status, histopathology types, grade, BR score as well as disease metastasis. No significant correlation of MMP7 was found with the lymph node status this was supported by study of Yamada Kaede, et al. (17). Also, with respect to the stage no significant correlation was observed and this was in concordance with the study of Sizemore Steven T., et al. (18).

A significant positive correlation with high tumor grade was observed in this study which suggests that the expression of MMP7 increases with the advancement of tumor. This was also observed by Zhu Linyan, et al. (19) and Han Ji-Chang, et al. (20). MMP7 expression also showed significant positive correlation with high BR score tumors, this finding was similar with the study of Jiang Wen G., et al (21).

In this study MMP7 expression was also correlated with the disease status and a statistically significant correlation was noted with the patients with disease relapse this was similar with many studies such as a study by Sun Da-wei, et al. (13) and also study by Polistena Andrea, et al. (22).

According to Kaplan and Meier univariate analysis, with respect to DFS, a significant higher incidence of disease relapse was noted in patients with MMP7 expression. This was in concordance with the study of Sun Da-wei, et al. (13); Also, no correlation was found between MMP7 and overall survival. This lack of correlation is in accordance with the findings of Pacheco Mercia M., et al. (23). In multivariate survival analysis with respect to disease free survival, MMP7 found to be independent prognostic factor in step one and Lymph node status and MMP7 in step two were found to be independent prognostic factor for predicting disease relapse. Also, Multivariate analysis of Szarvas Tibor, et al. (24) revealed that high MMP-7 tissue expression and serum concentration are stage and grade independent predictors of both metastasis-free and disease-specific survival. In this retrospective study, MMP7 protein expression associated with advancement of tumor grade, disease metastasis and reduced disease-free survival. This study suggests an oncogenic role of MMP7 in development of disease metastasis in triple negative breast cancer.

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