ORIGINAL ARTICLE

IMMUNOHISTOCHEMICAL EXPRESSION OF MATRIX METALLOPROTEINASE-1 (MMP-1) IN DIFFERENT TYPES OF BREAST CARCINOMA AND ITS COMPARISON WITH ER/PER AND HER2/neu

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Background: Breast cancer is a complex disease that comprises of various biological sub-types with different natural history. Matrix metalloproteinase-1(MMP-1) expression has been reported to inversely correlate with the survival. The current study was conducted to classify breast cancer into four subtypes based on IHC, to assess the immunohistochemical expression of MMP-1 in primary breast carcinoma, to compare its expression with ER/PR and HER2/neu. **Methods:** A descriptive study was conducted on 100 mastectomy specimens of breast carcinoma after being fixed in 10% formalin at Department of Histopathology, University of Health Sciences Lahore. Sections were taken on frosted slides for H&E and on lysine coated slides for immunohistochemistry (IHC). Haematoxylin and eosin (H&E) and IHC for oestrogen receptor/progesterone receptor (ER/PR), HER2/neu and Matrix metalloproteinase (MMP-1) were performed according to protocol. **Results:** The mean age was 48.44±11.79 Years (ranging 25 to 90 years). 97 cases were of Invasive ductal carcinoma (IDC) type, two showed the features of invasive lobular carcinoma and only one case was found to be of mucinous type, 19 cases were found to be in grade I, 74 in grade II and seven cases were in grade III. Breast cancer has been classified into four major groups based on IHC profile ER/PR and HER2/neu expression. The groups were: Triple positive, ER/PR+, HER2-, ER/PR-, HER2/neu+ and triple negative) cases. Among 100 cases of breast cancer, 9 were of triple positive type, (ER/PR +, HER2/neu+), 28 cases were of ER/PR +, HER2- type, 19 were ER/PR-, HER2/neu +ve and 44 cases were of triple negative type (ER/PR-, HER2/neu-ve). 13 cases were of score 1 MMP-1out of which 7 (25.0%) were ER/PR+, HER2-, 1 (5.3%) case was ER/PR-, HER2/neu +ve and 5 (11.4%) cases were triple negative. Among 30 cases of MMP-1 score 4, 5(55.6%) cases were of triple positive type, 3 (10.7%) cases were of ER/PR +, HER 2/neu-ve type, 12(63.2%) cases were of ER/PR-, HER 2/neu+ve type, and 10 (22.7%) cases were of triple negative type. A significant *p*-value of <0.05 was obtained. **Conclusions:** Among four IHC based subtypes, most frequent were triple negative breast cancers which showed high IHC score of MMP-I which is reported to be an important marker for metastatic potential in breast carcinoma.

Keywords: MMP-1; Immunohistochemistry; Triple negative breast cancers; ER; PR; HER2/neu

Citation: Shamsi FB, Anwar A, Lail RA, Bukhari MH, Naseem N, Nagi AH. Immunohistochemical expression of matrix metalloproteinase-1(MMP-1) in different types of breast carcinoma and its comparison with ER/PER and HER2/neu. J Ayub Med Coll Abbottabad 2022;34(1):12–6.

INTRODUCTION

Breast cancer is the most frequently diagnosed malignant cancer among women.[1] Despite the fact that many latest advancements have been made regarding prevention, early detection and treatment strategies, breast cancer portends a significant health burden.[2] According to Global Cancer Statistics 2018, about 2,088,849 new cases of breast cancer were diagnosed, of them about 626,679 women died of this deadly disease.[3] Incidence of breast cancer is increasing in Pakistan. One out of nine women living in Pakistan has a risk of developing breast cancer in her lifetime, which is one of the highest incidences recorded in Asia.[4] It has been established that breast cancer in many Asian countries presents in advanced stage with poorer prognostic features and has worst outcome when it was compared to their counterparts in the Western countries.[5]

Age of patient, axillary nodal status, size of tumour, histological grade, hormonal receptor status are the main factors used for stratification of breast cancer patients for evaluating the prognosis and adopting the right treatment plan.[6] Immunohistochemistry has an immense impact on the practice of diagnostic histopathology. It is a technique for identifying tissue constituents (antigen) by means of antigen-antibody interactions. Antibodies that bind to the epithelial cytoskeleton are highly selective for the demonstration of most malignant tumours.[7] The immuno-histochemical classification provides both therapeutic and

prognostic information.[5] Despite the remarkable achievements in diagnosis and management of breast cancer, clinicians still rely on the major clinico-pathological features and easily available tumour markers which involve ER, PR, HER2/neu. The status of oestrogen (ER), progesterone (PR) and human epidermal growth hormone (HER2) receptors affects course of breast cancer and patient survival.[8]

Matrix metalloproteinases (MMPs) are a group of enzymes consisting of 28 members which are capable of degrading essentially all macromolecules of the extracellular matrix (ECM). The activity of MMPs is tightly controlled extra-cellularly by tissue inhibitors of matrix metallo-proteinases (TIMPs). A number of studies have demonstrated a significant co-relation between MMP expression and the invasive potential of human cancers. MMP-1 has been described in a variety of advanced cancers with a prominent negative correlation with survival. MMP-1 is often up-regulated in breast cancer, especially in basal-type of breast tumours and is supposed to be critically involved in metastatic dissemination. Recent reports suggest that MMP-1 expression is associated with a shortened relapse free survival and poor outcome in breast cancer.[9]

This study is designed to find an association of breast carcinoma subtypes based on immunohistochemistry (ER, PR and HER2/neu) patterns with MMP-1 IHC expression in our community.

MATERIAL AND METHODS

This descriptive study was conducted in U.H.S laboratory of Morbid Anatomy and Histopathology Lahore. One hundred mastectomy specimens were brought after formalin fixation to U.H.S laboratory from Allied/DHQ hospitals and Punjab Medical College Pathology laboratory Faisalabad. Adult untreated female patients presenting with different grades and stages of breast carcinoma and patients presenting with a primary breast carcinoma were included in the study. Patient presenting with metastatic carcinoma to the breast, cases with history of co-morbidity, stromal tumours of the breast and male breast carcinoma were excluded.

Clinical record was obtained from concerned hospitals. A detailed gross examination of the specimen was carried out. Appropriate sections from the tissues were taken and paraffin embedded tissue blocks were made.

From each paraffin embedded tissue, section of 4 micrometer[10] was cut by a Leica Rotary Microtome, taken on a frosted slide while 4 sections of 4micrometer were taken on poly-L-lysine coated slides for ER/PR, HER2/neu and MMP-1 immunohistochemistry. The sections on the frosted slides were stained with conventional haematoxylin and eosin stain using method of Harris haematoxylin. H & E staining was performed to classify the tumours of breast and to assign the histological grades. Microscopy was done. Scarf Bloom Richardson grading system was used to grade the breast carcinomas. Immunohistochemical staining for MMP-1, ER/PR and HER2/neu was performed according to IHC protocol. ER and PR positive sections exhibited a brown nuclear staining of the tumour cells while HER2/neu positive sections exhibited complete brown cytoplasmic membranous staining of the tumour cells. MMP-1 IHC scoring was done according to Bostrom *et al*.[9] MMP-1 IHC positive cells exhibited brown cytoplasmic staining. Scoring system for MMP-I was adopted from study conducted by Baker *et al*.[9] The data was analysed by using SPSS 20. All the results were recorded in *pro forma*.

RESULTS

The minimum and maximum age was 25 and 90 years with the mean value of 48.44 and standard deviation of 11.79.(Table-1) The most common histological type was invasive ductal carcinoma (97% cases), two percent showed invasive lobular type and one had morphology of mucinous carcinoma (Table-2).19% cases were in grade I, 74% cases were in grade II and 7% cases were in grade III (Table-3). In this study, breast cancer has been classified into four major groups based on IHC profile ER/PR and HER2/neu expression, positive (+) and/or negative (-).

The groups were: ER/PR +, HER2/neu+ (triple positive), ER/PR+, HER2/neu-, ER/PR-, HER2/neu + and ER/PR-, HER2/neu-(triple negative) cases. Among 100 cases of breast cancer, nine were of triple positive type, 28 of ER/PR +, HER2/neu-type, 19 of ER/PR-, HER2/neu+ and 44 cases were of triple negative type. 13 cases were of score 1 MMP-1. Out of which 7 (25.0%) were ER/PR+, HER2/neu-, 1(5.3%) case was ER/PR, HER2/neu +, 5 (11.4%) cases were triple negative. Among 24 cases of MMP-1 score 2, two (22.2%) cases were of triple positive type, 13 (46.4%) of ER/PR+, HER2/neu – type and 9(20.5%) cases were of triple negative type. Among 33 cases of MMP-1 score 3, 2 (22.2%) case were of triple positive type, 5 (17.9%) of ER/PR/neu+, HER2/neu – type, 6 (31.6%) of ER/PR-, HER2+ type and 20(45.5%) cases were of triple negative type. Among 30 cases of MMP-1 score 4, 5 (55.6%) cases were of triple positive type, 3 (10.7%) of ER/PR +, HER 2/neu- type, 12(63.2%) of ER/PR-, HER 2/neu+ type, and 10 (22.7%) cases were of triple negative type. (Table-4) A significant p-value of <0.05 was obtained.

Table-1: Distribution of age

Parameter	N	Minimum	Maximum	Mean	SD. Deviation
Age	100	25	90	48.44	11.79

Table-2: Distribution of cases by histological types of breast carcinoma

Histological Type	Frequency	Percentage
Invasive Ductal Carcinoma	97	97.0
Invasive Lobular Carcinoma	2	2.0
Mucinous Carcinoma	1	1.0
Total	**100**	**100.0**

Table-3: Distribution of 100 cases by histological grades of tumours using Scarf Bloom Richardson grading system

Histological grade	Frequency	Percent
Grade I	19	19.0
Grade II	74	74.0
Grade III	7	7.0
Total	100	100.0

Table-4: Association between tumour subtypes based on ER/PR and HER2/neu and MMP-1

MMP-1	Group				Total
	ER/PR+,HER2+	ER/PR+,HER2-	ER/PR-,HER2+	ER/PR-,HER2-	
Score 1	0.00	7 (25.0%)	1 (5.3%)	5(11.4%)	13
Score 2	2 (22.2%)	13 (46.4%)	0.00	9 (20.5%)	24
Score 3	2 (22.2%)	5(17.9%)	6 (31.6%)	20(45.5%)	33
Score 4	5 (55.6%)	3 (10.7%)	12 (63.2%)	10(22.7%)	30
Total	**9**	**28**	**19**	**44**	**100**

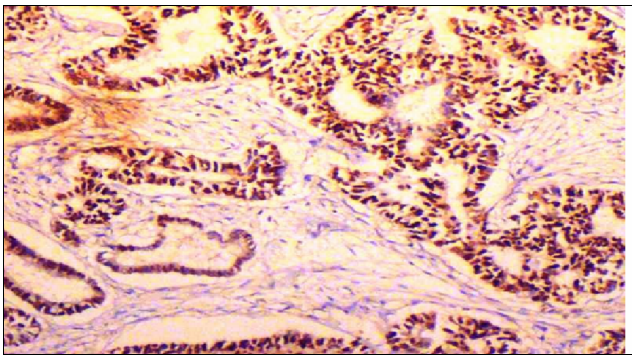

Figure-1: IHC staining (ER) in an ER/PR +, HER2/neu negative case of invasive ductal carcinoma (Grade I) of breast20x

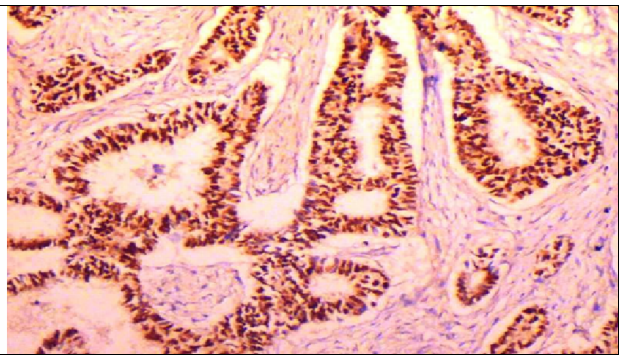

Figure-2: IHC staining (PR) in ER/PR +, HER2/neu negative case of invasive ductal carcinoma (Grade I) of breast 20x

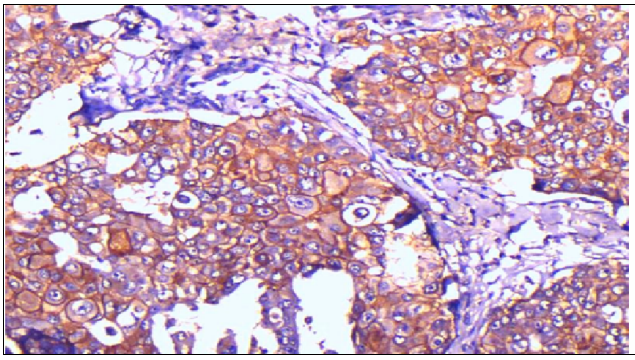

Figure-3: IHC staining (HER2/neu= 3+) in ER/PR -, HER2/neu positive case of invasive ductal carcinoma of breast (GradeIII) 40x

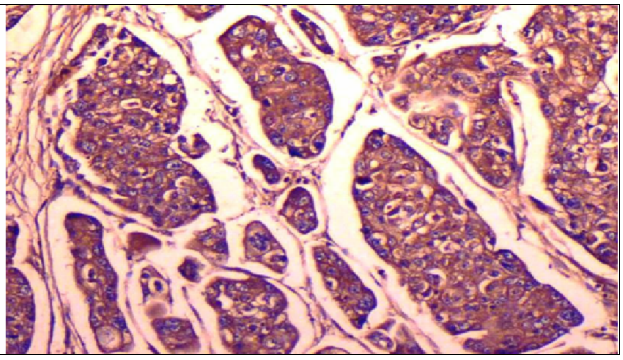

Figure-4: MMP-1 strong positivity in a triple negative case of invasive ductal carcinoma (Grade II) 20x

DISCUSSION

Classification of breast cancers based on ER/PR and HER2/neu status provides improved prognostic and therapeutic informations.[11] Although matrix metalloproteinases (MMPs) are found to be involved in breast cancer progression, the involvement of MMP-1 to this process has not been investigated in detail. Matrix metalloproteinases (MMPs) are important at several points during multi-stage neoplastic progression.

In this study, we evaluated immunohistochemical expression of MMP-1 using original tissue sections and observed its association with different breast cancer sub-types based on ER/PR and HER2/neu expression. Our results revealed statistically significant associations. We compared our results with previously published international data.

Among 100 cases, the minimum and maximum age was 25 and 90 years with the mean value of 48.44 and standard deviation of11.79. As carcinoma of breast has been widely studied area of cancer research, there are many reports from within Pakistan, Asia and from all over the globe which have documented the age at presentation for this disease. Our findings were consistent with most of them. According to one series reported in Kashmir valley showed mean age of its presentation was in 42.06±10.7 years and ranged from 22 to 60 years.[12] In other study the mean age of patients was 48.44±11.64 with 33.3% of patients younger than 40 years of age.[13]

The most frequent histological type observed in our study was invasive ductal carcinoma which comprised of 97 cases (97%) cases. Other studies like that of Naqvi *et el.*[14], Jaafar[15] and Rehman *et al.*[16] showed predominant morphology to be invasive ductal carcinoma.

Regarding histological grades, 19% cases showed the morphology of grade I, 74% cases were in grade II and 7% were grade III breast carcinomas. In the study conducted by Naqvi et al.,2017[14], 12(2.7%) patients presented with grade I, 281 (64.7%) were presented with grade-II and 145(32.5%) were diagnosed as grade III. (Oluogun *et al.*,)[17] showed in their research work that the majority of the cases were of grade II lesions. In another study conducted by Joensuu *et al.*[16], out of 72 breast cancer cases,42 cases were of grade-II, 22 of grade III and 8 cases were of grade I. So, regarding histological grading, our findings were consistent with all these studies.

Our study showed the expression of MMP-1 in different grades and different subtypes of breast cancer. Highest expression of MMP-1was observed in triple negative breast cancers. Our results were similar to Wang *et al.*[18] conducted a study that shows MMP-I over expression by those breast cancer patients who have triple negative and lymph node metastatic disease, and Bostrom *et al.*[9] who showed that MMP-1 expression carries a significant prognostic importance in breast cancer.

CONCLUSION

Triple negative (ER/PR-ve, HER2/neu –ve) immunohistochemical subtypes of breast cancer was the most frequent subtype of breast cancers showing high expression of matrix metalloproteinase. (MMP-1).

ACKNOWLEDGEMENT

We are thankful to Prof Rakhshinda Bajawa (Chairperson and HOD of Pathology Department) and Samina Qamar for helping us during the study.

AUTHORS' CONTRIBUTION

FBS: Data collection, write-up. AA: Literature search. RAL: Data analysis and interpretation. MHB: Proof reading. NN: Study design. AHN: Conceptualization of the study.

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Submitted: July 4, 2020	*Revised: September 27, 2021*	*Accepted: October 24, 2021*

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