DOI: 10.7860/JCDR/2018/34941.11540

Original Article

Pathology Section

Tumour Budding and MMP-2 Expression in Breast Invasive Ductal Carcinoma

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ABSTRACT

Introduction: Breast cancer is a major cause of cancer death among females. Tumour buds are clusters of undifferentiated malignant cells (one cell to less than or equal to five cells) at the invasive front of a tumour. They are believed to be the basis of tumour progression and metastasis. Over expression of MMP-2 {an Extracellular Matrix (ECM) proteolytic enzyme} is considered important for tumour invasion and metastasis.

Aim: This study is designed to evaluate tumour budding and expression of MMP-2 in breast invasive ductal carcinoma.

Materials and Methods: This cross-sectional observational study was conducted on 61 cases of female breast invasive ductal carcinoma. Cases were obtained from the Department of Pathology, Faculty of Medicine, Cairo University, Giza, Egypt and a private laboratory during the period from June 2015 until September 2016. Tumour budding detection using pan cytokeratin and expression of MMP-2 were evaluated immunohistochemically in 61 cases of female breast invasive ductal carcinoma. MMP-2 expression was evaluated in both neoplastic cells and accompanying stromal component.

Results: Significant positive correlations were found between tumour budding and ill defined borders, positive lymph node metastasis and low mitotic count. There were statistically significant positive correlations between expression of MMP-2 in invasive tumour and its expression in both in situ and stromal components. Significant positive correlations were found between expression of MMP-2 in tumour stromal cells and expression of MMP-2 in the in situ component and infiltrated resection margins. When budding was combined with MMP-2 expression in tumour cells; there were significant correlations with Oestrogen Receptor (ER) expression and MMP-2 expression in the in situ component and tumour stroma, and when combined with expression of MMP-2 in tumour stroma; there were significant correlations with expression of MMP-2 in invasive tumour and infiltrated resection margin.

Conclusion: Tumour budding might help in assigning subsequent treatment strategies in early breast cancer as in colorectal cancer. MMP-2 expression is seen in the in situ breast cancer and subsequent invasive components and the surrounding stroma, which points to its crucial role in tumour invasiveness and subsequent growth.

Keywords: Breast cancer, Extracellular matrix, Pan cytokeratin

INTRODUCTION

In Egypt, breast cancer is the most common malignancy among females [1]. Total number of breast cancers registered at the Pathology Department of National Cancer Institute of Egypt during the period 2000-2011 constituted 20% of total primary malignant tumours (ranked first); females with ductal carcinoma constituted the majority (83%) [2]. However, death rates from breast cancer in Egypt is rising (1.3% per year during 2000 to 2011), hence, we are in constant search for parameters for tumour behaviour and aggression and possible therapeutic targets to deal with that wide spread cancer in Egypt [3].

Tumour buds are clusters of undifferentiated malignant cells; single to less than or equal to five cells located in the invasive front border of a malignant tumour [4]. Tumour buds are linked to invasion and metastasis [5]. It is an independent aggressive prognostic parameter in operable colorectal cancer [6]. The down regulation of epithelial markers and up regulation of mesenchymal markers in tumour buds, suggests that tumour buds undergo partial Epithelia/Mesenchymal Transition (EMT), with a subset of tumours cells displaying a true hybrid epithelial/mesenchymal phenotype [5]. Regarding breast cancer, the studies done revealed that budding was correlated with larger tumour size, lymphovascular embolisation, lymph nodal metastasis and lower five year survival [7-9].

Matrix Metalloproteinases (MMPs) are proteolytic enzymes for breakdown of ECM and basement membranes [10]. MMP-2 and MMP-9 digest gelatins and denature collagens [11]. This proteolytic activity is inhibited by Tissue Inhibitors of Metalloproteinases (TIMPs) which can limit growth and metastasis of malignant tumours [12]. TIMP-2 is specific to MMP-2 and increased production/activation of MMPs revealed an important step for the invasiveness of the malignant tumours [13].

MMP-2 expression and MMP-2/MMP-9 co expression in association with other prognostic factors serve as a poor prognostic parameter in breast cancer [14]. Increased MMP-2 expression in seen in tumour stroma of breast cancers with aggressive behaviour, such as luminal HER2, HER2 enriched and triple negative tumours [15].

The aim of this study is evaluation of tumour budding and expression of MMP-2 in breast invasive ductal carcinoma and associated stroma, then correlate between them and other clinico-pathological features of patients to determine the possible role of tumour budding in local invasiveness and metastatic potential in breast cancer and any possible link between tumour budding and expression of MMP-2.

MATERIALS AND METHODS

This cross-sectional observational study was conducted on 61 cases of female breast invasive ductal carcinoma. Cases were obtained from the Department of Pathology, Faculty of Medicine, Cairo University, Giza, Egypt and a private laboratory during the period from June 2015 until September 2016. Tissue sections were obtained from specimens that included normal breast tissue at the tumour advancing front for assessment of budding. All cases were either mastectomy or conservative breast surgery specimens.

Data obtained from pathology sheets were: age of patients, size of the tumours, single tumour or multicentric, well or ill defined tumour

borders, presence of Paget's disease, lymph node status, status of surgical resection margins, available data regarding ER and Progesterone Receptor (PR) status (33 cases), Her-2/neu expression (32 cases), proliferation index for KI-67 (20 cases) and TN stage; based on breast cancer stage determination in the 7th edition of American Joint Committee on Cancer TMN system (AJCC).

Hematoxylin and Eosin (H and E) sections were prepared for histological evaluation of tumour grade, presence of necrosis, intraductal component, mitotic activity and lymphovascular embolisation. Scoring of the local inflammatory infiltrate was done according to Klintrup criteria [16]: Score (0) no inflammatory cells; (1) mild/patchy inflammatory cells; (2) band like inflammatory infiltration; (3) florid cup like inflammatory infiltrate at tumour edge.

Additional sections were mounted on charged slides for immunohistochemical staining for MMP-2 (polyclonal, rabbit IgG, dilution 1:200; Gene Tex) and pan cytokeratin (mouse antihuman cytokeratin (AE1/AE3), Dako) using a fully automated immunehistochemical system (DAKO/EnVision FLEX, high pH, (link) (code K8000). The positive control for MMP-2 was normal skin and for cytokeratin was normal breast epithelium. MMP-2 expression in normal skin was cytoplasmic in epidermal cells, hair follicles and upper dermal macrophages. Cytokeratin expression in breast epithelium was cytoplasmic.

Tumour budding was determined by counting foci of one to five cell clusters in ten high power microscopic fields (200X) at the invasive tumour border. Budding was considered either negative or positive by Salhia B et al., corresponding to <4 and ≥4 budding foci [8]. MMP-2 expression was evaluated in tumour cells (both invasive and in situ components) and in tumour stromal cells as conducted by Catteau X et al., shown in [Table/Fig-1] [15]. The cut-off value for positive expression in tumour stromal cells was for moderate or strong staining in ≥10% of cells.

MMP-2 intensity score	0 (absent)	1 (weak)	2 (moderate)	3 (strong)
MMP-2 extent score	0 (0-10%)	1 (11-25%)	2 (26-50%)	3 (>50%)
Total score (sum of intensity and extend)	0, 1, 2; negative expression		3,4,5,6 positive expression	

[Table/Fig-1]: Scoring of MMP-2 expression in breast ductal carcinoma tumour cells.

STATISTICAL ANALYSIS

Data were analysed using SPSS statistical package version 22.0. Numerical data were expressed as mean and Standard Deviation (SD), median and interquartile range or range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test was used to examine the relation between qualitative variables as appropriate. A p-value ≤0.05 was considered significant and all tests were two tailed.

RESULTS

This cross-sectional study was conducted on 61 female patients with breast invasive ductal carcinoma. Their ages ranged from 22 to 82 years with a mean±SD of 53.1±12.6 years. 30 (49.2%) out of 61 cases studied were >52 years and 31 (50.8%) cases were ≤52 years. The 52 (85.3%) out of 61 tumours were grade II and 36 (59%) out of 61 cases showed tumour size ≤3.5 cm while the rest were >3.5 cm. Regarding T stage, 37 out of 61 (60.7%) cases were T2 while 6 (9.8%), 11 (18%) and 7 (11.5%) cases were classified as T1, T3 and T4 respectively. 12 (19.7%) cases showed multicentric tumours and 48 (78.7%) cases showed ill defined tumour borders. Evidence of lymphovascular embolisation was documented in 35 (57.4%) out of 61 cases. Tumour necrosis was seen in 21 (34.4%) out of 61 cases while high mitotic rate {≥20/10 High Power Fields (HPFs)} was detected in 3 (4.9%) cases only .

Studying the inflammatory infiltrate according to Klintrup's criteria revealed prevalence of scores 1 and 2 in 24 (39.3%) and 22 (36.1%) cases respectively; while scores 0 and 3 were found in 11 (18%) and 4 (6.6%) cases only. An intraductal component was detected in 41 (67.2%) out of 61 cases and Paget's disease of the nipple was documented in only 4 (6.6%) cases. 42 (68.7%) cases were associated with metastatic axillary lymph nodes and only 10 (16.4%) out of 61 cases showed positive tumour infiltration to the resection margins.

Data regarding hormone receptor status of 33 cases available showed positive ER expression in 21 (63.6%) out of 33 and positive PR expression in 17 (51.5%) out of 33. An 8 (25%) out of 32 cases showed Her-2/neu overexpression (score 3+). Calculated mean for Ki-67 index in 20 cases was 22.5 with half the cases (50%) had labeling index ≤22.5 [Table/Fig-2,3].

Clinicopathological features		Tumour budding		Total	p-value
		Positive	Negative		
Age (years)	≤52	21 (67.7%)	10 (32.3%)	31 (100%)	0.127
	>52	26 (86.7%)	4 (13.3%)	30 (100%)	
Tumour grade	Grade I, II	41 (77.4%)	12 (22.6%)	53 (100%)	1
	Grade III	6 (75%)	2 (25%)	8 (100%)	
Tumour size (cm)	≤3.5	29 (80.6%)	7 (19.4%)	36 (100%)	0.540
	>3.5	18 (72%)	7 (18%)	25 (100%)	
Tumour stage (T)	T1	5 (83.3%)	1 (16.7%)	6 (100%)	0.634
	T2	30 (81.8%)	7 (18.9%)	37 (100%)	
	T3	7 (63.6%)	4 (36.4%)	11 (100%)	
	T4	5 (71.4%)	2 (28.6%)	7 (100%)	
Multicentricity	Multicentric	10 (83.3%)	2 (16.7%)	12 (100%)	0.715
	Single	37 (75.5%)	12 (24.5%)	49 (100%)	
Tumour border	Fairly defined	7 (53.8%)	6 (46.2%)	13 (100%)	0.035
	Ill defined	40 (83.3%)	8 (16.7%)	48 (100%)	
Lympho-vascular invasion	Positive	28 (80%)	7 (20%)	35 (100%)	0.553
	Negative	19 (73.1%)	7 (26.9%)	26 (100%)	
Necrosis	Positive	16 (76.2%)	5 (23.8%)	21 (100%)	1
	Negative	31 (77.5%)	9 (22.5%)	40 (100%)	
Mitosis	High	0 (0%)	3 (100%)	3 (100%)	0.010
	Low	47 (81%)	11 (19%)	58 (100%)	
Inflammation score	Score 0,1	27 (77.1%)	8 (22.9%)	35 (100%)	1
	Score 2, 3	20 (76.9%)	6 (23.1%)	26 (100%)	
Intraductal component	Positive	32 (78%)	9 (22%)	41 (100%)	1
	Negative	15 (75%)	5 (25%)	20 (100%)	
Nipple/Paget's disease	Positive	3 (100%)	1 (25%)	4 (100%)	1
	Negative	44 (77.2%)	13 (22.8)	57 (100%)	
Lymph node stage	N0	11 (57.9%)	8 (32.1%)	19 (100%)	0.169
	N1	15 (83.3%)	3 (16.7%)	18 (100%)	
	N2	9 (90%)	1 (10%)	10 (100%)	
	N3	12 (85.7%)	2 (14.3%)	14 (100%)	
Lymph node metastasis	Positive (N1, 2, 3)	36 (85.7%)	6 (14.3%)	42 (100%)	0.017
	Negative (N0)	11 (57.9%)	8 (42.1%)	19 (100%)	
Tumour resection margins	Free	39 (76.5%)	12 (23.5%)	51 (100%)	1
	Infiltrated	8 (80%)	2 (20%)	10 (100%)	
MMP-2 expression in intraductal component (n=41)	Positive	27 (77.1%)	8 (22.9%)	35 (100%)	1
	Negative	5 (83.3%)	1 (16.7%)	6 (100%)	
MMP-2 expression in invasive component	Positive	38 (79.2%)	10 (10.8%)	48 (100%)	0.450
	Negative	9 (69.2%)	4 (30.8%)	13 (100%)	

MMP-2 expression in tumour stromal component	Positive	18 (78.3%)	5 (21.7%)	23 (100%)	0.861
	Negative	29 (76.3%)	9 (23.7%)	38 (100%)	
ER (n=33)	Positive	17 (81%)	4 (19%)	21 (100%)	0.686
	Negative	9 (75%)	3 (25%)	12 (100%)	
PR (n=33)	Positive	13 (76.5%)	4 (23.5%)	17 (100%)	1
	Negative	13 (81.3%)	3 (18.7%)	16 (100%)	
Her-2 (n=32)	Positive	5 (62.5%)	3 (37.5%)	8 (100%)	0.327
	Negative	20 (83.5%)	4 (16.7%)	24 (100%)	
KI-67 (n=20)	≤22.5	9 (90%)	1 (10%)	10 (100%)	0.582
	>22.5	7 (70%)	3 (30%)	10 (100%)	

[Table/Fig-2]: Correlations between tumour budding and clinicopathological variables.
Significant p-value ≤0.05
ER: Oestrogen receptor; PR: Progestrone receptor

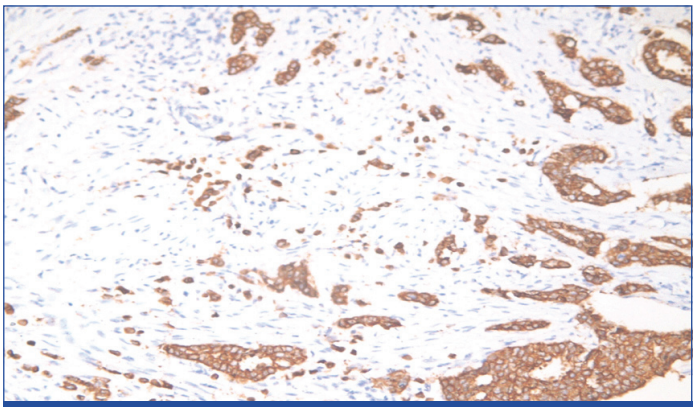
Clinico-pathological features		MMP-2 expression in invasive component		Total	p-value
		Positive	Negative		
Age (years)	≤52	22 (71%)	9 (29%)	31 (100%)	0.211
	>52	26 (86.7%)	4 (13.3%)	30 (100%)	
Tumour grade	Grade I, II	42 (79.2%)	11 (20.8%)	53 (100%)	1
	Grade III	6 (75%)	2 (25%)	8 (100%)	
Tumour size (cm)	≤3.5	27 (75%)	9 (25%)	36 (100%)	0.530
	>3.5	21 (84%)	4 (16%)	25 (100%)	
Tumour stage (T)	T1	6 (100%)	0 (0%)	6 (100%)	0.215
	T2	26 (70.3%)	11 (29.7%)	37 (100%)	
	T3	9 (81.8%)	2 (18.2%)	11 (100%)	
	T4	7 (100%)	2 (28.6%)	9 (100%)	
Multicentricity	Multicentric	10 (83.3%)	2 (16.7%)	12 (100%)	0.725
	Single	38 (77.6%)	11 (22.4%)	49 (100%)	
Tumour border	Fairly defined	9 (69.2%)	4 (30.8%)	13 (100%)	0.447
	Ill defined	39 (81.3%)	9 (18.8%)	48 (100%)	
Lympho-vascular invasion	Positive	27 (77.1%)	8 (22.9%)	35 (100%)	0.762
	Negative	21 (80.8%)	5 (19.2%)	26 (100%)	
Necrosis	Positive	16 (76.2%)	5 (23.8%)	21 (100%)	0.751
	Negative	32 (80%)	8 (20%)	40 (100%)	
Mitosis	High	3 (100%)	0 (0%)	3 (100%)	1
	Low	45 (77.6%)	13 (22.4%)	58 (100%)	
Inflammation score	Score 0, 1	25 (71.4%)	10 (28.7%)	35 (100%)	0.128
	Score 2, 3	23 (88.5%)	3 (11.5%)	26 (100%)	
Intraductal component	Positive	33 (80.5%)	8 (19.5%)	41 (100%)	0.741
	Negative	15 (75%)	5 (25%)	20 (100%)	
Nipple/ Paget's disease	Positive	4 (100%)	0 (0%)	4 (100%)	0.569
	Negative	44 (77.2%)	13 (22.8)	57 (100%)	
Lymph node stage	N0	14 (72.7%)	5 (26.3%)	19 (100%)	0.448
	N1	14 (77.8%)	4 (22.2%)	18 (100%)	
	N2	7 (70%)	3 (30%)	10 (100%)	
	N3	13 (92.9%)	1 (7.1%)	14 (100%)	
Lymph node metastasis	Positive (N1, 2, 3)	34 (81%)	8 (19%)	42 (100%)	0.521
	Negative (N0)	14 (73.7%)	5 (26.3)	19 (100%)	
Tumour resection margins	Free	39 (76.5%)	12 (23.5%)	51 (100%)	0.440
	Infiltrated	9 (90%)	1 (10%)	10 (100%)	
MMP-2 expression in intraductal component (n=41)	Positive	32 (91.4%)	3 (8.6%)	35 (100%)	<0.001
	Negative	1 (16.7%)	5 (83.3%)	6 (100%)	

MMP-2 expression in tumour stromal component	Positive	23 (100%)	0 (0%)	23 (100%)	0.002
	Negative	25 (65.8%)	13 (34.1%)	38 (100%)	
ER (n=33)	Positive	16 (76.2%)	5 (23.8%)	21 (100%)	0.249
	Negative	6 (50%)	6 (50%)	12 (100%)	
PR (n=33)	Positive	13 (76.5%)	4 (23.5%)	17 (100%)	0.282
	Negative	9 (56.3%)	7 (43.8%)	16 (100%)	
Her-2 (n=32)	Positive	4 (50%)	4 (50%)	8 (100%)	0.380
	Negative	18 (75%)	6 (25%)	24 (100%)	
KI-67 (n=20)	≤22.5	7 (70%)	3 (30%)	10 (100%)	0.650
	>22.5	5 (50%)	5 (50%)	10 (100%)	

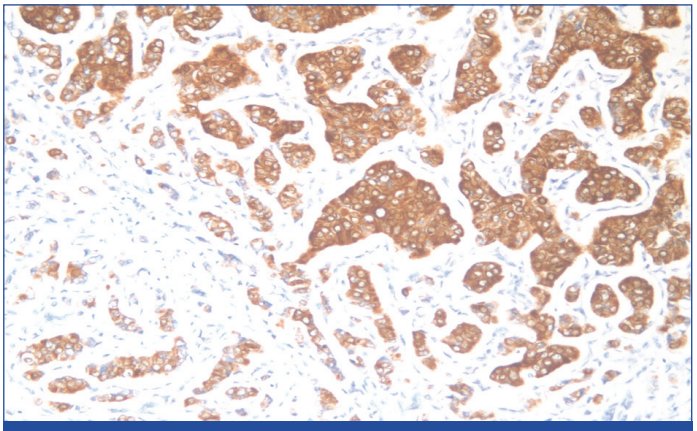
[Table/Fig-3]: Correlations of MMP-2 expression in invasive component and clinicopathological variables.
Significant p-value ≤0.05
ER: Oestrogen receptor; PR: Progestrone receptor

As regard tumour budding detection by cytokeratin immunostaining, 47 (77%) out of 61 of the cases were positive for budding [Table/Fig-2,4]. Immunohistochemical assessment of MMP-2 expression in invasive tumour revealed positive expression in 48 (78.7%) out of 61 cases [Table/Fig-3,5-7]. Positive MMP-2 expression was seen in the intraductal component of 35 out of 41 (85.4%) cases. MMP-2 expression in tumour stroma was positive in 23 out of 61 cases (37.7%) [Table/Fig-2,3,7].

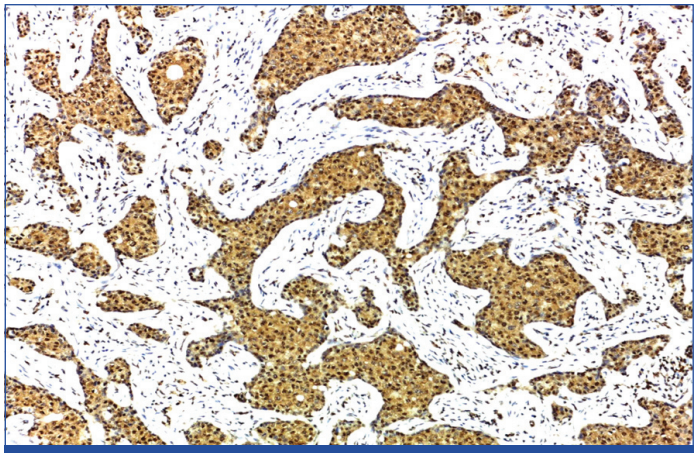
Statistical analysis using chi square test revealed statistically significant positive correlation between positive tumour budding and ill defined tumour borders, positive lymph node metastasis and low mitotic rate [Table/Fig-2]. There was statistically significant positive correlations between positive expression of MMP-2 in invasive tumour with positive expression of MMP-2 in both intraductal and stromal components with p-values <0.001 and 0.002, respectively [Table/Fig-3]. Statistically significant positive



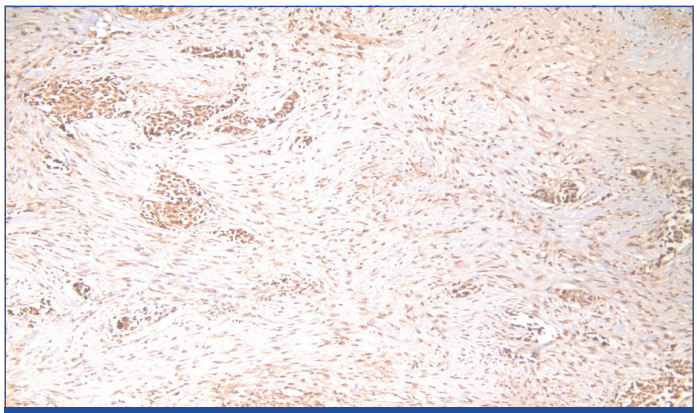
[Table/Fig-4]: Invasive duct carcinoma of the breast positive for tumour budding at the advancing tumour edge; detected by pan cytokeratin immunostaining (IHC 100X).



[Table/Fig-5]: Invasive duct carcinoma of the breast with positive MMP-2 expression, intensity score of 3 and a total score of 6 (IHC 200X).



[Table/Fig-6]: Invasive duct carcinoma of the breast with positive MMP-2 expression, intensity score of 2 and a total score of 5 (IHC 100X).



[Table/Fig-7]: Invasive duct carcinoma of the breast with positive MMP-2 expression in tumour cells, intensity score of 2 and a total score of 5 with concomitant positive stromal expression (IHC 100X).

correlations were found between positive expression of MMP-2 in tumour stromal component and positive expression of MMP-2 in intraductal component (p=0.023) and infiltrated resection margins (p=0.032).

Statistical analysis using chi square test revealed statistically significant positive correlations between concomitant positive expression of MMP-2 in tumour invasive component and positive tumour budding with positive expression of MMP-2 in both intraductal and stromal components, and ER status [Table/Fig-8]. Significant positive correlation was found between concomitant positive expression of MMP-2 in tumour stromal component and positive tumour budding with expression of MMP-2 in invasive component and infiltrated resection margin status [Table/Fig-9].

		MMP-2 expression in invasive component with tumour budding		Total	p-value
		Positive	Negative		
MMP-2 expression in Intraductal component		n=27	n=14		0.013
	Positive	26 (74.3%)	9 (25.7%)	35 (100%)	
	Negative	1 (16.7%)	5 (83.3%)	6 (100%)	
MMP-2 expression in tumour stromal component		n=38	n=23		0.045
	Positive	18 (78.3%)	5 (21.7%)	23 (100%)	
	Negative	20 (52.6%)	18 (47.4%)	38 (100%)	
ER		n=18	n=15		0.010
	Positive	15 (71.4%)	6 (28.6%)	21 (100%)	
	Negative	3 (25.0%)	9 (75.0%)	12 (100%)	

[Table/Fig-8]: Correlations between concomitant tumour budding and MMP-2 expression in tumour invasive component with MMP-2 expression in intraductal component, MMP-2 expression in tumour stroma and ER status.
Significant p-value ≤0.05
ER: Oestrogen receptor

		MMP-2 expression in stromal component with tumour budding		Total	p-value
		Positive	Negative		
Tumour resection margins		n=18	n=43		0.021
	Infiltrated	6 (60%)	4 (40%)	10 (100%)	
	Free	12 (23.5%)	39 (76.5%)	51 (100%)	
MMP-2 expression in invasive component		n=18	n=43		0.009
	Positive	18 (37.5%)	30 (62.5%)	48 (100%)	
	Negative	0 (0%)	13 (100%)	13 (100%)	

[Table/Fig-9]: Correlations between concomitant tumour budding and MMP-2 expression in tumour stromal component with MMP-2 expression in tumour invasive component and surgical resection margin status.
Significant p-value ≤0.05

DISCUSSION

The current study aimed to evaluate tumour budding and expression of MMP-2 (in both tumour cells and stromal components) in 61 resection specimens of female breast invasive ductal carcinoma and their correlations with each other and with other clinicopathological variables.

Tumour budding was observed in 47 (77%) out of 61 cases and showed statistical significant correlations with ill defined tumour borders (p=0.035), positive lymph node metastasis (p=0.017) and low mitotic rate (p=0.010). The correlations point to the possible role of budding phenomenon in cancer local spread and metastasis regardless of the proliferative rate of tumour cells. Gujam FJA et al., found results that point to the same role where high tumour budding (>20 tumour buds/five fields) was seen in 35% of their cases, with significant correlations with ER positive status (p=0.003), positive nodal metastasis (p=0.009), positive lymphatic vessel invasion (p=0.001) and high tumour/stroma ratio (p=0.001) and inverse correlation with local lymphocytic infiltration (p=0.002) [9]. Salhia B et al., proved significant correlations of high tumour budding in invasive ductal carcinoma with positive lymph node metastasis and lymphatic embolisation [8]. They studied ER positive and low proliferative cases and found significant correlation between lymph node metastasis and high tumour budding in ER positive cases (p=0.007) and in low proliferative cases (p=0.006). Liang F et al., proved near significant correlation between tumour budding and lymph node metastasis (p=0.050) and significant correlations with bigger tumour size (p=0.014) and lymphatic embolisation (p=0.001) [7]. Sriwidyani NP et al., found that tumour budding is an independent risk factor of metastatic disease in breast carcinoma [17]. The current study; unlike most others; didn't find significant relation with lymph vascular invasion or hormone receptor status that might be explained by inadequacy of available data regarding hormone status for all of present studied cases and the method of detecting vascular embolisation (H and E stained sections).

Immunohistochemical assessment of MMP-2 in invasive tumour revealed positive expression in 48 (78.7%) out of 61 cases. Positive MMP-2 expression was seen in the intraductal component of 35 (85.4%) out of 41 cases associated with intraductal carcinoma and in tumour stromal component of 23 (37.7%) out of 61 cases. Significant statistical correlations were found between positive MMP-2 expression in tumour invasive component with positive expression in the intraductal and stromal components. These correlations points to the important role of MMP-2 in progression of in situ cancer into invasive one, local cancer growth and the participation of stromal cells in this mechanism.

These results are in agreement with Nakopoulou L et al., who reported MMP-2 expression in 75.6% of tumours with significant positive correlation with tumour size >2 cm (p=0.022) reflecting the relation with cancer growth [13]. Sullu Y et al., had reported higher percent of tumoural MMP-2 expression; 93%, with strong expression in 75% of cases, it showed significant correlation only with high

grade tumours in ER negative and lymph node metastasis negative groups [18]. Min KW et al., and Ramos EA et al., evaluated 177 and 44 cases and reported lower positivity for expression of MMP-2 in breast cancer; 54.8% and 68% respectively [19,20]. Ramos EA et al., reported significant correlations with positive PR (p=0.0071), positive ER (p=0.0015), nodal metastasis (p=0.0082) and mortality (p=0.0082) [20], while Min KW et al., reported correlation only with positive Her-2 status (p=0.009) [19]. Vizoso FJ et al., documented MMP-2 expression in only 32.8% of tumours with no significant correlations at all [21].

As regard MMP-2 expression in tumour stromal component, significant correlations were documented only with MMP-2 expression in the in situ component and infiltrated resection margins. Positive stromal expression was higher than that reported by Nakopoulou L et al., 27.4%, who found significant correlation with TIMP-2, Ki-67 index and high p53 expressions [13]. Also, Vizoso FJ et al., reported low percentage of positive tumour stromal expression; 23.7%, with no significant correlations [21].

Correlating cases exhibiting concomitant positive tumour budding and MMP-2 expression in invasive tumour with other variables revealed positive significant correlations with MMP-2 expression in in situ ductal component (p=0.010), MMP-2 expression in stromal component (p=0.045) and positive ER status (p=0.010). Correlating cases exhibiting concomitant positive tumour budding and MMP-2 expression in tumour stromal component with other variables revealed highly significant correlations with MMP-2 expression in invasive component (p=0.009) and infiltrated resection margins status (p=0.021). This means that MMP-2 might play an important role in tumour budding, and both budding with MMP-2 expression might be modulated by tumour hormonal profile. No comparable studies emphasised these points before.

In conclusion, we emphasise that tumour budding in breast carcinoma is a parameter of aggressive tumour behaviour, growth and distant spread, thus can predict poor outcome of patients. It is under control of multiple factors; including possible hormonal factors and gene expressions, not only ECM proteolytic factors. Tumour budding might be of help in assigning subsequent treatment strategies in early breast cancer, as its presence is an indication of aggressive behaviour and its absence can help to avoid unnecessary aggressive interventions, the same as applied now in early colonic carcinoma. MMP-2 expression is seen in the in situ breast cancer and subsequent invasive components and surrounding stroma, which points to its crucial role in tumour invasiveness and subsequent growth. Thus, blocking its action might be useful in limiting invasion and growth.

LIMITATION

Further extensive studies with larger sample sizes, other histopathological subtypes of breast cancer included and follow-up for tumour progression and survival rates of patient's with positive tumour budding and MMP-2 expression could be more informative about their roles in tumour behaviour.

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FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Nov 25, 2017**
Date of Peer Review: **Jan 16, 2018**
Date of Acceptance: **Mar 17, 2018**
Date of Publishing: **May 01, 2018**