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Original Research Article

Relationship of Mast Cell Count with Hormonal Receptor Status in Breast Carcinoma: A Descriptive Study in A Tertiary Care Hospital In India

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Abstract

The incidence of breast cancer is increasing throughout the world as well as in India. Many risk factors and prognostic factors of breast cancer have been identified but the associations between mast cell infiltration and the clinical features of breast cancer remain unclear. The aim of the study was to estimate the mast cells number in malignant tumors and axillary lymph node and to find out any correlation between the number of mast cells and hormone receptor status. An institution based descriptive study was done in a tertiary care hospital. Total 44 specimens of post operative breast tissue with malignant breast lesion & dissected axillary lymph nodes were taken. Counting of mast cells was done in Toluidine blue stain. Immunohistochemical staining was done for ER, PR & HER2/neu using immunostaining kit. It was observed that when the tumor grade increases the number of must cell decreased per high power field. The mast cell count was significantly higher in cases of ER positive and PR positive breast cancers than that of the ER negative and PR negative respectively but there was no significant relation between mast cell count of HER2/neu. Mean mast cell count of triple negative cases was significantly low. This study indicated that the number of mast cells in breast cancer was inversely correlated with the grade of this tumor and higher mast cell count was related to good prognosis.

Keywords: Breast carcinoma, mast cell, estrogen receptor, progesterone receptor.

INTRODUCTION

Breast cancer is the most common type of malignancy diagnosed in women in developed countries and the second most common type diagnosed in developing countries. Breast cancer has been described as an alarmingly health problem in India [1]. Over the years, the incidences of breast cancer in India have steadily increased and as many as 100,000 new patients are being detected every year [2]. Despite the accumulation of data on the cellular and genetic properties of breast tumors, their heterogeneous structure and variable clinical behavior are not yet well understood [3]. Recent studies have focused on the modulating effects of cellular and extracellular matrix components that exist in the tumor microenvironment (TME) on tumor progression [4]. Eosinophils and mast cells are the inflammatory cells found in the stroma of malignant tissue. Mast cells, derived from the specific bone marrow progenitor cell and migrate to the tissue where they mature depending on the micro environmental condition. These cells have metachromatic granules containing heparin & histamine. Toluidine blue is one of the important metachromatic stains which stain the granules of the mast cells. Metachromasia depends on the dye and tissue components that unite with the dye to exhibit metachromasia. Heparin in mast cell granules is an acid polysaccharide, which permits the polymerization of basic dye molecule in Toluidine blue. Mast cells could facilitate the tumor angiogenesis through heparin like molecule and heparin could permit neovascularisation and metastasis through anticlotting effect [5]. Moreover mast cells secrete histamine and growth factors, such as VEGF, [vascular endothelial growth factor], PDGF [plate derived growth factor], SCF [stem cell factor],

and NGF [nerve growth factor].These cells are rich in metalloprotease which are necessary for tumor invasiveness [6].

Hormone receptor status [Estrogen receptor (ER) & Progesterone receptor (PR)] is the strongest prognostic factor in breast carcinoma and intensively studied as it guides to endocrine modulation therapy. Estrogen plays a central role in growth and differentiation of normal breast epithelium, stimulating cell proliferation and regulating the expression of gene, including the progesterone receptor. Tumor that express both ER, PR have the greatest benefit from hormonal therapy, but those containing only ER or only PR still have significant response [7]. Tumor with neither ER nor PR has a less than 10% likely hood of responding to hormone [8]. HER2/neu is a transmembrane glycoprotein with tyrosin kinase activity which belongs to the family of epidermal growth factor receptor (EGF-R). It's over expression can be measured by immunohistochemistry & found in 20-30% of breast carcinoma. Over expression of HER2/neu is associated with poor prognosis, but its evaluation is important as it is a very good predictor of response to Herceptin –a humanized monoclonal antibody to HER2/neu which specifically targets tumor cells. Even though, these markers, including the Luminal system, have limited contribution to clinical practice, more and more works are needed to improve current methods of diagnosis, treatment and prognosis prediction of breast cancer.

The prognostic significance of amount of stromal connective tissue is controversial. Few studies in stromal mast cells in the invasive breast carcinoma have been done and previous studies have indicated that many stromal mast cells are correlated to a favorable prognosis [9-11]. In some studies mast cell count is found to be correlated to the positivity of ER/PR. Dual role of mast cell in inhibiting or promoting tumor growth needs further investigation [12].

In this background the aim of the study was to estimate the mast cells number in malignant tumors and axillary lymph nodes and find out any correlation between the number of mast cells and hormone receptor status.

MATERIAL AND METHODS

The study was done at department of Pathology, Bankura Sammilani Medical College in collaboration with the Department of Surgery from Feb 2014 to Jan 2015. It was an institution based descriptive study with cross sectional design. Ethical clearance was obtained from institutional ethical committee. Study population was adult female having history of breast lump with or without enlarged axillary lymph nodes. Sample size was complete enumeration of patients attending hospital during study period. Total forty four specimens of post operative breast tissue & enlarged axillary lymph nodes of suspected breast malignancy was available for histopathological examination.

Sample was collected from the specimens sent for histological examination in the Pathology Department. Axillary tissues were separated from the breast if attached to it. External appearance was noted and the specimen was palpated for any mass or nodularity. With a marker a vertical line was drawn through the nipple and another line perpendicular to it ,also passing through the nipple .Thus the breast was divided in 4 quadrants –upper outer ,lower outer, upper inner , lower inner. Nipple and areola were removed. The entire breast was sliced in 2cm intervals longitudinally. Nipple, areola and all the slices were kept overnight for fixation. During the grossing of the first day the points were noted: (a) side of mastectomy (b) list of structures included in the specimen -skin, nipple, areola, breast, axillary tissue etc. (c) weight & dimensions of the specimen (d) external appearance, size, site, consistency and margin of the tumor and (e) presence of necrosis. Three sections from breast mass & one section each from four quadrants were taken. A section from from nipple & areola was taken in all cases. In lymph nodes sections, small nodes were mounted in full and nodes >0.5cm were sectioned and one section was given from each identified nodes. If axillary fat was grossly involved, a section from representative area was taken.

Tissue processing done in automated tissue processor and staining of sections done with hematoxylin & eosin (H &E) stain and Toluidine blue stain. Diagnosis and classification of the lesions to be done by H &E stain. Microscopic grading of malignant breast lesions according to Nottingham Modification of Bloom-Richardson System in H& E stained slides, under light microscope. Counting of mast cells was done in Toluidine blue stained slides. Mast cells in six high power fields were counted and the average was taken. The mast cells were counted in the areas where they were more in number.

Immunohistochemical staining was done for ER, PR & HER2/neu using immunostaining kit. Protocol for IHC was for polymer detection system. Two parameters evaluated in immunohistochemical preparation of hormone receptors are the number of tumor cell nuclei stained and the intensity of reaction. Interpretation of IHC staining was done by using 'Allred' scoring system.

After getting all the data, it was analyzed by SPSS-10 – by applying Paired Samples T test and correlation study

RESULTS AND DISCUSSION

The study was conducted on 44 patients admitted in Bankura Sammilani Medical College & Hospital. Age range of the patients was between 18-75

years with mean age of 45.32 years (SD 11.06 years). On clinical examination, over lying skin fixed in 65.9%, nipple retracted in 63.6%, palpable lymph nodes were found in 61.4% of cases and lump size > 5cm in 52.3% of cases (Table-1).

According to histological types, infiltrating ductal carcinoma – no special type (IDC-NST) (Figure-1) was diagnosed in 77.3% cases, mucinous carcinoma 11.4%, medullary carcinoma 4.4%, invasive lobular carcinoma (Figure-2) 2.3%, metaplastic carcinoma 2.3% and papillary Carcinoma 2.3%. According to Modified Bloom Rechardson histological grading, grade I tumor was 6.8%, grade II found in 38.6% and grade III was 54.6% of the tumors.

Among 44 patients palpable lymph nodes were found in 27 cases and out of them 70.37% were metastatic and 29.63% were reactive.

Table-1: Distribution of study population according to some variable of breast lump (n=44)

Variables		Frequency	Percentage
Over lying skin	Fixed	29	65.9
	Free	15	34.1
Nipple	Retracted	28	63.6
	Not retracted	16	33.4
Lymph node	Palpable	27	61.4
	Not palpable	17	38.6
Size of breast lump	< 2cm	3	6.8
	2-5 cm	18	40.9
	>5 cm	23	52.3

Mean mast cell count in breast lump was 5.76/ high power field (HPF) (SD 3.51) and median 5.25 /HPF. 27 clinically palpable lymph nodes were present and mean mast cell count in lymph node was 11.20/ HPF (SD 12.07). Mean mast cell count in breast lump of grade I was 13.03/ HPF (SD 4.5), in grade II 6.3/ HPF (SD 3.5) and in grade III 4.47/ HPF (SD 1.9). One way analysis of variance shows there was significant difference between three groups. With the increasing tumor grade the number of must cells was decreased per high power field but there was no significant association between tumor grade and mean must cell count/ HPF in lymph node.

Median value of mast cell count in breast lump in case of palpable lymph node was 5.00 /HPF and non palpable lymph node was 5.40 /HPF. Mann-Whitney U test showed that there was no significant association between these two groups.

Table-2: Distribution of study population according to hormone receptors status and mast cell count/hpf (n=44)

Hormone receptors	Mean and SD of mast cell count/hpf	P value
ER positive (n=17)	8.87 ± 3.67	0.001
ER negative (n=27)	3.82 ± 1.35	
PR positive (n=21)	7.30 ± 4.11	0.006
PR negative (n=23)	4.37 ± 2.1	
HER2/neu positive (n=19)	5.22 ± 3.02	0.365
HER2/neu negative (n=25)	6.20 ± 3.84	
Both ER & PR positive (n=13)	9.00 ± 4.34	0.000
Both ER & PR negative (n=19)	3.56 ± 1.30	
ER & PR negative & HER2/neu positive (n=9)	3.66± 1.28	0.86
ER & PR negative & HER2/neu negative (n=10)	3.48± 1.38	

The median value of mast cell count in breast lump with metastasis was 4.5/HPF, and in case of reactive lymph nodes it was 5.5/HPF. Mann-Whitney U test showed that there was no significant association between these two groups.

The median value of mast cell count in lymph node with metastasis was 5.1/HPF, and in case of reactive lymph nodes it was 19.15/HPF. Mann-Whitney U test found that there was significant association between these two groups.

Histological types for good prognosis were infiltrating ductal carcinoma, Mucinous carcinoma, invasive lobular carcinoma and Papillary Carcinoma. Medullary carcnoma and Metastatic carcinoma had poor prognosis. Mean of m**ast** cell in breast lump in case of bad prognosis type (4.06/HPF) was less than mean of good prognosis type (6.03/HPF) and this difference was statistically significant.

Among 44 cases of breast carcinoma, 17 cases were ER (Estrogen receptor) positive, 21 were PR (Progesterone receptor) positive, 19 cases were HER2/neu positive and 10 cases were triple negative.

ER & PR, both positive found in 13 cases and both ER & PR were negative in 19 cases.

The mean mast cell count in ER positive cases was 8.87/HPF and in ER negative cases was 3.82/HPF (Table-2). The mast cell count was significantly higher in cases of ER positive and PR positive breast cancers than that of the ER negative and PR negative respectively but there was no significant relation between mast cell count of HER2/neu positive and HER2/neu negative cases. Mean mast cell count of triple negative cases was significantly low (p=0.026) than that of the others which were not triple negative (Table-2). Mean mast cell count in case of both ER & PR positive patients were significantly higher than that of both ER & PR negative. Among 19 both ER & PR negative patients, 9 were HER2/neu positive and 10 were HER2/neu negative (triple negative) but no significant relation was found between two groups.

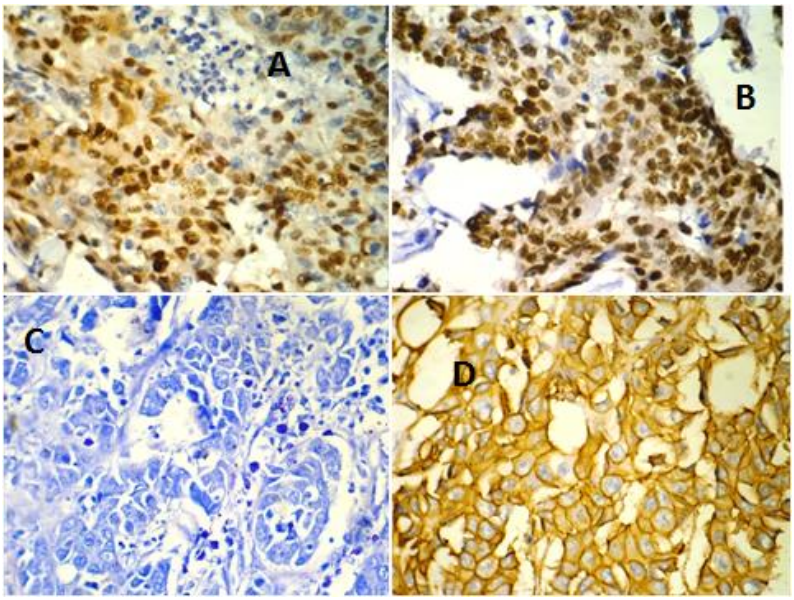

Fig-1: A) Progesterone receptor (PR) expression (Score 7) in Invasive ductal carcinoma (IHC, high power view). B) Estrogen receptor (ER) expression (Score 8) in Invasive ductal carcinoma (IHC stain, High power view). C) Mast cell presence in Invasive ductal Carcinoma (Toluidine blue stain, high power view). D) HER2/neu 3+ (positive membrane stain) expression in IDC (IHC stain, High power view)

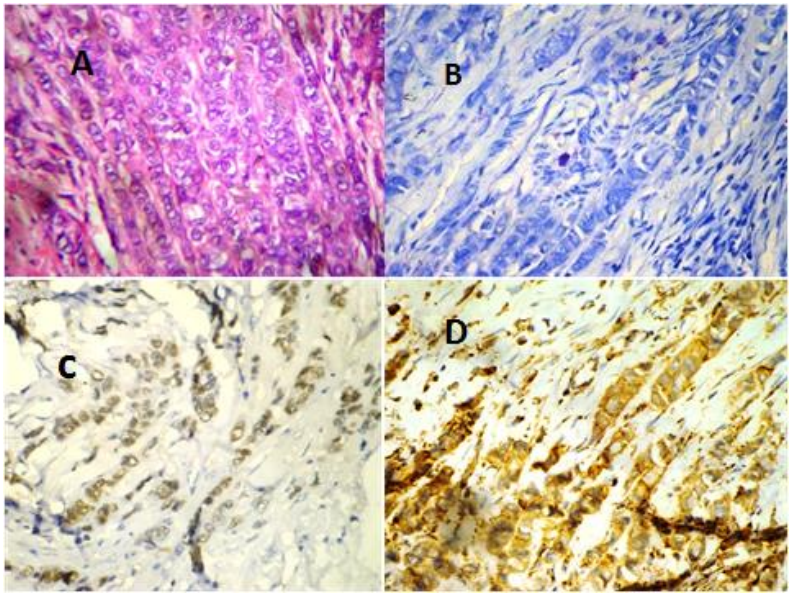

Fig-2: A) Histology showing infiltrating lobular carcinoma in Indian file pattern (H&E stain, high power view). B) Section show mast cells within the stroma in invasive lobular carcinoma (Toluidine blue stain, high power view). C) Estrogen receptor (ER) expression (Score 4 nuclear stain) in Invasive lobular carcinoma (IHC stain, High power view). D) Section shows HER2/neu (negative membrane stain) not expressed in invasive lobular carcinoma (IHC stain, High power view)

DISCUSSION

The occurrence, development and prognosis of breast cancer are related to gender, age, tumor size, histological type and grade, axillary lymph node metastasis, female hormone receptors, HER-2 expression. It is important to identify new markers for this subgroup of patients who might then not need adjuvant chemotherapy. Few studies of stromal mast cells in invasive breast carcinomas have been done and two studies have indicated that many stromal mast cells are correlated to a favorable prognosis [13].

Age distribution of the patients in this study was between 18-75yrs with a mean age of 45.3 yrs similar to the other previous studies by Siddique MS in 2000 (48 yrs) in Pakistan [14] and by Sunita Saxena *et al*., in 2005 (45- 54yrs). Indian females present with breast cancer at an earlier age than Western patients.

In this study mean mast cell count in breast lump in grade I was highest than grade II and grade III. When the tumor grade was increasing the number of must cell decreasing per high power field. Bowers HM *et al.*, described that a higher number of mast cells were found in the non-involved axillary lymph nodes in those women with a better prognosis. In 2008, Mitra Heidarpour *et al.*, done a study on 108 cases with invasive breast cancer where grading was done according to the Nottingham Modification of the Bloom-Richardson system. Fifty-four (50%) women had grade 1, 16 (14.8%) had grade 2 and 38 (35.2%) had grade 3 tumor [15]. The presence of stromal mast cells correlated significantly to low grade tumors.

The median value of mast cell count in lymph node with metastasis was lower than reactive lymph nodes in this study. In women with axillary lymph node metastasis, more mast cells were found in the non-involved axillary lymph nodes the similar result also found by Naik *et al*., Horny HP *et al*., [16-18].

In this study counting of mast cells in peritumoral fibrous tissue was done. Sumoszuk M and Della Rovere F also counted mast cells in peritumoral area [5, 19]. In this study histological types for good prognosis (IDC.NST, Mucinous carcinoma, lobular carcinoma & Papillary Carcinoma) had lower mean must cell count than bad prognosis types (Medullary carcinoma & Metastatic carcinoma). In a study by Gui Young Known *et al*., in 2005, found that there was a statistically significant relation between ER & PR positive cases and higher mast cell infiltration in peritumoral area [19]. According to Dell Rovere F *et al*., mast cell count was significantly higher in case of ER & PR positive [20]. Mitra Heiderpour found in their study that presence of stromal mast cells correlated significantly to ER positivity [15]. In this study it was found that higher mast cell count significantly related with ER & PR positivity but there was no significant relation between HER2/neu and mast cell count. Among 44 cases 10 cases were triple negative means ER, PR, HER2/neu negative. Their average mast cell count was 3.48/hpf. It was found that mast cell count of triple negative breast cancer cases was not significantly related to both ER and PR negative.

CONCLUSION

In this study it was found that higher mast cell count significantly related with ER & PR positivity but there was no significant relation between HER2/neu and mast cell count estrogen receptor, progestogen receptor. Recently the triple negative cancers are taken as of bad prognosis, where the mast cell count was significantly low.

So it can be said that higher mast cell count is well related with good prognosis of breast cancers and in rural setup where these type of immune staining is not available the mast cell count by to Toludine blue stain can be used as a screening procedure to determine the prognosis of breast cancers and their sensitivity to hormonal therapy.

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