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AXILLARY LYMPH NODE STATUS IN PRIMARY BREAST CARCINOMA;

ASSOCIATION OF AXILLARY LYMPH NODE STATUS WITH ER/PR, HER2/NEU AND MMP-I IN PRIMARY BREAST CARCINOMA

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ABSTRACT…Objectives: The most important prognostic factor in patients of breast carcinoma is axillary lymph node metastasis. Current study was conducted to find the frequency of lymph node metastasis in hundred cases of primary breast carcinoma and association of lymph node status with immunohistochemical expression of ER/PR,HER2/neu and MMP-1(matrix metalloproteinase-1).**Design:** Descriptive study. **Period:** Aug 2012 to Jun 2013. **Setting:** U.H.S laboratory of Morbid Anatomy and Histopathology Lahore. **Materials and methods:**One hundred mastectomy specimens with axillary lymph node dissection were included. After gross examination, tissue processing and microtomy the tissue slices of 4 micrometer were taken on frosted and lysine coated slides. H/E and IHC for ER/PR,HER2/neu and MMP-1 were done according to protocol. **Results:** Among 100 breast cancer subjects, 72 were positive for lymph node metastasis while 28 subjects were negative. A significant association between lymph node status and ER IHC expression was noticed. When Chi square test was applied with p-value of 0.001 was observed.Also a significant association between lymph node status and PR IHC expression was noticed. Chi square test was applied and the p-value of 0.004 was obtained.Association between lymph node status and HER2/neu IHC expression was analysed. Chi square test was applied with a p-value of 0.467 was obtained (not significant).A significant association between lymph node status and MMP-1 IHC expression was observed. Chi square test was applied anda p-value of 0.004 was obtained. **Conclusions:** Most of the primary breast carcinomas were presented with axillary lymph node metastasis (72%).Significant associations were observed between lymph node status and ER/PR immuonohistochemical expressions however the association between HER2/neu IHC and lymph node status was not statistically significant. The immunohistochemical expression of MMP-1 is significantly associated with lymph node status. It shows that it is an important marker for metastatic potential in breast carcinoma.

Key words: Lymph node status, immunohistochemistry, ER/PR,HER2/neu, MMP-1

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INTRODUCTION

Breast carcinoma is the most frequent malignancy in women and therefore the most important cause of cancer-related death worldwide, accounting for 23% (1.38 million) of all new cancer cases and 14% (458, 400) of all deaths due to cancer in 2008.[1] Pakistan has one of the highest incidence rates in Asia. Unlike the West epidemiology, the disease tends to occur at a younger age, has larger tumour size, and shows a higher frequency of metastases to regional lymph nodes.[2]

The most important prognostic factor in patients of breast carcinoma is axillary lymph node metastasis.[3] Nodal metastasis is not only a marker of diagnosis at a later point in the natural history of breast cancer but also a marker of an aggressive phenotype[4]It is reported that ER/PR and HER2/neu have an independent prognostic value.[3] Other studies also report that MMP-1 (matrix metalloproteinase-1) also has an independent prognostic value.[5] Association of lymph node status with MMP-1 immunohistochemical expression along with that of ER/PR and HER2/neu has not been described yet.

Current study was conducted to find the frequency of lymph node metastasis in hundred cases of

primary breast carcinoma and association of lymph node status with immunohistochemical expression of ER/PR, HER2/neu and MMP-1(matrix metalloproteinase-1).

MATERIALS AND METHODS

This descriptive study was conducted during August2012 to June 2013 in U.H.S laboratory of Morbid Anatomy and Histopathology Lahore. One hundred mastectomy specimens with axillary lymph node dissection 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 was cut by a Leica Rotary Microtome, taken on a frosted slide while 4 sections of 4 micrometer 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. Microscopy was done. 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 Bostromet al[5] and Baker et al.[6] MMP-1 IHC positive cells exhibited brown cytoplasmic staining. The data was analysed by using SPSS 20. All the results were recorded in proforma.

RESULTS

A total of 100 mastectomy specimens of primary breast carcinoma were included in the study. These cases were analyzed as regards to lymph node status, ER, PR, HER2 and MMP-1 immunohistochemical score. 72 were positive for lymph node metastasis while 28 subjects were negative.Among the positive lymph nodes, 58(80.6%) were ER negative and 14(19.4%) were ER positive. Among the 28 lymph nodes that were negative for metastasis, 13(46.4%) were ER negative and 15(53.6%) were ER positive. A significant association between lymph node status and ER IHC expression was noticed. When Chi square test was applied, the p-value of 0.001 was observed.

Among 72 cases which were positive for lymph node metastasis, 57(79.2%) were PR negative and 15(20.8%) were PR positive. Among 28 cases that were negative for lymph node metastasis, 14(50.0%) were PR negative and 14(50.0%) were PR positive. A significant association between lymph node status and PR IHC expression was noticed. Chi square test was applied and a p-value of 0.004 was obtained.

Among 72 cases which were positive for lymph node metastasis, 51(70.8%) were HER2/neu negative and 9(12.5%) were HER2/neu score 2+ while 12(16.7%) were HER2/neu score 3+. Among 28 cases that were negative for lymph node metastasis, 22(78.6%) were HER2/neu negative and 4(14.3%) were HER2 score 2+ while 2(7.1%) were HER2/neu score 3+. An association between lymph node status and HER2/neu IHC expression was analysed. Chi square test was applied and a p-value of 0.467 was obtained that was not statistically significant.

Among 72 positive cases for lymph node metastasis, 10(13.9%) were of MMP-1 score 1, 11(15.3%) were of score 2, 24(33.3%) cases were of score 3 and 27(37.5%) were MMP-1 score 4. Among 28 cases that were negative for lymph node metastasis, 3(10.7%) were MMP-1 score1, 13(46.4%) were score 2 while 9(32.1%) were MMP-1 score 3 and 3(10.7%) cases were of score 4. A significant association between lymph node status and MMP-1 IHC expression was observed.

Chi square test was applied and a p-value of 0.004 was obtained.

Lymph node Status	Frequency	Percentage
Negative	28	28.0
Positive	72	72.0
Total	**100**	**100.0**

Table-I. Distribution of cases by lymph node status

Lymph Node Status	ER Score		Total
	Negative	Positive	
Negative	13 (46.4%)	15 (53.6%)	28
Positive	58 (80.6%)	14 (19.4%)	72
Total	**71**	**29**	**100**

Table-II. Association between immunohistochemical expression of ER and lymph node status

Lymph Node Status	PR Score		Total
	Negative	Positive	
Negative	14 (50.0%)	14 (50.0%)	28
Positive	57 (79.2%)	15 (20.8%)	72
Total	**71**	**29**	**100**

Table-III. Association between immunohistochemical expression of PR and lymph node status

Lymph Node Status	HER2/ neu Score			Total
	Negative	Positive	Strong Positive	
Negative	22 (78.6%)	4 (14.3%)	2 (7.1%)	28
Positive	51(70.8%)	9 (12.5%)	12 (16.7%)	72
Total	**73**	**13**	**14**	**100**

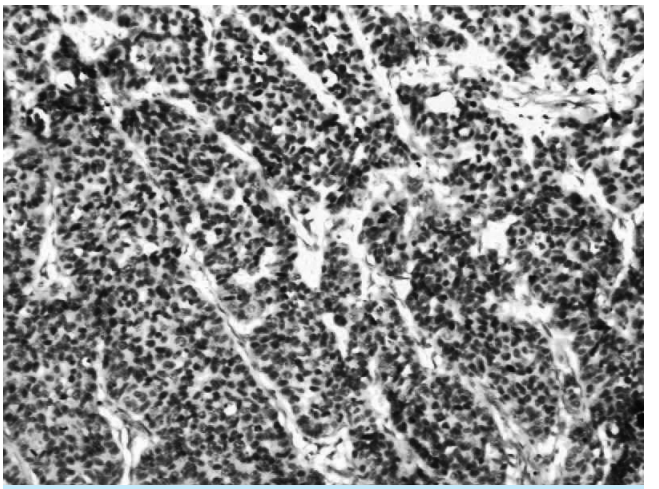
Table-IV. Association between immunohistochemical expression of HER2/neu and lymph node status

Lymph Node Status	MMP 1 Score				Total
	Score 1	Score 2	Score 3	Score 4	
Negative	3 (10.7%)	13 (46.4%)	9 (32.1%)	3 (10.7%)	28
Positive	10 (13.9%)	11 (15.3%)	24 (33.3%)	27 (37.5%)	72
Total	**13**	**24**	**33**	**30**	**100**

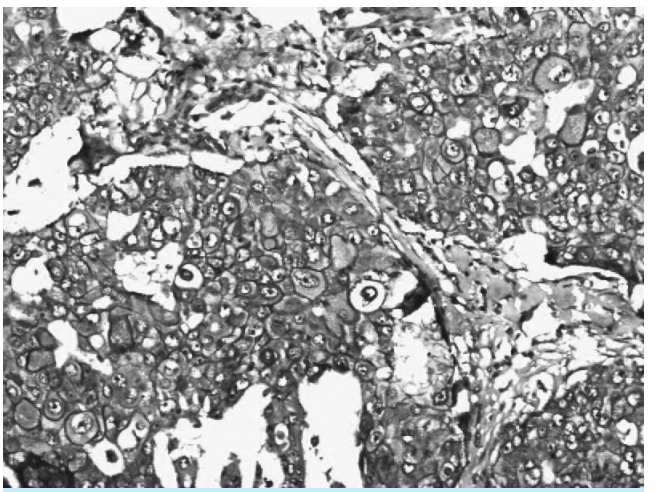
Table-V. Association between immunohistochemical expression of MMP-1 and lymph node status

DISCUSSIONS

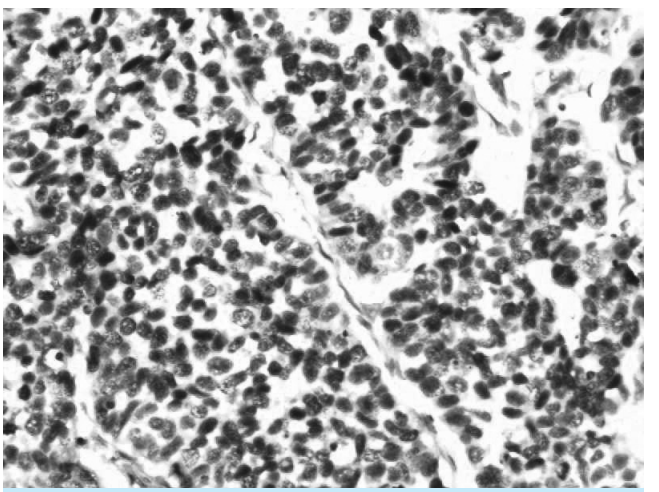
Breast cancer prognosis is related to a variety of clinical and pathological factors. Axillary lymph node involvement is believed to be an important factor for predicting prognosis.



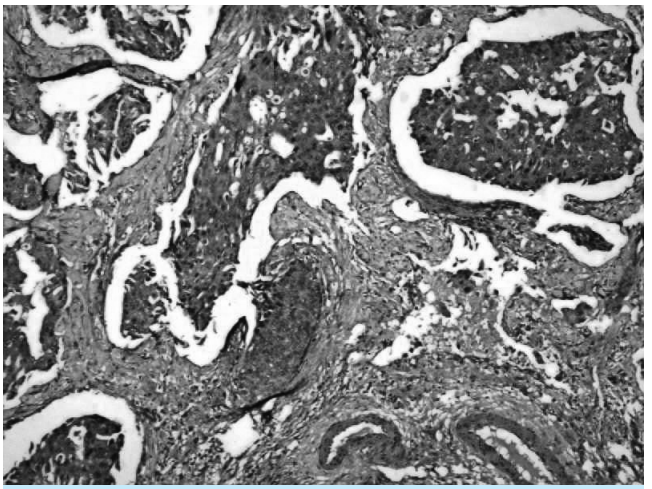
IHC staining(ER= 3+) in a case of invasive ductal carcinoma breast (GradeII)20x



IHC staining (HER2/neu= 3+) in acase of invasive ductal carcinoma of breast(GradeIII)40x



IHC staining (PR= 3+) in a case of invasive ductal carcinoma breast (GradeII)40x



IHC staining (MMP-1 strong positivity) in a case of invasive ductal carcinoma breast(Grade III) 20x

Both the hormone receptors and MMP-1 are reported to be independent prognostic factors for breast carcinoma.[3,5] Also we know that axillary lymph node status is one of the best independent prognostic factors for disease free survival and overall survival of breast cancer.[3] Our study highlights the significant association of immunohistochemical expression of hormone receptors and matrix metalloproteinase-1 with axillary lymph node status.

In our study, among 100 breast cancer subjects, 72 were positive for lymph node metastasis while 28 subjects were negative. In a study carried out by Mona et al. (2005)[7], all the 50 cases (100%) were presented with lymph node metastasis. While Moses Ambroise and colleagues[8] showed 58.19% lymph node involvement. Joensuu et al. (2013)[9] reported in their study that out of 72 cases of breast cancer, 38 were positive for lymph node involvement.Study conducted in Yemen by Ahmad et al.(2011)[10] showed that 75.3 % cases of breast cancer were positive for lymph nodes.

We found that among the positive lymph nodes, 58(80.6%) were ER negative and 14(19.4%) were ER positive. Among the 28 lymph nodes that were negative for metastasis, 13(46.4%) were ER negative and 15(53.6%) were ER positive. A significant association between lymph node status and ER IHC expression was noticed. Among 72 cases which were positive for lymph node metastasis, 57(79.2%) were PR negative and 15(20.8%) were PR positive. Among 28 cases that were negative for lymph node metastasis, 14(50.0%) were PR negative and 14(50.0%) were PR positive. A significant association between lymph node status and PR IHC expression was noticed.

In a recent study conducted by Elsayed et al[3] in Egypt in 2014, the expression status of ERand PR showed inverse significant association with Axillary lymph node metastasis. Those tumours which were ER+/PR+ were the least likely to have metastatic deposits. Over-expression of HER2 was significantly associated with presence of lymph node metastasis.

In the present study we found that among 72 cases which were positive for lymph node metastasis, 51(70.8%) were HER2/neu negative and 9(12.5%) were HER2/neu score 2+ while 12(16.7%) were HER2/neu score 3+. Among 28 cases that were negative for lymph node metastasis, 22(78.6%) were HER2/neunegative and 4(14.3%) were HER2 score 2+ while 2(7.1%) were HER2/neu score 3+. An association between lymph node status and HER2/neu IHC expression was analysed. Chi square test was applied with a p-value of 0.467 was obtained(not significant).

Almasri and Al Hamad, 2005[11] showed no statistically significant correlation between HER2 over expression and axillary lymph node status. AlsoPrati et al, 2005[12] also did not show significant association between HER2 over expression and axillary lymph node status.

CONCLUSIONS

Analysis of lymph node status as well as various pathological parameters such as IHC expression of ER/PR, HER2 and MMP-1 is important because it provides valuable prognostic and predictive information. Our results showed that axillary lymph node status has significant association with hormone receptor expression as well as with MMP-1 IHC expression.In our study we did not find significant association between HER2 over expression and lymph node status.

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