DOI: 10.7860/JCDR/2021/48946.15155

Original Article

Pathology Section

Expression of Biomarkers Estrogen Receptor, Progesterone Receptor, HER2 in Primary Breast Tumour and Synchronous Metastatic Axillary Lymph Node

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ABSTRACT

Introduction: Estrogen Receptor (ER), Progesterone Receptor (PR), Human Epidermal growth factor Receptor 2 (HER2) status are routinely used to guide treatment decision for breast cancer. Treatment protocol in breast cancer is currently based on biomarker characteristic of primary tumour. But this biomarker status may change as the tumour progresses from primary to synchronous metastatic lymph node. Hence, it is important to know the biomarker status of these synchronous metastatic lymph nodes as it may serve as an important tool to guide management, evaluate prognosis and to anticipate the possibility of recurrent risk of primary invasive breast cancer.

Aim: To study the expression of ER, PR, HER2 status in primary breast carcinoma and synchronous metastatic lymph node and to evaluate concordance and discordance between them.

Materials and Methods: This study was observational, retrospective and prospective study conducted over a period of one and half years from February 2015 to October 2016 at ESI-PGIMSR, Maniktala, Kolkata, India, where 50 cases of breast carcinoma with positive axillary lymph node metastasis were studied. Haematoxylin and Eosin (H&E) sections were reviewed and representative paraffin blocks were selected. Immunostains were performed and scoring was done following standard protocols. Standard statistical methods were applied for analysis of data using chi-square test and kappa statistics and data was analysed using Statistical Package for the Social Sciences (SPSS) version 6.1.3 software.

Results: Out of 50 cases the mean age of the patients was 50.56±10.5 years. Amongst ER and PR status, 24 out of 50 (48%) and 18 out of 50 (36%) were ER and PR positive respectively. HER2 positive cases were 48% i.e., 24 out of 50 cases. The overall discordance rates of ER, PR and HER2 was found to be 10%, 8%, 18%, respectively. The discordance rates of ER positive and ER negative cases were 4.2% and 15.4%, respectively. The discordance rates of PR positive and PR negative cases were 5.6% and 9.4% respectively, whereas 29.2% of HER2 positive cases were discordant in lymph node metastases.

Conclusion: There was discordance between ER, PR, HER2 status of primary tumour and metastatic lymph node. Hence, assessment of these biomarker status in axillary lymph node metastases may be considered along with primary tumour in breast carcinoma work-up.

Keywords: Concordance, Discordance, Immunohistochemistry, Tumour progression

INTRODUCTION

There has been a growing increase in the incidence of breast cancer, which is still the most significant cancer-related cause of female mortality [1]. The prognosis and the therapeutic modalities of a tumour, to a large extent depend on its biological characteristics. Recently, newer biomarkers and immunohistochemical markers have been introduced after the emergence of molecular techniques. Immunohistochemical markers aid in classification of breast cancer into biologically distinct subtypes, guide treatment decisions and also serve as potential prognostic and predictive factors [2].

Treatment protocol in breast cancer is currently based on biomarker characteristic of primary tumour. But this biomarker status may change as the tumour progresses from primary to synchronous metastatic lymph node. The positivity of these markers, could be altered by number of reasons including, technical factors or preanalytical components. Any biological modifications during the metastatic process or tumour progression may also influence the positivity of these markers [3]. Hence, it is important to know the biomarker status of these synchronous metastatic lymph nodes as it may serve as an important tool to guide management, evaluate prognosis and possibility of recurrent risk of primary invasive breast cancer.

The ERs and PRs are two different groups of proteins that are activated by oestrogen and progesterone respectively. Expression of these receptors is an important parameter for determining therapy and predicting prognosis in patients who are receiving hormone therapies.

HER2 is a tyrosine kinase protein, amplification and over expression of which is seen in approximately 25-30% of breast cancers and it confers poor prognosis [4].

Axillary lymph node metastasis is one of the important metastatic routes of breast carcinoma, but whether the expression of biomarkers in the synchronous axillary lymph node metastasis, is in concordance with the primary breast tumour remains controversial [5]. Several retrospective studies demonstrated instability between primary breast tumour and synchronous axillary lymph node metastasis in expression of ER, PR, and HER2 status [6]. Current targeted therapy in breast carcinoma is guided by biomarker status of primary tumour. But in due course of time as the tumour progresses from primary to metastatic clone, the biomarker status of these metastatic lymph node may, not reflect the same biomarker status, as it is expressed in the primary tumour. Tumour heterogeneity is an important factor which affects the clinical efficacy of breast cancer, other factors are inherent host and biological factors along with adjuvant therapy. Studies have shown variable concordance and discordance rates between primary breast tumour and synchronous metastatic axillary lymph nodes [7]. Hence, it becomes necessary to study the relationship between primary and synchronous axillary nodal metastasis. According to National Comprehensive Cancer

Network (NCCN) clinical practice guidelines for breast cancer, there may be discordance between the ER and/or PR determination between the primary and metastatic tumours, and the NCCN panel recommends biopsy in cases of metastasis at presentation or first recurrence [8].

The present study was undertaken to investigate the expression of biomarkers ER, PR, HER2 in primary breast tumour and synchronous metastatic axillary lymph nodes with an objective of evaluating whether there is any concordance or discordance of these biomarker status between primary breast tumour and lymph node metastases.

MATERIALS AND METHODS

The present study was observational, retrospective and prospective study. The study population were histopathologically diagnosed cases of breast carcinoma with positive lymph node metastases. Fifty cases of breast carcinoma with lymph node metastasis both from past records and histopathological specimens received in the hospital during the study period were included. The study was conducted over a period of one and half years from February 2015 to October 2016 at ESI-PGIMSR, Maniktala, Kolkata, India, bearing Institutional Ethics Committee no -ESI/PGIMSR/MKT (474)/IEC. Retrospective blocks of past one-year cases of breast carcinoma with axillary lymph nodes were retrieved for the study. Patients were selected irrespective of age and sex with clearly defined inclusion and exclusion criteria.

Inclusion Criteria

i) Patients undergoing modified radical mastectomy with evidence of tumour without any prior treatment and also those with residual tumour postchemotherapy were included in the study.

ii) Any number of lymph nodes with macro metastasis (>2 mm) or with micro metastasis (>0.2 mm to 2 mm and/or >200 cells) and with isolated tumour cells (≤0.2 mm and ≤200 cells) were included in the study. In case of multiple metastasis, lymph node with largest deposit were considered in the study.

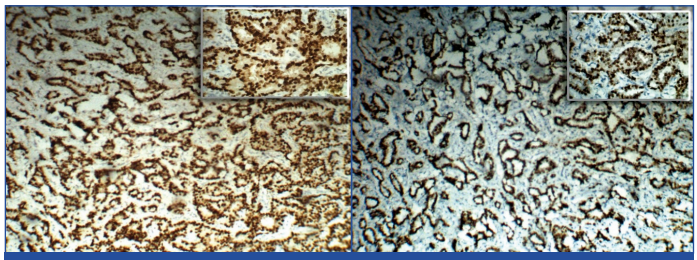
Exclusion criteria: The cases with sections having fixation artifacts were excluded from the study.

Study Procedure

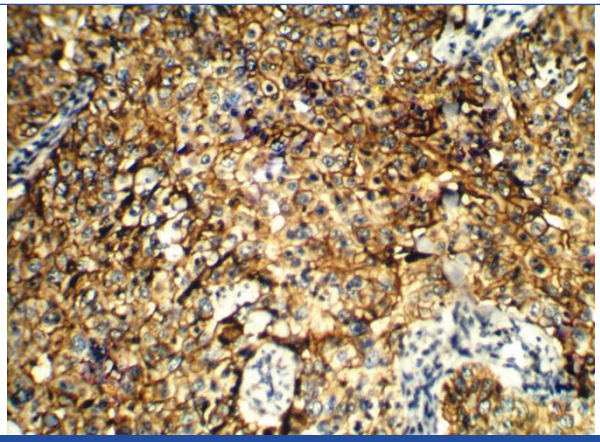
Relevant clinical data was obtained from medical records. Haematoxylin and Eosin (H&E) stained slides were examined, a representative block for each case was selected for immunohistochemistry. Four micrometer sections were cut and mounted on poly L lysine coated slides taking care to mount the section flat and wrinkle free. Slides were incubated for 30 minutes at 60ºC. Slides were deparaffinised and then rehydrated in graded alcohol and then put to water. Antigen retrieval was done using Tris EDTA buffer at pH-9 in a microwave in three cycles for about 20 minutes. After cooling down to room temperature, slides were washed in wash buffer (pH 7.4) endogenous peroxidase blocking was done with 3% hydrogen peroxide for ten minutes. Immunohistochemistry was done using primary antibody (Rabbit monoclonal antibody SP1 for ER, Rabbit monoclonal antibody Y85 for PR and Rabbit monoclonal antibody SP3 for HER2) and slides were incubated for 60 minutes at room temperature in a moist chamber. Again, after wash with wash buffer; slides were incubated in the moist chamber with secondary antibody (horse radish peroxidase labeled). The reaction product was detected with 3,3- diaminobenzidine chromogen. Counter staining was done by haematoxylin. Sections were again dehydrated in graded alcohol and mounted by DPX.

Immunohistochemistry slides were scored by two pathologists individually using Allred scoring system for ER and PR [3]. A score of 3-8 was considered positive.

Immunoreactivity for ER and PR was assessed by estimating the percentage of tumour cells showing nuclear staining. More than 10% of the tumour cells showing immunoreactivity were considered as positive, as depicted in [Table/Fig-1,2]. HER2 staining was scored according to the College of American Pathologists guidelines. Moderate to strong complete membrane staining of 10% or more of the tumour cells was considered to be positive (3+) as shown in [Table/Fig-3]. Equivocal score (2+) was considered weak to moderate complete membrane staining in >10% of tumour cells and HER2 expression was labeled as negative (score 1, 0) when incomplete membrane staining that was barely perceptive in >10% tumour cells or incomplete barely perceptible staining in <10% of tumour cells respectively [5].



[Table/Fig-1,2]: (ER and PR, 100X, Inset 400X): Photomicrographs showing ER and PR strong intensity with Allred score of 8, respectively. (Images from left to right)



[Table/Fig-3]: (HER2, 400X): Photomicrograph shows HER2 score 3+ with complete membrane staining in more than 10% tumour cells.

STATISTICAL ANALYSIS

Statistical significance between biomarker status in primary tumour and lymph node metastasis were investigated using the Chi-square test and data was analysed using SPSS version 6.1.3 software. The p-value <0.05 was considered to be statistically significant. The concordance rate of ER, PR and HER2 between the primary breast carcinoma and synchronous metastatic lymph nodes was assessed by Fleiss-Cohen weighted kappa statistics.

RESULTS

The present study was conducted on 50 breast carcinoma cases with positive lymph node metastasis. The mean age of the patient was 50.56±10.5 years with an age range of 27 to 80 years. Only one case in the study population was male breast carcinoma and 11 out of 50 cases received neoadjuvant chemotherapy. The clinicopathological characteristics are depicted as shown in [Table/Fig-4].

Distribution of cases according to pathological Tumour-Node-Metastasis (pTNM) staging is depicted in [Table/Fig-4]. In this study group majority of cases 37 out of 50 i.e., 74% belongs to T2, while according to N status majority of cases 21 out of 50 i.e., 42% belongs to N1. Case distribution according to Nottingham histological grade has been depicted in [Table/Fig-4]. In the present study, majority of the cases 21 out of 50 i.e., 42% are grade 2. Cases were also sorted according to molecular subtype which is depicted as [Table/Fig-4].

With regard to ER, PR, HER2 immunohistochemistry, [Table/Fig-5] ER status, 24 (48%) were ER positive and 26 (52%) were ER negative. Amongst PR status majority of cases, i.e., 32 (64%) were PR negative and 18 (36%) were PR positive. While for HER status

Parameters	No. of patients (N=50)	Percentage (%)
Chemotherapy		
Chemotherapy received	11	22
Chemotherapy not received	39	78
Stage		
Primary Tumour (PT)		
T1	06	12
T2	37	74
T3	06	12
T4	01	02
Lymph node status (pN)		
N1	21	42
N2	18	36
N3	11	22
Nottingham grade		
Grade 1 (G1)	10	20
Grade 2 (G2)	21	42
Grade 3 (G3)	19	38
Molecular subtype		
Luminal A	14	28
Luminal B	13	26
HER2 enriched	13	26
Triple negative	10	20
Biomarker status		
Estrogen receptor status		
ER positive	24	48
ER negative	26	52
Progesterone receptor status		
PR positive	18	36
PR negative	32	64
HER2 receptor status:		
Negative (1+)	24	48
Equivocal (2+)	02	04
Positive (3+)	24	48

[Table/Fig-4]: Distribution of cases according to the clinicopathological characteristics: Age 27-80 years (Mean 50.56 years).

there was equal distribution in negative (1+) and positive (3+) with 48% each. Only 2 among these cases (4%) were equivocal (2+).

Biomarkers	Expression in PBC n, %	Node concordance n, (%)	Node discordance n, (%)	Kappa statistics (k)	p-value
ER expression					
Positive	24 (48)	23 (95.8)	1 (4.2)	0.801 (almost perfect agreement)	0.1865
Negative	26 (52)	22 (84.6)	4 (15.4)		
PR expression					
Positive	18 (36)	17 (94.4)	1 (5.6)	0.831 (almost perfect agreement)	0.6327
Negative	32 (64)	29 (90.6)	3 (9.4)		
HER2 expression					
Positive	24 (48)	17 (70.8)	7 (29.2)	0.667 (substantial agreement)	0.0382
Negative	24 (48)	23 (95.8)	1 (4.2)		
Equivocal	02 (04)	01(50)	1(50)		

[Table/Fig-5]: Expression of different biomarkers with node concordance and discordance rate.
ER: Oestrogen receptor; PR: Progesterone receptor; PBC: Primary breast carcinoma

Distribution of cases according to nodal concordance and discordance along with expression of ER, PR and HER2 in primary breast carcinoma are depicted in [Table/Fig-5]. When ER, PR, HER2 status of synchronous metastatic lymph nodes was considered, the authors found that overall concordance rate of ER, PR, HER2 was 90%, 92% and 82%, respectively. While overall discordance rate was 10%, 8% and 18%, respectively [Table/Fig-5]. Specifically, 23 cases were concordantly ER positive in primary breast cancer and in nodal metastasis. While 22 cases were ER negative in both primary and metastatic tumour. Changes in ER status between primary breast carcinoma and corresponding synchronous metastases were evident in five out of 50 cases (10%) as demonstrated in [Table/Fig-5]. Where one of the cases (4.2%) changed from ER positive to ER negative, while 4 cases (15.4%) changed from ER negative to ER positive (p-value 0.1865).

As shown in [Table/Fig-5] with respect to PR status 17 cases were concordantly PR positive while 29 cases were PR negative in both primary and metastatic tumour. Discordance was observed in 4 out of 50 cases (8%). One out of the discordant cases (5.6%) was PR positive in primary tumour and PR negative in lymph node metastases, while 3 cases (9.4%) were PR negative in primary tumour and PR positive in lymph node metastases (p-value 0.632). [Table/Fig-5] also displays HER2 status where it was found that 41 out of 50 cases were concordant while 9 cases were discordant. Out of these 9 cases (4.1%) changed from negative (1+) to equivocal (2+), 7 (29.2%) changed from positive to negative while 1 case changed from equivocal to negative (p-value 0.038).

The concordance rate of ER, PR and HER2 between the primary breast carcinoma and synchronous metastatic lymph nodes was assessed by Fleiss-Cohen weighted kappa statistics, where k values of 0, were regarded as no agreement, 0.21-0.4 as fair agreement, 0.41-0.6 as moderate agreement, 0.61-0.8 as substantial agreement, and 0.81-1 as almost perfect agreement. The kappa (k) value for concordance in ER and PR status between the primary tumour and lymph node metastases was 0.801 and 0.831, respectively which is almost in perfect agreement, while for HER2 status k value is 0.667 which is regarded as substantial agreement. Statistical significance of ER, PR & HER2 concordance and discordance were analysed by Chi-square test. The p-value significance level was placed at 0.05. No statistically significant difference between expression of ER and PR in primary breast carcinoma and axillary lymph node metastasis was found and p-values were 0.186 and 0.632 respectively. The p-value was statistically significant for HER2 discordance with value of 0.0382.

DISCUSSION

Synchronous axillary lymph node metastasis, considered as local metastasis, is one of the important metastatic routes of breast carcinoma. It may represent the metastatic breast cancer or the recurrent disease much better in comparison to primary breast tumour [9]. The postoperative systemic treatment for breast cancer is aimed at the potentially metastatic cancer cells and the biomarkers of primary tumours may not accurately represent the potential metastases, due to lack of stability throughout the tumour progression. Besides this, the adjuvant therapy for the tumour might lead to the variable expression of biomarkers [7].

This study showed overall discordance rate in estrogen receptor status as 10% as opposed to study conducted by Nedergaard L et al., which was 21% [9]. In this study, 4.2% changed from ER positive to negative while 15.4% changed from ER negative to positive, in contrast to this study their discordance rate was greater in positive to negative (33%) than negative to positive (6.5%). This discordance of estrogen receptor status may be due to loss of receptor status or certain mutation (deletions of 16q and gains of 1q) that might cause expression of ER in these metastatic clone [4]. This discordance can partly explain why some patients with ER positive primary tumour are reported to respond poorly to endocrine therapy, or

some patients with ER negative phenotype with ER positive lymph node metastasis might be benefited from this therapy.

Aitken SJ et al., demonstrated overall discordance rate of PR to be 23.4%. The discordance rate was higher amongst PR positive tumours (35%) whereas lower among PR negative tumour (15.2%) [10]. Whereas, in this study the overall discordance in PR status is 8% and discordance among PR negative tumour is higher (9.4%) than PR positive tumour (5.6%). In the same study, overall HER2 discordance was 8.9%. Their discordance rate was higher in HER2 positive tumour (10.7%) and lower in HER2 negative tumour (8.9%), which is consistent with findings of this study that showed higher discordance in HER2 positive status (29.2%%) and lowest in HER2 negative status (4.1%).

In a study done by Sujarittanakarn et al, the discordance rate observed for ER, PR, and HER2 was 11.1%, 20.2% and 10.1% respectively [6]. This is almost close to our overall discordance rate of 10%,8%, and 18%for ER, PR and HER2 respectively. Expression of different clone of cancer cells either due to genetic or epigenetic mechanism might be the reason for this frequent shift of ER and PR status. Thus, patients changing from hormone receptor primary tumour to hormone receptor negative axillary metastasis can be resistant to endocrine therapy and their treatment needs to be altered accordingly. HER2 status in synchronous lymph node may strongly influence therapeutic management and impact the prognosis of patients. Loss of HER2 amplification in post relapse and on other hand for the patient, changing from HER2 negative primary tumour to HER2 positive synchronous axillary metastasis may allow possibility of targeted trastuzumab therapy.

In a study by Georgescu R et al., it was found that the discordance rate for ER and PR expression was 31.7% and 41.5%, respectively and for HER2 it was 24.4% which was higher in comparison to this study with the discordance rate of 10% for ER, 8% for PR and 18% for HER2 expression [11]. This supports the heterogeneity of the primary breast tumours and the unstable molecular profile through the axillary lymph node metastases process in all breast carcinomas.

Limitation(s)

Present study included 11 postchemotherapy cases out of 50 and remaining 39 were non chemotherapy cases, the adjuvant therapy might lead to variable expression of studied biomarkers. For the equivocal cases (2+) of HER2 FISH was done wherever possible, but for those cases where FISH could not be performed due to limited resources, were kept as equivocal only.

CONCLUSION(S)

This study has demonstrated discordance in expression of biomarkers namely ER, PR, HER2 in synchronous metastatic lymph nodes. Synchronous axillary lymph node metastases may represent the potential of metastatic breast cancer better than the primary tumour [8]. Indeed this may strongly influence therapeutic management, which indicates the necessity of assessing biomarker status of these metastatic clones as well along with primary tumour. So, the authors concluded that, in future assessment of ER, PR, HER2 status in synchronous metastatic lymph node might be considered in clinical practice along with primary tumour in breast cancer work up to guide the therapy management and evaluate the recurrent risk of primary invasive breast cancer patient with synchronous axillary lymph node metastasis.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]
- Plagiarism X-checker: Feb 11, 2021
- Manual Googling: May 06, 2021
- iThenticate Software: Jun 02, 2021 (18%)

ETYMOLOGY: Author Origin

AUTHOR DECLARATION:
- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? Yes
- For any images presented appropriate consent has been obtained from the subjects. Yes

Date of Submission: **Feb 10, 2021**
Date of Peer Review: **Mar 17, 2021**
Date of Acceptance: **May 27, 2021**
Date of Publishing: **Jul 01, 2021**