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Original Research Article

Influence of Marginal Adipose Tissue Invasion in Prognosis of Ductal Carcinoma of the Breast and on Lymph Node Metastasis and Its Correlation with Hormone Receptor Status

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Abstract:

Introduction: Marginal adipose tissue invasion (ATI) may lead to a larger contact area between cancer cells and the peritumoral functional lymphatic endothelium increasing the chances for lymphovascular invasion. The prognostic significance of adipose tissue invasion at the tumor margins has not been evaluated fully. Present study is intended to evaluate the adipose tissue invasion at tumor margins, lymphovascular invasion and its prognostic significance in carcinoma breast.

Material and Methods: This prospective observational study contain 100 cases of breast cancers, diagnosed as invasive ductal carcinoma NOS were analysed. Clinical and morphological details of cases were recorded according to the proforma. The observations were compared with other studies and inferences drawn.

Results: Out of 100 cases analysed, 74 cases showed presence of adipose tissue invasion (ATI). Out of the total cases, 66.2 % belonged to 41-60 years of age. 78.4 % of cases had Bloom-Richardson grade – II. 59 case out of 100 showed lymph node metastasis .62.7 % of node positive cases had adipose tissue involvement. 31.5 % and 22.2 % cases showed positivity for estrogen receptor and progesterone receptor respectively and no statistical correlation was observed between receptor status and adipose tissue invasion. 100% cases of ATI belonged to average to high-risk group according to St Gallen criteria. Case without ATI showed a higher mean 10-year survival rate compared to cases with ATI. 43 % of the present study cases studied showed lymphovascular invasion.

Conclusion: Cases which are positive for ATI showed significant increase in lymph node metastasis and a lower 10 years survival rate. Thus, its presence indicates tumour aggressiveness and adverse outcome. Marginal adipose tissue invasion and lymphovascular invasion can be used together as a prognostic marker to predict the tumor aggressiveness and to formulate therapeutic strategies. More studies and investigations are required for substantiating the significance of ATI and its influence in the prognosis of breast cancer.

Keywords: Breast carcinoma, ATI – Adipose tissue invasion, LVI – Lymphovascular invasion.

Introduction

Breast carcinoma is the most common malignant tumor and the leading cause of carcinoma death in females. It accounts for 23% of all cancers in females globally. Infiltrating ductal carcinoma is the most common type of invasive breast carcinoma (75–80% of all mammary invasive carcinomas) [1].

Various studies have been carried out to assess the prognostic parameters [2] of breast cancer patients. Prognostic factors as such can be categorised into two groups- traditional and molecular. Traditional factors include tumor size [3], histological grade [4,5], vascular invasion, stromal characteristics, lymphatic tumor emboli [6] and angiogenesis [7]. Molecular factors include hormone receptors, p53 and HER2/neu expression.

Functional lymphatics at the tumor margins are responsible for lymphatic metastasis. Tumor cell morphology and proliferation are seen to affect lymph node metastasis. Ability of the tumor to

invade the adjacent tissue has been proposed to be an important prognostic factor in many recent articles. The prognostic significance of adipose tissue invasion at the tumor margins has not been evaluated fully.

Generally, when intraductal carcinoma cells infiltrate the breast stroma, the cells initially penetrate the fibrous tissues followed by the fibro adipose tissues and finally the adipose tissues in breast cancer. Marginal adipose tissue invasion (ATI) may lead to a larger contact area between cancer cells and the peritumoral functional lymphatic endothelium increasing the chances for lymphovascular invasion. Yamaguchi et al., [8] found ATI to be an independent factor influencing nodal metastasis. Patients with ATI had a poorer prognosis than patients without ATI (10-year disease free survival, 76% and 94%, respectively). In addition, patients without ATI or LVI had neither lymph node metastasis nor recurrent disease. The study concluded that ATI was one of the biologic indicators of tumor aggressiveness.

Lymphovascular invasion is a crucial step in the complex process of tumor metastasis [9]. The presence of carcinoma cells in either lymphatic vessels, blood vessels or both is a significant prognostic factor in invasive breast cancer, with respect to local and distance recurrence and poorer survival. Node-negative patients with lymphovascular invasion had higher breast cancer mortality rate compared with patients with no lymphovascular invasion. The combination of these two microscopic features may be a useful predictive factor in identifying patients who require axillary dissection, chemotherapy or hormonal treatment. The Present study intended to evaluate the adipose tissue invasion at tumor margins, lymphovascular invasion and its prognostic significance in carcinoma breast.

Materials and Methods

The present study was a prospective institution-based observational study conducted at the Department of Pathology, Madurai medical college during the period of July 2015 to August 2017. Ethical clearance for the study was obtained from the Ethical Committee of Madurai medical college, Madurai. A total sample of 100 cases of breast cancers, diagnosed as invasive ductal carcinoma NOS were analyzed during this period.

Inclusion Criteria: All cases of mastectomy with axillary lymph node dissection in invasive ductal carcinoma.

Exclusion criteria:

- Special histology type – mucinous, lobular, medullary or squamous cell carcinoma
- Distant Metastasis
- Malignancy at other sites
- Patient who received neoadjuvant chemotherapy

Methodology and Techniques

The study material included 100 breast cancer specimens. Clinical and morphological details of cases were recorded according to the proforma which included patients name, age, biopsy number, clinical history, investigations and treatment done, gross description, histological factors, hormone receptor status, St. Gallens risk stratification, 5 and 10 year survival rate prediction by PREDICT.

Modified radical mastectomy specimens were collected and fixed in 10% neutral buffered formalin for 12 hours. After adequate fixation, condition of the nipple skin was noted; the specimens were photographed and sliced with 5–10 mm intervals. Tumour size was measured as the largest dimension of the invasive component and the distance of the tumour from the surgical margins were recorded. Number of nodes in the axillary pad of fat was recorded and they were sectioned through the hilum. Representative bits were taken from the specimen and processed routinely to obtain 3-5 μm thin paraffin sections. Staining was done by Hematoxylin and Eosin staining technique.

Histo-morphological evaluation:

Histological grading: All tumours were graded according to modified Bloom – Richardson Histological grading criteria. The grade is calculated by scoring architectural differentiation, nuclear pleomorphism and mitotic index. All the three components are scored from 1 to 3. Sum of the three scores then determines the final score. Scores 3-5 are then categorised into well differentiated (grade I), 6-7 as intermediate (grade II) and 8-9 as poorly differentiated (grade III). Adipose tissue was defined as a pure aggregate consisting of more than 20 fat cells without intervening fibrous tissue in the breast. The adipose tissue included tissues surrounding the mammary ducts or lobules and those in the subcutaneous layers. Fibro adipose tissue (fat cells mixed with various fibrous tissues) was strictly distinguished from adipose tissue.

Marginal ATI was defined as the presence of more than 20 cancer cells in direct contact with the adipose tissue or the location of cancer cells in the adipose tissue. Only cases with unequivocal ATI were considered positive (ATI+). Doubtful cases were considered negative (ATI–). LVI was defined as the presence of peritumoral lymphatic emboli in endothelium-lined spaces, according to the literature. Samples with obvious emboli were considered positive (LVI+) and doubtful samples were considered negative (LVI-).

Hormone Receptor Status: Due to paucity of fund, 38 cases for estrogen receptor and 37 cases for progesterone receptor were randomly chosen by lottery system. The status of estrogen and progesterone receptors was evaluated by immunohistochemistry using commercially available antibodies (Dako Cytomation, Kyoto, Japan). Cases with more than 10% of the tumor cell nuclei stained were defined as positive.

Risk Stratification: Based on St. Gallen calculator (10), the cases whose hormone receptor status was available were divided into low and average-high risk categories and thus helped identify high risk patient who would benefit from systemic adjuvant therapy. The patients with all four criteria's- 1. Node negative patients older than 35 years, 2. ER and or PR positive 3. Well differentiated tumor 4. Tumor size equal or smaller than 2 cm in diameter were grouped under minimal low risk and those who did not fulfil these criteria were grouped as average-high risk.

Survival Rate Prediction - Predict (11).

Five- and ten-years survival rate of the breast cancer patients were calculated using PREDICT, which is a computer-based program by taking into account the information on age, tumor size, tumor grade, estrogen receptor (ER) status, HER2, Ki67 and nodal status.

Statistical analysis was carried out to analyse the association between different parameters using epi-info version 7.2.6.0 and MS excel. P value less than 0.05 were considered statistically significant.

Results

Out of 100 cases analysed,74 cases showed presence of ATI. Out of the total cases of adipose tissue invasion, 49 cases belonged to the age group of 41-60 years. 41(55.4 %) of the case with ATI had tumour size in the range of 2-5cms. 58(78.4%) cases of adipose tissue invasion had Bloom-Richardson grade – II. 43 out of 100 cases showed lymphovascular invasion, of which 37(86 %) cases showed ATI. 59 case out of 100 showed lymph node metastasis. 37(62.7%) of node positive cases had adipose tissue involvement.

31.6 % (6 out of 19) cases with adipose tissue invasion showed positivity for estrogen receptor. 22.2 % (4 out of 18) cases with adipose tissue invasion showed positivity for progesterone receptor. 100% (19) cases of ATI belonged to average to high-risk group according to St Gallen criteria. Case without ATI showed a higher (54.6 %) mean 10-year survival rate compared to cases with ATI (42.5%).

Table 1: Correlation between ATI and various parameters of carcinoma breast

Parameters	ATI present	ATI absent	P value
Mean Age (years)	51.04	53.96	0.8424
Mean Tumor Size (cms)	5.19	4.79	0.953
T1	5	2	-
T2	41	15	
T3	28	9	
Bloom-Richardson Grade			
Grade I	8	3	
Grade II	58	16	0.130
Grade III	8	7	
Lymphovascular Invasion			
Present	37	6	0.0170
Absent	37	20	
Lymph Node Metastasis			
Present	37	22	0.002
Absent	37	4	
Estrogen Receptor (38)			
Positive	6	11	0.102
Negative	13	8	
Progesterone Receptor (37)			
Positive	4	4	0.931
Negative	14	15	
St Gallen Risk Stratification			
Average-High Risk	19	17	0.298
Minimum-Low Risk	0	1	
Survival rate prediction-PREDICT			
5 Years Survival Rate	58.5%	69.6%	0.776
10 Years Survival Rate	42.5%	54.6%	

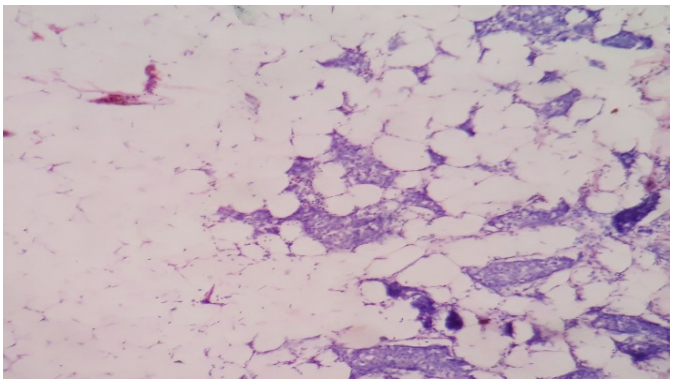

Figure 1: Marginal Adipose Tissue Invasion (100x)

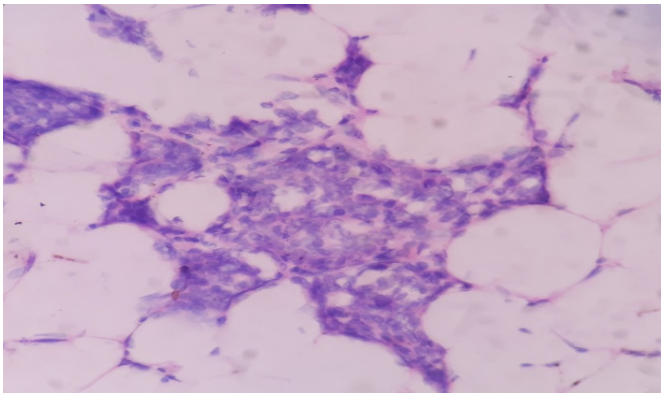

Figure 2: ATI (400x)

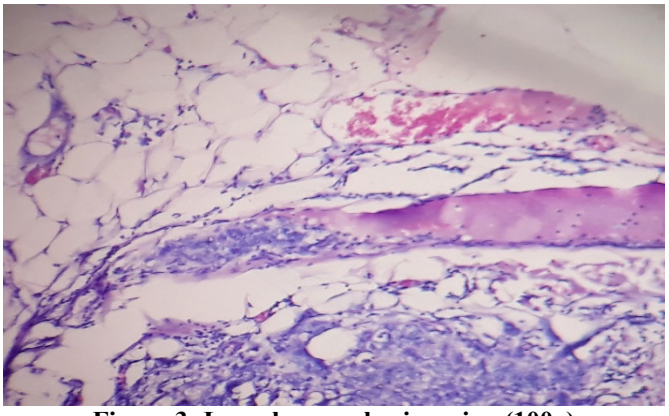

Figure 3: Lymphovascular invasion (100x)

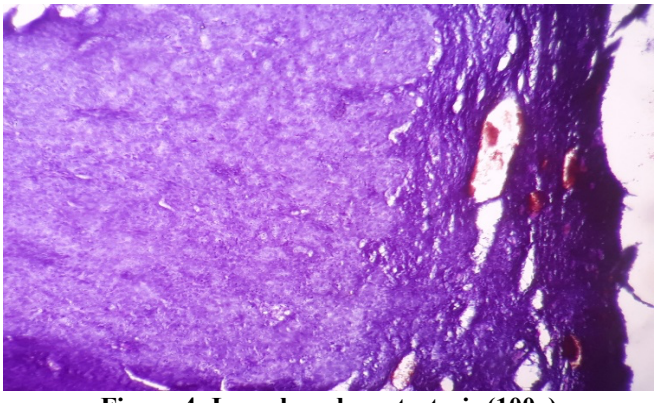

Figure 4: Lymph node metastasis (100x)

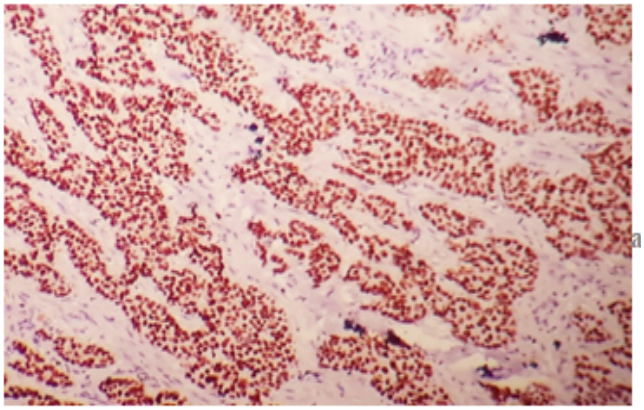

Figure 5: Estrogen Receptor nuclear positivity (100x)

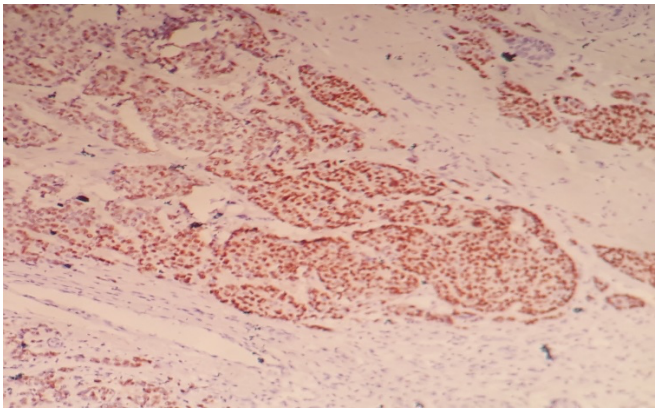

Figure 6: Progesterone receptor positivity (100x)

Discussion

Out of the total 136 mastectomy with axillary lymph node dissection specimens received, 114 cases (83.8 %) were diagnosed as infiltrating ductal carcinoma. Out of these 100 cases were evaluated for marginal adipose tissue invasion, lymphovascular invasion and lymph node metastasis. Age distribution, tumor size, Bloom-Richardson grade, estrogen and progesterone receptor status were also assessed and compared for cases with and without adipose tissue invasion.

In this study the age distribution of infiltrating ductal carcinoma cases with and without adipose tissue invasion were evaluated. 66.2% (49 out of 74) of the cases belonged to age group of 41-60 years followed by almost similar incidence of 18.9% (14 out of 74) for 20-40 years and 14.9 % (11 out of 74) for >61 years. The results were similar to the study conducted by Christoper I et al., [12] on infiltrating ductal carcinoma which showed an increase in rate by 1.26 fold among age group 50-59 years old and no increase in rates after 60 years of age.

Numerous studies have shown that survival decreases with increasing tumor size and that there is a coincidental rise in the frequency of axillary nodal metastases. Roger et al., [13] in his study conducted on 534 cases noted significant association between tumor size and axillary node involvement with T_{1a} (0-0.5 cm, 3%); T_{1b} (0.6-1.0 cm, 10%); (1.1-1.5 cm, 21%); and (1.6-2.0 cm, 35%). In this study the frequency of axillary node involvement and tumor size were T1 (<2cm, 7.1%), T2 (2-5cm, 42.9%), T3 (>5cm, 50%).

Grade of the tumor was calculated using Bloom-Richardson score. In this study 11 % of the cases belonged to grade I, 74 % to grade II and 15 % to grade III. 5% (3 out of 59) of cases with lymph node metastasis belonged to grade I, 47.5 % (28 out of 59) to grade II and 15.3 % (9 out of 59) to grade III. In this study conducted on 100 cases, 74 cases showed presence of ATI which is almost similar to the results of Yamaguchi et al. with 79 % ATI [8].

41 cases showed no nodal involvement and 59 showed nodal metastasis. Among the cases with adipose tissue invasion, 25.7 % (19 out of 74) had upto 5 lymph nodes involvement and 24.3 % (18 out of 74) more than 5 lymph nodes involvement. This observation is in concurrence with study by Woo et al. where 59.8 % of the cases showed lymph node metastasis [14].

Yamaguchi et al., [8] in his study found a significant association between age and adipose tissue invasion. In this study with 63 % cases in the age group of 41-60 years, no significant association was found between age and adipose tissue invasion.

55.4 % (41 out of 74) of the cases with adipose tissue invasion belonged to the tumor size group of 2-5 cms. 37.8 % (28 out of 74) of ATI cases had tumor size more than 5 cms. However no statistically significant association was found between tumour size and ATI in this study (p value 0.9531) unlike the study by Yamaguchi et al [8] which showed significant association. ($p<0.0001$)

Among the adipose tissue invasion cases 31.6 % (6 out of 19) showed positivity for estrogen receptor and 68.4% (13 out of 19) were estrogen receptor negative. Estrogen receptor status was non-significantly related to adipose tissue invasion (p value: 0.102). The study by Kimijima et al., [15] showed a significant relationship in older patients (60 years) with a correlation between estrogen receptor positivity and fat invasion. Lymphovascular invasion in the present study was observed in 43 cases out of 100.

The presence of tumor emboli within peritumoral endothelial lined spaces both lymphatic and vascular was considered positive for invasion. 56.1 % (32 out of 57) of the lymphovascular positive cases showed axillary lymph node metastasis. The cases of the present study when grouped according to St Gallen risk stratification criteria showed 100 % of the cases with adipose tissue invasion belonged to average to high-risk group. However, no statistical association was observed between these factors.

Five- and ten-year survival rate of the breast cancer patients were calculated using PREDICT, which is a computer-based program by taking into account the information on age, tumour size, tumour grade, estrogen receptor (ER) status, HER2, KI67 and nodal status. Mean 5- and 10-years survival rate obtained in this study were 58.5 % and 42.5 % in cases with ATI and 69.5 % and 54.6 % in cases without ATI respectively. It shows that 5- and 10-years survival rate of patients with ATI is lower as compared to those without ATI. Data for 237 ATI+ cases and 64 ATI– cases were analysed for the survival by Yamaguchi et al. The 5- and 10-year disease-free survivals were 83% and 76% respectively; in ATI+ cases and both values were 94% in ATI– cases.

The clinical relevance of marginal ATI when combined with peritumoral LVI was investigated. Lymph node metastasis was found in 59.45% (22/37) of ATI+/LVI+, 83.33 % (5/6) of ATI-/LVI+,40.54 % (15/37) of ATI+/LVI- and 85 % (17/20) of ATI-/LVI- .

The observations were partly comparable with the study of Yamaguchi et al., where lymph node metastasis was found in 74.6 % of ATI+/LVI+ cases (53/71) and 70% of ATI–/LVI+ cases (7/10). In ATI+/LVI–cases, the frequency was 26.7% (46/172). However, no nodal metastasis was observed in ATI–/LVI– cases (0/52).

Conclusion

As ATI positive cases showed significant increase in lymph node metastasis and show lower 10 years survival rate, so we suggest that marginal adipose tissue invasion and lymphovascular invasion can be used together as a prognostic marker to predict the tumor aggressiveness and also to formulate therapeutic strategies. More studies and investigations are required for substantiating the significance of ATI and its influence in the prognosis of breast cancer.

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