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Original Research Article

Topographical distribution and histological characterization of stromal fibrosis in invasive breast carcinoma

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ABSTRACT

Background: Carcinoma breast is associated with various changes in organ and at the tissue level. The desmoplastic response in a tumor due to tumor hypoxia may result in dense or sparse, homogenous or focally heterogeneous fibrosis of the breast tissue. Peritumoral and stromal fibrosis has been assessed as a marker of intratumoral hypoxia and is being studied as histological surrogate prognostic parameter in breast cancer.

Materials and Methods: We studied the topographical distribution and histological characterization of fibrosis and correlated it with various other histopathological parameters. 50 patients of carcinoma breast presenting to our institute were included in the study.

Results: We obtained a statistically significant correlation of presence of fibrosis with presence of lymphovascular invasion (p value =0.043), while we could not obtain any significant difference with age, menopause, tumor size, tumor grade, pathological stage, lymph node involvement, presence of necrosis, adipocytic invasion, duration of tumor, laterality, presence of inflammatory infiltrate and with ER, PR, HER2Neu status.

Conclusions: This is a limited study in a small number of patients focussing on various stromal changes in breast cancer. This study has further characterized the heterogeneous nature of fibrosis in breast carcinoma using special staining which may facilitate the physician in making patient diagnosis, prognostication and outcome.

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1. Introduction

Breast cancer is the commonest cancer among women in urban registries of Delhi, Mumbai, Ahmedabad, Kolkata and Trivandrum according to ICMR-PBCR data.¹ Carcinoma breast is associated with various changes in organ and at the tissue level. Microscopic changes such as histological grade, presence of inflammatory infiltrate, lymphovascular invasion, adipocytic invasion, type of borders and margins, presence or absence of necrosis, degree of nuclear pleomorphism, nuclear and cellular

atypia, hormone receptor status and various other well-established factors have been known to affect the prognosis of patient.^{2,3} In our present study we attempted to establish a correlation between relatively less established parameter i, e stromal fibrosis and other well known prognostic indicators of carcinoma breast. In our study, we studied the topographical distribution, histological characterization of fibrosis and correlated it with various other histopathological parameters.

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2. Materials and Methods

The study was a cross sectional descriptive study conducted in the Departments of Pathology and Surgery, Maulana Azad Medical College and associated Lok Nayak Hospital, New Delhi-110002 over a period of 1.5 years from October 2012 to April 2014.

50 patients of carcinoma breast presenting to our surgery department who had not undergone any preoperative chemotherapy were included in the study. All subjects were selected from the patients visiting the surgery OPD, LokNayak Hospital after taking their consent

Gross examination was done with special reference to tumor size, quadrant of breast involved, involvement of nipple areola, skin, deep resected plane and resected margin (Figure 1). Sections were routinely processed in histokinette, paraffin embedded and 4 μ sections were stained with H & E. Immunohistochemical staining was done for ER, PR and HER2Neu and the percentage of immunopositive cells was determined under light microscopy at 40X.



Fig. 1: Gross appearance of tumor on serial slicing of MRM specimen

Expression of ER and PR was scored⁴ between 0 and 2 as follows: 0: less than 5% of nuclei staining; 1: 5%-19% of nuclei staining; 2: >20% of nuclei staining and interpreted as positive when more than 10% of tumor cells showed positive nuclear staining. Expression of HER2/neu was from scored 0 to 3. It was considered positive when a weak to strong complete membranous staining in at least 10% of tumor cells was seen⁴ (score 2 and 3).

Fibrotic focus is defined in literature as a scar-like area replacing necrosis in the center of a carcinoma.⁵ It appears as a radially expanding fibrosclerotic core and consists of loose, dense or hyalinized collagen bundles and a variable number of fibroblasts. FF (fibrotic focus) smaller than 3mm in diameter do not contain carcinoma cells, while larger FF sometimes do.⁵⁻⁷ For absolute assessment of the size of fibrotic focus, its largest diameter was measured on H& E slides and special stains under microscope and distribution of fibrosis was recorded. Grading of fibrosis was done as follows.⁸

1. No fibrosis

2. Grade 1-large number of fibroblasts with very few collagen fibres
3. Grade 2- admixed fibroblasts and collagen in various ratios
4. Grade 3-mainly collagen fibres, mostly hyalinized.

Fibrosis was correlated with clinicopathological profile in these cases.

2.1. Statistical analysis

Association between the presence of fibrosis with other clinicopathological parameters and any statistical significant association among various histopathological characteristics were examined by Chi-square test or Fischer's exact probability test. A p-value <0.05 was taken significant.

3. Observations

Of these 50 cases, 48 were of infiltrating duct carcinoma and 2 cases were of mucinous carcinoma. The patient's age ranged from 30 to 80 years. Mean age of the patients was 52 years with majority being in the age group of 41-50 years. The most common presenting complaint was a lump, seen in 48 patients, followed by pain (30%) and nipple discharge (6%). Other symptoms like ulceration of skin, weight loss, and fatigue were also seen. Besides a palpable lump, other important clinical findings were palpable axillary lymph nodes (24), nipple/skin involvement (10) and nipple discharge (3). Among 50 cases, various risk factors which were present were history of oral contraceptive pill intake in 5 cases(10%), history of no breast feeding in 4 cases(8%), family history of breast cancer in 2(4%) cases and history of concurrent malignancy in 1 case which was well differentiated type of endometrial carcinoma in a patient aged 75 years. 52% patients had left side while 48% had right sided tumor. Most common quadrant involved in carcinoma breast was upper outer quadrant (46%) followed by central quadrant (20%). The cases were diagnosed by aspiration cytology, mammography or trucut biopsy before surgery Mammography was performed in 10 (20%) cases. Majority of the cases belonged to BIRADS 4/5. Besides mammography, the other common modality included FNAC (76%) and trucut biopsy (44%). For 1 case, diagnosis was made on excision biopsy. Modified radical mastectomy was performed in 49(98%) of the cases while lumpectomy was done in 1 case. Majority of the cases were of clinical stage T2 N0 M0 (48%) followed by T2N1M0 (18%).

The tumor size varied from 0-10 cm, with the mean of 4 cm. 8 cases (16%) had a tumor size \leq 2cm while 5 cases (10%) were >5 cm in size. Majority 37 (74%) of tumors were in the size range of 2 to 5cm. There was a strong and significant association between lymph node involvement and stage of tumor (p value =0.001).

3.1. Breast cancer staging

Staging was done according to the AJCC guidelines⁹. Majority of the cases belonged to stage II (72%), followed by stage III (26% cases). The cases in stage III increased from 24% to 26% with a decline in stage II cases from 74% in clinical stage to 72% in pathological staging. This is explained by the fact that not all metastatic lymph nodes are clinically palpable

Table 1: Presence of various histopathological features

Parameters assessed	Present	Absent
Necrosis	27 (54%)	23 (46%)
Calcification	14(28%)	36 (72%)
Inflammatory infiltrate	35(70%)	15 (30%)
Adipocytic infiltration	42 (84%)	8 (16%)
Angio-lymphatic invasion	31(62%)	19 (38%)
Fibrotic Focus (FF)	30(60%)	20(40%)
Elastosis	30(60%)	20(40%)

The intensity of pattern of inflammatory infiltrate was graded as - absent (0), mild (1), moderate (2) marked (3) and with germinal centre (4). The intensity of inflammatory infiltrate was further correlated with necrosis and grade of the tumor. On evaluating the inflammatory infiltrate with clinicopathological parameters, association was found with tumors showing adipocytic invasion (p value =0.006). Also higher number of cases with necrosis and lymphovascular invasion exhibited inflammatory component though the difference was not significant statistically (Figure 2).

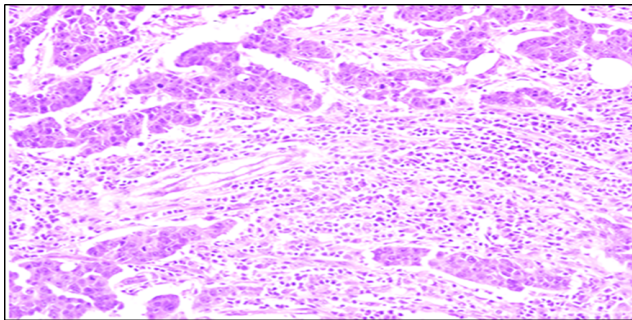


Fig. 2: IDC Grade 2: Inflammatory infiltrate composed of chiefly lymphocytes: Grade 3 (H&E;100X)

3.2. ER/PR/HER2Neu status

Out of 50 patients, 25 were positive for ER (50%) and 23 cases were positive for PR expression (46%), 31 cases showed ER/PR positivity, 17 cases were both ER and PR positive and 19 cases were both ER and PR negative. Out of 50 cases, HER2Neu was positive in 17 cases (34%). Out of total 50 cases, 12 were triple negative (Figures 3 and 4).

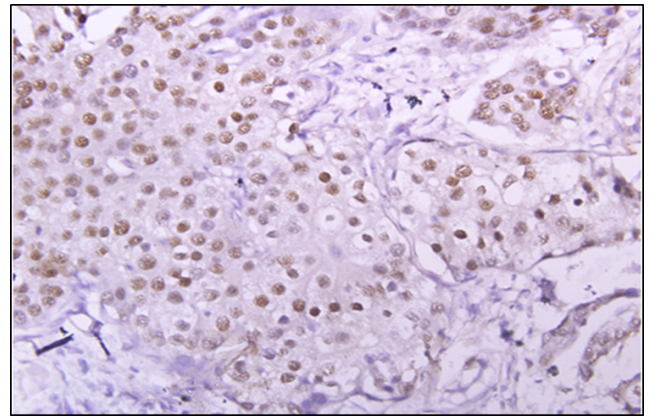


Fig. 3: Strong nuclear expression of Estrogen Receptor IDC grade 2

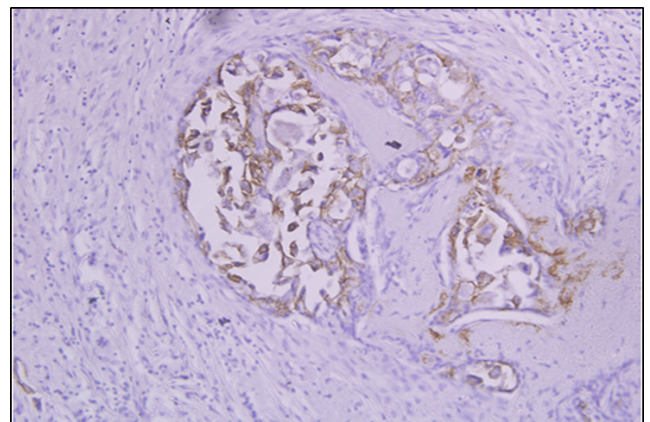


Fig. 4: Strong membranous positivity with Her 2 neu in Grade III (600x)

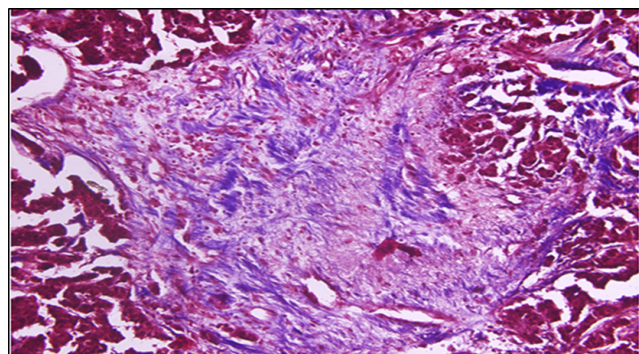
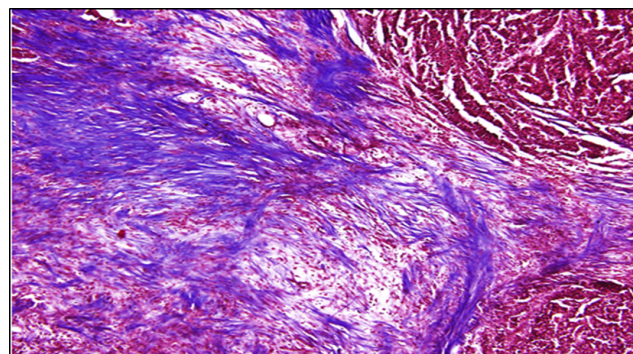
3.3. Fibrosis

All the specimens were assessed thoroughly for presence of fibrosis and sections were taken from the centre of tumor and special stains Masson's trichrome and Von Gieson Elastica were performed in addition to routine Hematoxylin Eosin sections. A focus of >1 mm in size was called as a fibrotic focus (FF).⁵⁻⁸ Cases were screened for the number of fibrotic foci and they were graded from 0 to 3. In cases where there were more than one fibrotic foci, the highest grade of FF was taken up for statistical analysis.

Out of 50 cases, 30 cases showed presence of fibrotic foci. Out of these 30 cases, 7 had single foci, 12 had 2 foci and 11 cases showed >2 foci. Out of 30 cases, 7 had single foci, 12 had 2 foci and 11 cases showed >2 foci. Fibrotic foci were measured under microscope in mm. Results showed most of the foci were between 1-2 mm. 4 cases had fibrosis but less than 1 mm so were not counted as fibrotic foci, 9 cases showed a fibrotic focus >8 mm in diameter (Figures 5, 6, 7 and 8).

Table 2: Correlation of fibrosis with clinicopathological parameters

		Fibrosis+	Fibrosis-	p value
Age group	≤40years	8	5	0.895
	>40years	22	15	
Tumor size	≤5 cm	27	17	0.672(fischer's)
	>5 cm	3	3	
Lymph node	Neg	20	15	0.529
	Pos;	10	5	
Tumor grade	1	4	2	0.907 (fischer's's)
	2	20	15	
	3	6	3	
Tumor necrosis	Present	16	11	0.908
	Absent	14	9	
Lymphovascular emboli	Present	22	9	0.043
	Absent	8	11	
Adipocytic Invasion	Present Absent	25 5	17 3	1.000 (fischer's)
Pathological stage	1	19	14	0.626
	2	11	6	
	3	11	6	
ER status	Pos	17	8	0.248
	Neg	13	12	
PR status	Pos	15	8	0.487
	Neg	15	12	
HER2Neu status	Pos	10	7	0.903
	Neg	20	13	
ER/PR	Positive	20	11	0.405
	Both negative	10	9	
ER/PR/HER2Neu	Positive	22	16	0.589
	Triple negative	8	12	
Duration of lump	≤ 6months	21	15	0.700
	>6 months	9	50	
Inflammatory infiltrate	Present	22	13	0.529
	Absent	8	7	

**Fig. 5:** Loose fibrovascular fibrotic focus: Grade 2 (MT; 400x)**Fig. 6:** Fibrotic focus grade 2 with loose oedematous areas and scattered lymphocytes. (Masson's trichrome; 100x)

Correlation between size of tumor size and size of fibrotic focus was not significant statistically.

Only statically significant correlation was obtained between presence of fibrotic foci and presence of lymphovascular invasion (p value < 0.05).

4. Discussion

Tumor hypoxia may result in desmoplastic response in the form of dense or sparse, homogenous or focally heterogenous fibrosis of the breast tissue.² This desmoplastic response mainly consists of collagen fibres in varying amounts and arrangements. It is termed as fibrotic focus when it is more

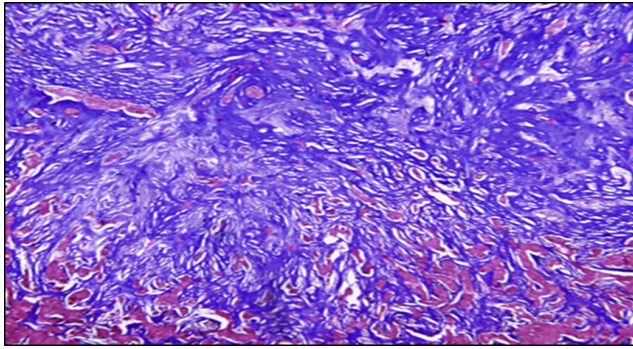


Fig. 7: Fibrotic focus grade 3 entrapping tumor cells at the periphery (MT x100)

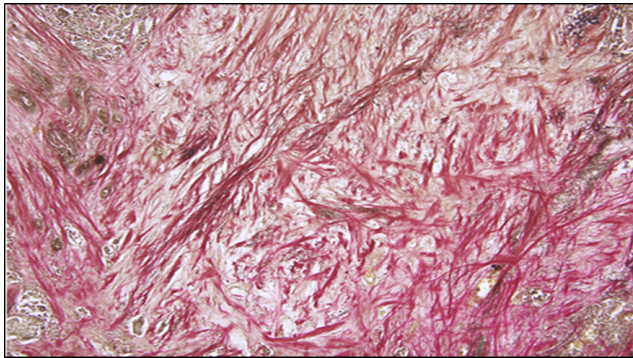


Fig. 8: Criss cross arrangement of collagen bundles in grade 3 fibrotic focus (VGE; 400x)

than 1 mm in diameter. Peritumoral and stromal fibrosis has been assessed as a marker of intratumoral hypoxia and is being studied as a histological surrogate prognostic parameter in breast cancer.^{9–16}

The study of stroma adjacent to tumor cells comprising of fibroblasts, collagen fibres, elastic fibres, and inflammatory cells may help in understanding the difference in prognosis of similar grade and stage patients.¹⁷

34 out of 50 patients in our study showed presence of intratumoral fibrosis. Out of those 34, 30 had a presence of fibrotic focus defined as an area more than 1 mm in largest diameter. so fibrosis was present in 68 % cases and fibrotic focus was demonstrable in 60% of total cases which is in accordance with Hasebe et al(1996) who demonstrated fibrotic focus in 80 out of 152 patients(52.63%).¹⁸

Tumor cells and infiltrating macrophages around the tumor cells initiate the release of profibrotic and proangiogenic growth factors. VEGF has been proposed to play a central role.¹⁹ We obtained a statistically significant correlation of presence of fibrosis with presence of lymphovascular invasion (p value =0.043), while we could not obtain any significant difference with age, menopause, tumor size, tumor grade, pathological stage, lymph node involvement, presence of necrosis, adipocytic invasion,

duration of tumor, laterality, presence of inflammatory infiltrate and with ER, PR, HER2Neu status. Contrary to our results, Hasebe et al(1996) reported a statistical significant association of presence of FF with increasing grades of tumor (p value < 0.05) and axillary lymph node involvement(p value <0.05). He also failed to obtain any significant correlation of fibrosis with age, menopausal status and hormonal receptor status contrary.¹⁸ In his further study, Hasebe et al (2002) stated the parameters significantly associated with the presence of FF were an invasive tumor size of < 20 mm, histological Grade 3, the presence of lymphatic invasion, the presence of vascular invasion, the presence of adipose tissue invasion, the presence of skin invasion, and the presence of nodal metastasis.²⁰ Among these parameters, those that still significantly increased the relative risks of the presence of FF were the presence of adipose tissue invasion, invasive tumor size, the presence of vascular invasion, and the presence of nodal metastasis. The significant associations of the presence of FF with lymphovascular invasion probably suggest that IDCs with FF have a greater aggressive invasive potential than do IDCs without FF. The significant associations of the presence of FF with lymphovascular invasion most likely reflect IDCs with FF having a higher metastatic potential than IDCs without FF.

Out of 30 cases showing fibrotic focus, 19 cases showed grade 3 fibrosis, 11 showed grade 2 fibrosis. Hasebe et al 2001 demonstrated a significant correlation between grade of fibrosis and lymph node metastasis with a p value < 0.05, whereas in our case p value was 0.346(>0.05) and not significant.²¹

Hasebe²⁰ et al, 2002 stated in IDCs with FF, FF dimension of >8 mm significantly increased the risk of initial distant organ metastasis. His prospective study clearly demonstrated FF dimension to be a very useful prognostic parameter for patients with IDCs with FF

5. Conclusions and Recommendations

This is a limited study in a small number of patients focussing on various stromal changes in breast cancer. This study has further characterized the heterogeneous nature of desmoplasia in breast carcinoma using special staining which may facilitate the physician in making patient diagnosis, prognostication and outcome. The results of our study add to the growing literature that attempts to classify and categorize various stromal changes and tries to find out their prognostic significance. The study of stroma adjacent to tumor consisting of fibroblasts, collagen bundles, elastic fibres, microvessels and inflammatory cells, in addition to study of tumor cells may help in prognostication of breast cancer patients.

The presence of fibrotic focus as a marker of intratumoral hypoxia and as a surrogate of angiogenesis is easily identifiable pathological feature, which can be easily added

to pathology report. Its independent prognostic value merits confirmation by future trials.

6. Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

7. Conflicts of Interest

The authors declare that they have no conflicts of interest.

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