ORIGINAL RESEARCH PAPER

Histopathology

RETRACTION CLEFTS IN INVASIVE CARCINOMA BREAST- CORRELATION WITH HISTOPATHOLOGICAL FACTORS AND HORMONAL STATUS

KEY WORDS: Retraction cleft, Breast carcinoma

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ABSTRACT

Retraction clefts (RCs) in breast neoplasm have recently piqued pathologists' interest owing to the ease of identification in hematoxylin-eosin (H&E) stained sections wherein they can be further classified. It can be a predictor of nodal metastasis in breast carcinomas. RCs are defined as the cavity in tumour sections with no endothelial cell lining around tumour glands or nests. Some of the studies proposed that the RC can be a marker of disease progression and nodal metastasis. We studied the amount of RCs in 50 invasive breast cancer specimens, as well as their relationships with histopathological factors. Objectives of the study was 1.To assess the relationship of RC with histopathological factors. 2.To determine the relationship of RC with hormonal status. Materials and methods: 50 formalin-fixed paraffin-embedded samples of Invasive breast carcinoma no specific types were included. Conventionally stained H & E sections of 4 μm and 2μm thick were studied for RC, and compared with histopathological factors and hormonal status. Results: Out of 50 cases included in this study, 42 cases showed presence of RC. It showed significant correlation with the size of tumour (p-0.001), Lymphovascular invasion (p-0.004), Perineural invasion (p-0.010), stage (p-0.001), Lymph node metastasis (p=0.03) and HER2 positive status (p-0.03). No statistically significant association was identified between the RCs and tumor grade (p-0.667), ER (p-0.643), PR status (p–0.345), Ductal carcinoma in situ (DCIS) (p-0.0.775), Necrosis (p-0.804). Conclusion: Retraction clefts in invasive breast carcinoma can be an important histopathological marker for detecting cases with poorer outcome based factors.

INTRODUCTION:

Breast cancer is the most prevalent malignant tumour in women and one with a high degree of heterogeneity[1]

Breast cancer staging is greatly influenced by nodal metastasis, which is an important early event during tumour progression in most solid tumours[2]. Although the lymphatic system plays a significant role in the initial spread of cancers, little is known about how tumour cells interact with and enter the lymphatic system[3]. Whether lymphatic spread is an active or passive process and whether it depends on tumor-induced lymphangiogenesis or invasion of pre-existing lymphatic vessels[4].

Retraction clefts (RCs) in breast neoplasms have recently gained the attention of pathologists since they are simple to recognise and classify in hematoxylin-eosin (H&E) stained sections when seen under an optical microscope[5]. The space around tumour glands or nests in tumour sections that has no endothelial cell lining was identified as RC[6]. Uncertainty surrounds the mechanics driving RC development. According to some studies, RCs were connected to the loss of basal cells in breast and prostate adenocarcinomas, while other studies claimed that aberrant stroma around the tumour was to blame for RCs[6,7]. Additionally, the lymphatic vessels, or "pre-lymphatic channels," may possibly aid in the development of RC[7]. Some studies showed the importance of RCs for diagnosis and prognosis[8-13].

Geza Acs et al. made significant contributions to the clinical value of RCs in breast cancer, which they referred to as "retraction artefact" at that time[8-11]. Investigations on core needle biopsy materials and resected specimens revealed that 55.7% to 64.8% of samples included RCs[8-11]. In both types of their samples, a significant correlation between extensive RCs and nodal metastases was found. Additionally, their research found a strong correlation between the severity of RCs and a bad prognosis[8-11]. They therefore think that extensive RCs was not just a chance artefactual occurrence brought on by poor fixation and processing, but rather represented real prelymphatic space that changed tumor-stromal interactions and contributed to lymphatic spread, tumour progression, and a poor prognosis[8-11].

AIM

To study retraction clefts in invasive carcinoma breast and its relationship with histopathological factors and hormonal status

MATERIALS AND METHODS

In this study, 50 formalin-fixed paraffin-embedded (FFPE) samples were collected from the patients with no special type invasive breast carcinoma, received at the Department of Pathology, of a tertiary care hospital. All samples included were of patients without prior chemotherapy or radiotherapy. Surgically resected specimens were fixed in 10% neutral buffered formalin and processed according to standard protocol. 4 μm thick sections were cut from paraffin embedded sections and routinely stained with hematoxylin & eosin (H&E).

Morphologic Evaluation of Retraction Clefts: RCs are similar to lymphatic or blood vessels, surrounding tumor glands or nests, but without lining endothelium. The extent of RCs was determined by evaluating the proportion of clefts that affected the tumor nests in the whole section. For example, tumors with clefts that affected approximately 10% of tumor nests were classified as 10% RCs. Hormonal status reports were accessed from the institution data system. To further reduce the evaluation bias, lower than 5% RCs were counted as RC negative in this study.

RESULTS:

Table 1: Frequency table of clinicopathological features (n=50)

	n=50	Percentage
AGE		
30-40	6	12%

41-50	18	36%
51-60	16	32%
61-70	7	14%
71-80	3	6%
Laterality		
right	24	48%
left	26	52%
Focality		
Unifocal	48	96.0%
multifocal	2	4.0%
LVI		
Positive	30	60.0%
negative	20	40.0%
PNI		
Positive	18	36.0%
negative	32	64.0%
DCIS		
present	21	42.0%
Absent	29	58.0%
Necrosis		
Present	27	54.0%
Absent	23	46.0%
LN		
Positive	29	58.0%
negative	21	42.0%
GRADE		
1	9	18.0%
2	32	64.0%
3	9	18.0%
STAGE		
1	5	10.0%
2	32	64.0%
3	7	14.0%
4	6	12.0%
ER		
Positive	34	68.0%
Negative	16	32.0%
PR		
Positive	30	60%
Negative	20	40%
HER2		
Positive	23	46%
Negative	27	54%
Hormonal status		
Luminal A	16	32.0%
Luminal B	16	32.0%
HER2 enriched	9	18.0%
TNBC	9	18.0%
Retraction cleft		
Present	42	84.0%
absent	8	16.0%

After considering the inclusion and exclusion criteria, the clinicopathological variables were studied.

Patients with breast carcinoma in our study were seen predominantly in the 5thdecadeof life (n=18) with left sided lesions being more i.e.52%.Unifocal lesions predominated with 96% cases (n=40).Amongst all the cases majority of tumors were of 2-5cms size (68%) and belonged to stage II (n=32, 64%).32 cases were grade 2 tumors followed by 9 cases each of grade 1 and grade 3. 30cases (60%) showed the presence of Lymphovascular invasion (LVI) and perineural invasion (PNI) was present in 18 cases (36%) while in 32 cases (64%) it was absent. Ductal carcinoma in situ (DCIS) component was present in 21 cases (42%) and absent in 29 cases (58%). Necrosis was present in 27 cases (54%) while absent in 23 cases (46%). Lymph node (LN) metastasis was seen in 29 cases (58%) and absent in 21 cases (42%). Out of 50 cases, majority cases were of luminal A type (n=16) and Luminal B (n=16) type followed by triple negative (n=9) and Her 2 enriched (n=9) each. Retraction cleft (RC) was found to be present in majority cases of 84 % (n=42) and 33 cases showing >75% retraction clefts (table 1).

Table 2: Comparison of retraction cleft with histopathological features (n=50)

Variables	Retraction cleft					
	Present	Absent	P value	<75%	>75%	P value
Tumorsize						
< 2 cm	1(20%)	4 (80%)	0.001	4(80%)	1(20%)	0.072
2-5 cm	31(91.1 %)	3(8.9%)		10(29.4 %)	24(70.6%)	
≥ 5 cm	10(90.9 %)	1 (9%)		3(27.2%)	8(72.8%)	
Grade						
Grade 1	7(77.7%)	2(22.3%)	0.667	2(22.2%)	7(77.8%)	0.282
Grade 2	28(87.5 %)	4(12.5%)		10(31.3 %)	22(68.8%)	
Grade 3	7(77.8%)	2(22.2%)		5(55.6%)	4(44.4%)	
LVI						
Absent	14 (66.6%)	7(33.3%)	0.004	13(61.9 %)	8 (38.1%)	0.001
Present	28(96.5 %)	1(3.5%)		4 (13.7%)	25(86.2%)	
PNI						
Absent	25(78.1 %)	7(21.8%)	0.131	15(46.8)	17(53.2)	0.010
Present	17(94.4 %)	1(5.6%)		2(11.1)	16(88.9)	
DCIS						
Absent	24(82.7 %)	5(17.2%)	0.778	12(41.4 %)	17(58.6%)	0.195
Present	18(85.7 %)	3 (14.2%)		5(23.8%)	16(76.2%)	
Necrosis						
Absent	19(82.6 %)	4 (17.4%)	0.804	10(43.5 %)	13(56.5%)	0.191
Present	23 (85.2%)	4 (14.8%)		7(25.9%)	20(74.1%)	
LN						
Absent	15(71.4 %)	6 (28.6%)	0.039	10(47.6 %)	11(52.4%)	0.084
Present	27(93.1 %)	2 (6.9%)		7(24.1%)	22(75.9%)	
TNM stage						
Stage 1	1(20%)	4(90%)	0.001	5(100%)	0(0%)	0.008
Stage 2	29(90.6 %)	3(9.4%)		8(25%)	24(75%)	
Stage 3	6(85.7%)	1(14.3%)		3(42.9%)	4(57.1%)	
Stage 4	6(100%)	0(0%)		1(16.7%)	5(83.3%)	

When histopathological features were compared with Retraction cleft (RC), taking the cut off value to be 10% it was found that the RC correlated significantly with tumor size, LVI, nodal metastasis and stage of tumor (p value <0.05). When the cut off was increased to 75%, RC correlated significantly also with PNI, LVI and TNM stage (p value<0.05). Although it did not correlate significantly with grade of tumor, majority of grade 2 tumors showed RC (87.5%) with 68.8% of the cases showing >75% RC. With the presence of DCIS component 85.7% cases (n=18) showed RC with 76.2 % (n=16) showing >75% RC. Necrosis also showed a correlation with RC though a statistically significant correlation was not established. (Table 2).

Table 3: Comparison of retraction cleft with hormonal status (n=50)

Variables	Retraction cleft					
	Present	Absent	P value	<75%	>75%	P value
ER						
Negative	14(87.5 %)	2(12.5 %)	0.643	7(43.8 %)	9(56.3 %)	0.318
Positive	28(82.4 %)	6(17.4 %)		10(29.4 %)	24(70.6 %)	
PR						
Negative	18(90 %)	2(10%)	0.345	9(45%)	11(55%)	0.180
Positive	24(80 %)	6(20%)		8(26.7 %)	22(73.3 %)	
HER2						
Negative	20(74.1 %)	7(25.9 %)	0.038	14(51.9 %)	13(48.1 %)	0.004
Positive	22(95.7 %)	1(4.3%)		3(13%)	20(87%)	
Molecular classification						
Luminal A	11(68.8 %)	5(31.3 %)	0.239	6(37.5 %)	10(62.5 %)	0.070
Luminal B	15(93.8 %)	1(6.3%)		4(25%)	12(75%)	
HER2 enriched	8(88.9 %)	1(11.1 %)		1(11.1 %)	8(88.9 %)	
TNBC	8(88.9 %)	1(11.1 %)		6(66.7 %)	3(33.3 %)	
	NS: Non significant (p>0.05). * indicates p<0.05 (significant); ** indicates p<0.01 (highly significant); ***indicates p<0.001 (very highly significant) TNBC: Triple negative breast cancer					

Majority of ER positive cases (n=28)(82.4%) showed presence of RC with 70.6% cases(n=24) showing>75%. PR positivity also showed a trend in the occurrence of RC (Table 2) with 80% (n=24) cases being positive for RC and 73% (n=22) cases showing >75% RC in the tumor sections. HER 2 status showed a statistically significant correlation **(p=0.038)** with the presence of RC and with >75% percentage (Table 3).Luminal A, B and HER2 enriched group showed maximum cases with RC (Table 3).

DISCUSSION:

Carcinoma breast is one of the leading global causes of female mortality and morbidity. The existing scenario resulted in extensive research towards adverse prognostic indicators. Several clinical and histopathological features have been identified towards this aim. The ease of 'retraction cleft' identification has interested research into delving deep to investigate this factor. This study on 50 cases, similar to Geza ACS et al, showed a preponderance of women affected being in the 5th decade (36%) followed by 6th decade (32%)[10], and of grade 2. Majority of cases in his study[9-11](74%) had absence of Lymphovascular invasion (LVI) unlike our study which had 50 % cases with LVI. Our study constituted 68%, 60% and 46% of positive cases of ER, PR and HER2 respectively which was similar to study by Geza ACS et al[10]. In our study retraction cleft was seen in about 84% cases which was comparable to study by Geza ACS et al in 2012, 2015 and 2009[9-11] but not in consensus with the study by Huang liangliang[13] who had only 15% cases with RC.

When the occurrence of RC in invasive breast carcinoma was compared with clinicopathlogical features, significant correlation was noted with tumour size (p=0.001), LVI (p=0.004), PNI, Lymph node (LN) metastasis (p=0.03) and TNM stage (p=0.001), when the cases were divided into 2 groups of high and low RC, with a cut-off of 75%. These findings were consistent with the 3 studies by Geza ACS et al [9-11].

On comparison of RC with hormonal status a positive correlation was found (p=0.03) with HER2 status which was consistent with study by Geza Acs et al in the year 2015 and Huang Liang et al[13] study. Geza ACS et al [9-11] also found no correlation of ER and PR status with RC in his two studies which is consistent with our study. In the study by Huang Liang et al [13] when the RC was divided into 2 groups of >75% RC and <75% RC, they found ER to be correlating with presence of RC which was not the case in our study while it correlated significantly with HER2 positive status. The molecular subtypes also did not show any statistical significance in our study similar to study by Huang Liang et al [13].

CONCLUSION:

The study conducted on 50 cases of breast carcinoma revealed that presence of retraction clefts (RC) had direct relationship with an increasing tumour size and stage, presence of PNI, LVI, lymph node metastasis and HER2 positive status. However there was no significant correlation found between RC and various other histopathological features, though a positive trend was seen with them. Hence to better understand their relationship with clinicopathological variables, the prognosis of the disease and for the development of new targeted therapies, more research on retraction cleft and its correlation with disease progression is required with a larger study group.

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