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Original Article

Association of clinico-pathological and immunohistochemical prognostic parameters with presence of ductal carcinoma in-situ in invasive ductal carcinoma of breast

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Keywords:

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ABSTRACT

Background: The clinical outcome of breast carcinoma varies in each individual due to its molecular heterogeneity. There is a rising interest in whether the associated ductal carcinoma in-situ in invasive ductal carcinoma of breast affects the prognosis and overall survival of the patient. This study evaluates the difference in clinico-pathological and immunohistochemical prognostic factors between invasive ductal carcinoma with associated in-situ component, and invasive ductal carcinoma alone.

Materials and Methods: The study was conducted at a tertiary care hospital in South Tamilnadu, India. Two study groups were categorized based on the presence/absence of in-situ component in invasive ductal carcinoma of breast. Clinico-pathological variables and immunohistochemistry [Estrogen receptor (ER), Progesterone receptor (PR), HER2/neu and ki67] findings were compared between the two groups and statistical analysis was performed.

Results: There were 25 cases in each group. A significant statistical difference in tumor size was observed between invasive ductal carcinoma associated with in-situ component (mean-3.6cm) and without in-situ component (mean-5.0cm). A higher proliferative index (60%) was seen in invasive ductal carcinoma alone. There was no difference in the expression of Her2neu between the two groups. A proportionate increase in premenopausal population (60%) and hormone receptor positivity (ER, PR) was observed in invasive ductal carcinoma associated with in-situ component.

Conclusions: invasive ductal carcinoma associated with ductal carcinoma in-situ shows a less aggressive behaviour compared to invasive carcinoma alone. Further studies of a larger scale need to be done which might help in identifying the subgroup for targeted therapy.

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INTRODUCTION

Breast carcinoma is the most common malignant tumor among women. According to GLOBOCON 2020 estimates, female breast cancer is the most commonly diagnosed cancer worldwide, with an estimated 2.3 million new cases (11.7%) and 6.9% of cancer deaths.[1] The major risk factors associated with breast cancer are heredity, estrogen exposure, lifestyle and environmental factors.[2] Increased mammographic screening has led to the early detection of in-situ carcinoma, comprising 20-45% of all newly detected mammographic breast lesions.[3]

Many studies have shown that about 50% of untreated ductal carcinoma in-situ (DCIS) progresses to invasive breast cancer. The time duration and the factors predicting the progression of in-situ components to invasive carcinoma is largely unknown.[4] In tumors with both invasive and in-situ components, it is believed that the invasive cancer develops from in-situ component. In tumors without DCIS component, the invasive ductal carcinoma is presumed to arise from atypical ductal hyperplasia or de-novo.[5,6] Recently, studies have tried assessing the effect of presence of in-situ component in invasive ductal carcinoma on the prognosis and overall survival of the patients.

It is also debated whether IDC alone (IDC without DCIS) is distinct from IDC associated with DCIS (IDC-DCIS).[5-7] Steinman et al conclude that DCIS is a precursor for IDC-DCIS based on the similar expression of molecular markers in both in-situ and invasive components.[8] Studies by Aubele et al and Alexe et al also supported the above-said concept based on genomic data.[9,10] But on the contrary, Farabegoli et al suggested that DCIS may not have been a precursor of the invasive component.[11]

Several molecular markers have also been implicated in the development and prognosis of the breast cancer, including hormone receptors Estrogen receptor (ER) and Progesterone receptor (PgR), HER2/neu, Ki-67 etc. In addition to predicting the prognosis, these markers are also used for targeted therapy and to assess the response to treatment. 10-34% of breast carcinomas show overexpression of HER2/neu protein.[12,13] HER2/neu is an independent prognostic indicator for the overall survival of patients with breast carcinoma.[14,15] Ki- 67, a proliferation marker also acts as both prognostic and predictive factor. The effect of these markers in IDC-DCIS is yet to be evaluated, which might affect the therapeutic approach to breast carcinoma.

This study was undertaken to document the association of clinicopathological and immunohistochemical characteristics in invasive ductal carcinoma with and without in situ component.

MATERIALS AND METHODS

The present study is a comparative study conducted in the Department of Pathology at Govt Tirunelveli Medical College, Tirunelveli, Tamilnadu, India for a period of two years. Institutional Ethical clearance was obtained from Institutional Ethical Committee.

Modified radical mastectomy specimens with histopathological diagnosis of Invasive ductal carcinoma were included in the study. Breast carcinomas with a histopathological diagnosis other than IDC (Like Invasive lobular carcinoma of breast, Metaplastic carcinoma etc.) and patients who had received neoadjuvant chemotherapy were excluded from the study.

All resected Modified radical mastectomy specimens were adequately fixed in 10% neutral buffered formalin. Extensive sampling was done to search for DCIS and representative tissue sections were taken. After tissue processing and paraffin embedding, 4-5 µ thickness sections were taken and routinely stained with haematoxylin and eosin. The slides were examined and tumors with in-situ components were taken as one group (IDC-DCIS) and invasive ductal carcinoma without DCIS is taken as another group (IDC alone).

The invasive component of the tumor was graded according to Modified Bloom and Richardson grading system. Relevant clinical history like age, and menopausal status were recorded for all patients from the clinical case records. Other prognostic variables like tumor size, presence/absence of DCIS component, nipple invasion, presence/absence of necrosis, lymphovascular invasion, and number of lymph nodes showing metastasis were also assessed.

Immunohistochemistry was performed for ER/PgR, Her2/neu and Ki-67 in formalin-fixed and paraffin embedded tissue sections using standard protocol. Allred score was used to interpret ER and PgR staining and a score of ≥ 3 was considered positive. Her2/neu was interpreted as per ASCO guidelines and membrane staining of 3+ was considered positive.[16] In both the groups, HER2/neu amplification was assessed only in the invasive component. Expression of Ki 67 was reported as the percentage of positive tumor cells by observing 500 tumor cell nuclei in areas of the section with the highest labeling frequency. As per St Gallens Consensus 2013, a cut-off point of 20% was taken to separate the cases into two groups: High proliferative rate (>20%) and Low proliferative rate ($\leq$ 20%).[17]

Statistical analysis was performed by SPSS software Version 17 (SPSS, Chicago, IL, USA). Pearson's chi-square, Fisher's exact test and student's t-test were used to evaluate the statistical differences between the two groups. p-value <0.05 is considered significant.

RESULTS

Our study had 25 cases in each group. In the both IDC alone (median 53 years) and IDC-DCIS group (median-54 years), the most common age group affected was 41-50 yrs. Postmenopausal women were most affected in both the groups. Among the premenopausal population, 60% of cases were in IDC-DCIS category. (Table 1)

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Table 1: Clinical variables of invasive ductal carcinoma with associated in-situ component (IDC-DCIS) and invasive carcinoma without associated in-situ component (IDC alone)

	Variable	IDC-DCIS No of cases (%)	IDC alone No of cases (%)	p value
Age in years	≤ 40	1 (4%)	-	0.819
	41-50	9 (36%)	11 (44%)	
	51-60	9 (36%)	7 (28%)	
	61-70	5 (20%)	6 (24%)	
	71-80	1 (4%)	1 (4%)	
Menopausal Status	Premenopausal	12 (48%)	8 (32%)	0.386
	Postmenopausal	13 (52%)	17(68%)	

In both the groups, majority of the patients had a tumor size between 2-5 cm. IDC-DCIS showed a small tumor size in 16 % of cases compared to that of 8 % in IDC alone. Increased tumor size (> 5cm) was seen in IDC group. In both groups, grade 2 was most common comprising 64% in IDC-DCIS and 68% in IDC alone. In both IDC with and without DCIS, 16% of the cases showed tumor necrosis. In both IDC with and without DCIS, one case in each group presented with nipple invasion. (Table 2)

Table 2: Pathological variables of IDC-DCIS and IDC

	Variable	IDC with DCIS No of cases (%)	IDC No of cases (%)	p value
Tumor size	Mean & SD	Mean-3.66 SD -1.559	Mean -5.09 SD -2.868	0.046 p value-< 0.05
	<2cm	4 (16%)	2 (8%)	
	2-5cm	15 (60%)	14 (56%)	
	>5cm	6 (24%)	9 (36%)	
Tumor grade	Grade 1	6 (24%)	4 (16%)	0.651
	Grade 2	16 (64%)	19 (76 %)	
	Grade 3	3 (12%)	2 (8%)	
Necrosis	Present	4 (16%)	4 (16%)	1.000
	Absent	21 (84%)	21 (84%)	
Nipple invasion	Present	1 (4%)	1 (4%)	1.000
	Absent	24 (96%)	24 (96%)	

The DCIS component in IDC-DCIS group was predominantly comedo type constituting 68%. Among other patterns, solid type comprised 12%, cribriform type 8%, mixed (all non comedo types) 8% and micropapillary type 4%. 80% of the IDC-DCIS was associated with high-grade DCIS and the rest 20% of the tumors showed low-grade DCIS. (fig. 1).

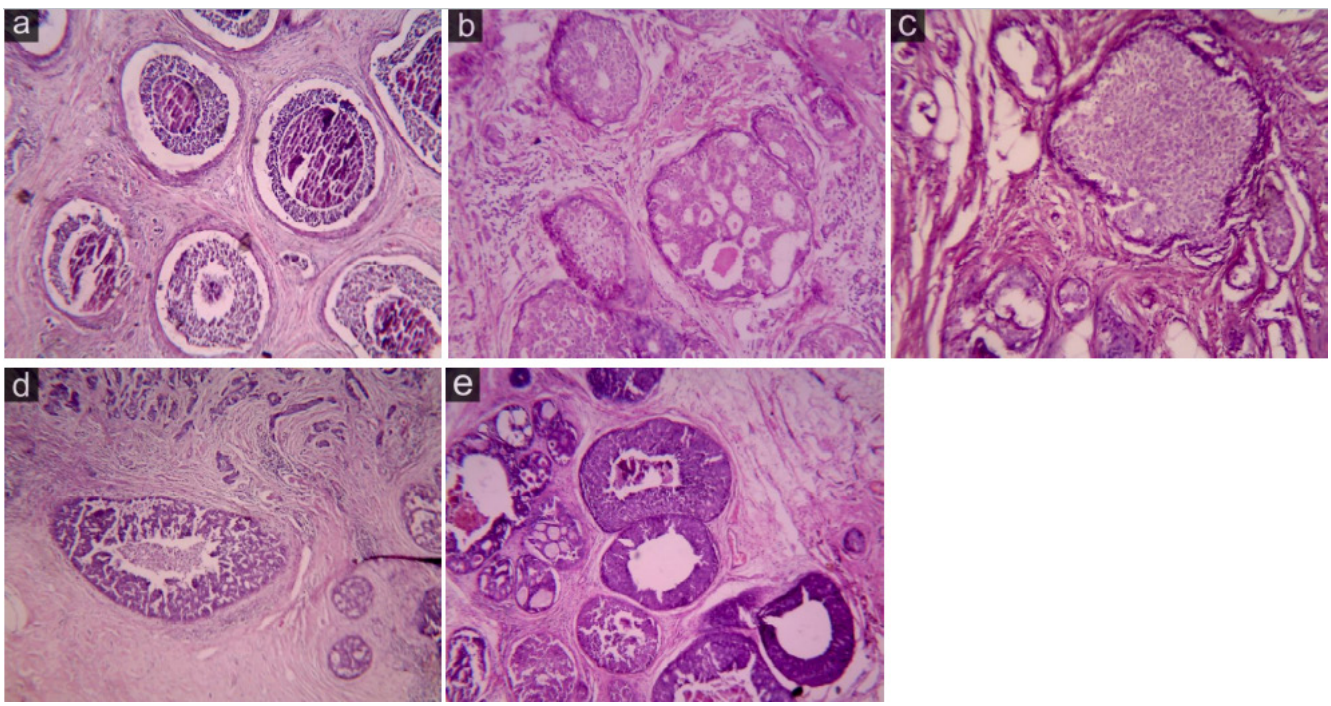

Figure 1: *Ductal carcinoma in-situ (DCIS). a- Comedo type DCIS (H & E stain,100X), b-Cribriform type DCIS (HE stain,100X) , c-Solid type DCIS (H & E stain,100X), d - Micropapillary type DCIS (H & E stain,100X), e-Mixed type DCIS (HE stain,100X).*

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Lymph node involvement was not seen in 44 % and 28% of cases in IDC-DCIS and IDC alone groups respectively. Higher number of nodal involvement (>9 nodes) was seen in 8% of the cases in IDC-DCIS group and 16% of cases in IDC alone group. 6 cases of IDC-DCIS and 8 cases of IDC alone showed lymphovascular invasion. There was no statistical significance between two groups. (Table 3)

Table 3: Lymphovascular invasion and lymph node status of invasive ductal carcinoma with in-situ component (IDC-DCIS) and invasive carcinoma without in-situ component (IDC)

		IDC-DCIS	IDC	p value
Lymphovascular invasion	Present	6 (24%)	8 (32%)	0.753
	Absent	19 (76%)	17(68%)	
Lymph node status	Negative	11(44%)	7 (28%)	0.522
	1-3	6 (24%)	5 (20%)	
	4-9	6 (24%)	9 (36%)	
	>9	2 (8%)	4 (16%)	

Estrogen receptor was found to be positive in 16 cases (64%) among IDC-DCIS group and 13 cases (52%) in IDC alone group. Progesterone positivity was seen in 11 (44%) in IDC-DCIS group and 9 (36%) in IDC alone group. ER/PR positivity was higher in IDC-DCIS compared to IDC alone. In IDC-DCIS 5 cases (20%) showed HER2/ neu positivity. In IDC alone, 8 cases (32%) showed HER2/ neu positivity. There was no significant difference in HER2/neu status between IDC with and without DCIS. In IDC-DCIS, only 7 (28%) cases showed a high proliferative index. However, in IDC alone group 15 (60%) showed a higher proliferative index which was statistically significant (p-value- 0.022) (Table 4 and fig. 2 and 3).

Table 4: Immunohistochemical studies in invasive ductal carcinoma with associated in-situ component (IDC-DCIS) and invasive carcinoma without associated in-situ component (IDC alone)

		IDC-DCIS	IDC alone	p-value
Estrogen receptor	Positive	16(64%)	13 (52%)	0.390
	Negative	9 (36%)	12 (48%)	
Progesterone receptor	Positive	11 (44%)	9 (36%)	0.390
	Negative	14 (56%)	16 (64%)	
Her2/neu	Positive	5(20%)	8(32%)	0.949
	Negative	19(76%)	16(64%)	
	Equivocal	1(4%)	1(4%)	
Ki-67	Low(≤ 20)	18(72%)	10(40%)	0.022
	High(>20)	7(28%)	15(60%)	

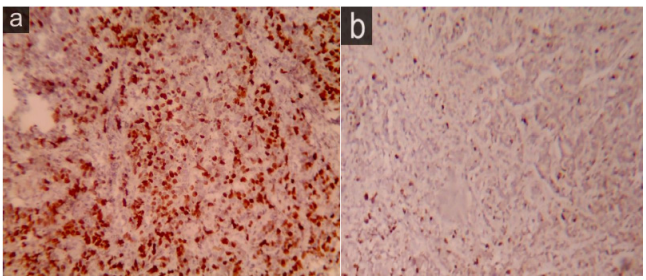

Figure 2: *Ki67 proliferation index. a.High Ki-67 index (DAB, 100X). b.Low Ki-67 index (DAB, 100X)*

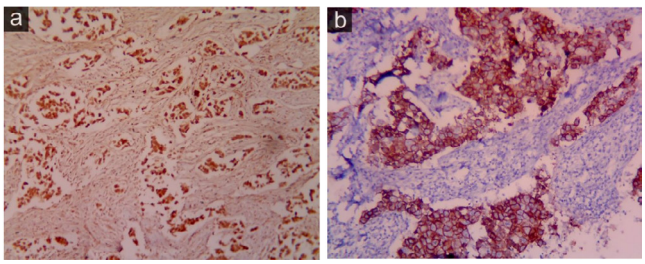

Figure 3: *Immunohistochemistry. a.Estrogen receptor positivity (DAB, 100X). b.Her2/neu positivity - Score 3.+ (DAB, 100X)*

DISCUSSION

The clinical outcome of breast carcinoma varies with every individual due to the heterogeneous nature of the tumor. Recently, studies have been exploring whether IDC-DCIS and IDC alone might be biologically distinct and also if the associated DCIS affects the prognosis of the patient.[5-9] (Table 5)

Table 5: Number of cases studied in various studies.

Studies	IDC-DCIS	IDC alone
B H Jo et al[5]	144	84
H Wong et al[6]	616	543
Mylonas et al[7]	36	130
Rana S Aziz et al[20]	12	7
Present study	25	25

Study done by Wong et al suggested a hypothesis that IDC alone acquired invasive potential at the early stage of carcinogenesis and so there is no features of pre-invasive potential. The tumors with delayed progression from pre-invasive DCIS to IDC present as IDC-DCIS, which indicates that IDC-DCIS is of decreased biological aggressiveness.[6]

Previous studies have demonstrated that when compared to tumors with IDC alone, IDC-DCIS tumors are associated with smaller tumor size, lower tumor grade, lower Ki-67 staining, a higher number of ER-positivity and reduced risk of local recurrence when compared to IDC alone.[5-7,18,19] Also, a few studies also observed an improved overall survival (OS) in IDC-DCIS as compared to IDC alone.[5,6,15] Similarly Kole et al observed an improved survival in IDC-DCIS in node negative and tumor size of <4cm. Hence he suggested that the presence of DCIS component may be a

marker of reduced aggressiveness and it can be included as a prognostic factor in future.[21]

In the present study, the median age of IDC-DCIS was 54 years and IDC alone is 53 years. Median age was higher compared to studies of B H Jo et al[5], H Wong et al.[6] Most cases in both groups are in the postmenopausal status. However, premenopausal population is proportionately higher in IDC-DCIS (48%) compared to that of IDC alone (32%). Studies by B H Jo et al[5], H Wong et al[6] also showed a higher proportion of premenopausal women in IDC-DCIS.

In our study the median tumor size of IDC-DCIS was 3.6cm, while that of IDC alone was 5cm. There is significant statistical difference in tumor size between both groups which is in concordance with other studies.[18-20] But the median size of the present study was comparatively higher than the results of H Wong et al[6] and Chagpar et al.[18] (Table 6) Carter et al suggested that tumor size is an independent risk factor in node negative cases and as tumor size increased, survival decreased regardless of lymph node status.[22] So it can be suggested that tumors with IDC-DCIS can have a better prognosis compared to that of IDC alone.

Table 6: Comparison of tumor size of IDC-DCIS vs IDC alone, with other studies

Studies	IDC-DCIS size	IDC alone
Wong et al[6]	1.8 cm	1.8 cm
Dietrich et al[14]	1.7cm	2.3 cm
Chagpar et al[15]	1.37 cm	1.44 cm
Present study	3.66 cm	5.09 cm

In our present study most patients presented with histological grade 2 with 68% in IDC-DCIS and 76% in IDC alone groups. This is in concordance to the study done by Dietrich et al who showed 58.9% of IDC-DCIS and 60.4% of IDC alone group to be of grade 2.[18] Higher proportion of grade 3 cases were seen in the study done by Wong et al in both IDC-DCIS (48.9%) and IDC (54.7%) and Chagpar et al showed higher tumor grade in IDC-DCIS.[19] In the present study, an equal number of cases showed tumor necrosis and nipple invasion in both groups. But these prognostic variables were not assessed in other studies.

Axillary lymph node ìnvolvement is the most important prognostic factor in operable breast cancer.[23] In the present study, 72% of the IDC cases showed lymph node positivity compared to that of 56% of IDC-DCIS. This is concordant with the results of Dietrich et al where high percentage of lymph node involvement seen in IDC alone (28.7%) compared to IDC-DCIS (13.3%). The prognosis is also dependent on the number and the level of regional lymph nodes. Fisher et al suggested that the prognosis will be poor if a greater number of nodes is involved.[24] In our study, there was a higher number of lymph node involvement in IDC alone compared to that of IDC-DCIS which is similar to that of H Wong et al.[6] So it can be suggested that IDC-DCIS have a better prognosis compared to IDC alone.

Expression of molecular markers not only determine the prognosis of the patient but also aids in deciding the treatment modality. In this study, in IDC-DCIS there is a higher rate of ER positivity with 64% compared to 52% in IDC alone and PR positivity is seen in 44% of IDC-DCIS and 36% of cases in IDC alone. This was in concordance with previous studies.[5,7,19] Jo et al also find a significant difference among the two groups.[5]

Her2/neu overexpression confers a worse biological behaviour but it is also a good predictor of response to trastuzumab. In the present study, 20 % of IDC-DCIS showed Her2/neu expression compared to 32 % in IDC alone. (Table 4) This result was concordant with the results of Mylonas et al, which showed 31% positivity in IDC-DCIS and 49.6% in IDC alone but contrary to the results of H Wong et al where higher HER2/neu amplification was seen in IDC-DCIS (25.5%) compared to IDC alone (16.2%).[6,7]

In the present study, IDC alone showed a higher Ki-67 index of 60% compared to that of 28% in IDC-DCIS. Our study showed a significant difference in Ki-67 expression between IDC-DCIS and IDC alone with a p-value of 0.02. This was similar to the studies of H Wong et al[6], Mylonas et al[7] who showed a significant difference between the two groups. A high Ki 67 index is correlated with decreased overall survival and relapse free survival irrespective of the nodal status.[25] This shows the presence of DCIS is associated with less aggressiveness. Mylonas et al also suggested that since the expression of Her 2/neu and Ki-67 is lower in IDC-DCIS compared to that of IDC alone, IDC-DCIS could implicate a less malignant behavior than IDC alone.[7]

In summary, IDC-DCIS tumors show a small tumor size, decreased number of lymph node involvement, a proportionate increase in hormone receptors positivity and low ki-67 expression. This infers that the presence of DCIS confers ductal carcinoma to be less aggressive.

CONCLUSIONS

Invasive ductal carcinoma with an associated DCIS component can be considered as less aggressive as compared to those without an associated DCIS component. However, authors acknowledge small sample size of the present study and lack of long-term follow-up. Hence, to consider the presence of DCIS in an invasive ductal carcinoma as a distinct and favorable prognostic factor, further studies of a larger scale should be conducted. This might help in identifying the subgroup for personalized therapy.

Conflict of Interest: None

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