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Immunohistochemical expression of CD44 in invasive breast carcinoma

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ABSTRACT

Background: In India, breast cancer forms the most common malignancy, accounting for 28.2% of all female cancers. Breast cancer consists of phenotypically diverse group of diseases. Due to different relapse abilities, doubling times, and different infiltrative capacities, it is important to identify potential biomarkers that could be used to screen high-risk patient and predict breast cancer prognosis in conjunction with classical pathological parameters. **Aims and Objectives**: The present study was conducted to evaluate immunohistochemical expression of CD44 in breast carcinoma and to correlate CD44 expression with other clinicopathological parameters and molecular classification of carcinoma breast. **Materials and Methods:** The present study was a prospective descriptive study conducted on 100 cases of carcinoma breast diagnosed on modified radical mastectomy specimens that were submitted to the Department of Pathology, Pt. B. D. Sharma, PGIMS, Rohtak, Haryana, over the period of 2 years. **Results:** Out of 100, 64 cases (64%) were CD44 positive. Out of these 64 cases, 9 (9%) cases were 1+, 34 (34%) cases were 2+, and 21 (21%) cases showed 3+ positive expression of CD44 in tumor cells. Loss of CD44 expression was seen in 36 (36%) cases. A statistically significant association of CD44 was seen with multifocality of the tumor, negative estrogen receptor/progesterone receptor (ER/PR) expression, and molecular subtypes. No statistically significant association of CD44 was seen with age, side of tumor, lymph node metastasis, lymphovascular invasion, ductal carcinoma *in situ* and Ki67, and HER2neu expression. **Conclusion:** A high CD44 expression in breast carcinoma correlates with aggressive tumor characteristics such as multifocality, negative ER/PR expression, and belonging to the basal subtype which is associated with poor prognosis. Conversely, low CD44 expression is linked to Luminal A and Luminal B subtypes which have good prognosis. These indicate the role of CD44 as a prognostic biomarker in the carcinoma breast.

Key words: Carcinoma breast; Immunohistochemistry; CD44; Molecular expression; Invasive

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INTRODUCTION

In India, cancer of the breast is the most common cancer among women in many regions and has overtaken cervix cancer, which was the most frequent cancer a decade ago. The histomorphological types seen in breast cancer patients indicate that invasive ductal carcinoma not otherwise specified (IDC NOS) was found to be the most common type (88%), followed by infiltrating lobular carcinoma (3.7%), colloid carcinoma (1.1%), ductal carcinoma *in situ* (DCIS) (1.1%), and metaplastic types (0.9%).[1]

Breast cancer represents a biologically and phenotypically heterogeneous collection of diseases with different clinical behaviors. In this era of modern medicine, only the morphological classification (nuclear grade, tubular grade, mitotic index, histological grade, and morphological characteristics) and the clinicopathological parameters:

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Tumor size, lymph node involvement, and metastasis are insufficient to predict the real behavior of breast tumor pathophysiology. Thus, many studies focus on analyzing the molecular patterns of breast cancer to group these tumors into classes to assist in clinical management.

Based on comprehensive gene expression profile studies, four clinically relevant molecular subtypes were revealed: Luminal A, Luminal B, enriched human epidermal growth factor receptor 2 (HER2) (HER2+), and triple negative. The groups of genes responsible for the segregation of the molecular subtypes of breast carcinoma are genes related to the expression of estrogen receptors (ERs), progesterone receptors (PRs), HER2, and cell proliferation regulator (Ki-67). The immunohistochemical (IHC) panel with these four biomarkers (ER/PR/HER2/Ki-67) has been considered efficient and significant in the stratification of these molecular entities[2] (Table 1).

CD44 is a cell surface transmembrane protein and is the principal receptor for hyaluronic acid (HA), a major component of ECM. CD44 was identified as the first integral hyaluronan HA binding receptor. HA is the main component of the extracellular matrix and its abundance is associated with aggressive tumor type, metastasis, and cancer progression.[3] CD44 also binds to HER2. HER2 overexpression can activate singling pathways, promoting cell survival, tumor growth, and metastasis. The CD44-HER2 complex by HA increases the growth of malignant cells.[4]

CD44 plays essential roles in the cancer progression of multiple tumor types, including breast cancer, lung adenocarcinoma, ovarian cancer, and glioblastoma. Invasion from *in situ* to adjacent tissues of tumor cells occurs before metastasis and contributes to cancer development. It has been found that CD44 cancer cells are among the chief subgroup of collectively invading luminal breast tumor cells with distinctive gene profile of mesenchymal gene and pivotal functional regulators of invasion.[5]

The studies conducted on the invasive breast cancer patient using CD44 and their correlation with molecular classification, clinicopathological parameters, and lymph node metastasis had shown variable conflicting results. The present study was being conducted to evaluate these parameters in the population in our part of the country.

Aims and objectives

Aim

To evaluate immunohistochemical expression of CD44 in breast carcinoma.

Objectives

1. To correlate CD44 expression with molecular classification of breast carcinoma.
2. To correlate CD44 expression with clinicopathological parameters of breast carcinoma.

MATERIALS AND METHODS

Case selection

The present study was conducted in the Department of Pathology at PGIMS, Rohtak, Haryana, over 100 breast carcinoma specimens. It was a prospective observational study conducted over a period of 2 years from January 2022 to December 2023.

Inclusion criteria

All cases of breast carcinoma were included in the study.

Exclusion criteria

Cases with *in situ* carcinoma, lumpectomy specimen, tru-cut biopsy, cases with incomplete information, and inadequate biopsies were excluded from the study.

Morphological evaluation

All the specimens received in the department of pathology were subjected to careful and detailed gross examination. Specimens were fixed in 10% neutral-buffered formalin, routinely processed, paraffin embedded, and the sections were stained with hematoxylin and eosin stain.

Histologic grading

Histopathological diagnosis was established on routine H and E stains. Histologic grading was done using the Modified Bloom-Richardson (MBR) grading system and Nottingham prognostic index (NPI) taking into account the scores for tubule formation, nuclear pleomorphism, and mitotic count.[6]

The details of scoring of individual parameters are as follows:

- Tubule formation
  - Score 1: >75% tumor area showing tubule formation

Table 1: Molecular classification of breast carcinoma

Immunoprofile	Luminal A	Luminal B	HER2 Enriched	Basal-like
ER, PR	ER+PR+	ER+ and PR+	ER-PR-	ER-PR-
HER2	HER2-	HER2±	HER2+	HER2-
Others	Low Ki-67 (≤15%)	Ki-67 >15%		CK5/6and/or EGFR+

ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2

  - Score 2: 10–75% tumor area showing tubule formation
  - Score 3: <10% tumor area showing tubule formation.

- Nuclear pleomorphism
  - Score 1: Mild Variation in shape and size
  - Score 2: Moderate pleomorphism with visible nucleoli
  - Score 3: Marked pleomorphism with prominent nucleoli.

- Mitotic count
  - Score 1: 0–11 mitotic count (per 10 high power field at tumor periphery)
  - Score 2: 12–22 mitotic count (per 10 high power field at tumor periphery)
  - Score 3: ≥23 mitotic count (per 10 high power field at tumor periphery).

- Histologic grade was assessed by adding up the scores of the three parameters
  - Grade I (well differentiated)=3–5
  - Grade II (moderately differentiated)=6–7
  - Grade III (poorly differentiated)=8–9.

- NPI[7]

Using tumor size, MBR histologic grade, and lymph node stage, NPI was calculated.

NPI=(0.2×size of tumor)+lymph node stage+histologic grade

NPI thus calculated was interpreted as under:
- <3.4=good prognosis
- 3.4–5.4=moderate prognosis
- >5.4=poor prognosis.

IHC analysis

Immunohistochemistry was assessed by subjecting one section each from representative block to CD44 and other IHC markers (ER, PR, HER-2neu, and Ki-67) for molecular profiling. IHC stains were performed using standard technique.

Data interpretation

Cases showing membranous staining for CD44 in tumor cells were graded as under:[8]
- Score 0: No+ve cells
- Score 1: <25%+ve cells
- Score 2: 25–75%+ve cells
- Score 3: >75%+ve cells

Positive control

The adjacent normal breast tissue and the fibrocystic disease.

Negative control

Negative control was obtained by substituting the primary antibody with an antibody of nonspecific relevance.

ER/PR staining

Brown diffuses or grainy nuclear staining was taken as positive for ER/PR and assessed by Allred scoring based on the assessment of proportion and intensity.

Score for proportion (PS)
- 0=no staining
- 1=<1% nuclei stained
- 2=1–10% nuclei stained
- 3=11–33% nuclei stained
- 4=34–66% nuclei stained
- 5=67–100% nuclei stained

Score for intensity (IS)
- 0=no staining
- 1=weak staining
- 2=moderate staining
- 3=strong staining

The scores were summed to give a maximum of 8. Patients with tumors scoring 2 or less were regarded as ER/PR negative.

HER2neu staining

HER2/neu was assessed by HER2/neu scoring system. Brown membranous staining was taken as positive. IHC analysis showing uniform, intense membrane staining of >10% of the tumor cells was taken as positive.

The CD44 expression was correlated with various clinicopathological parameters such as age, tumor size, tumor type, axillary lymph node status, histological grade, NPI score and ER, PR and HER2/neu.

Statistical analysis

The results obtained were interpreted and correlated statistically. Mean and standard deviations were calculated. When the data were qualitative, a Chi-square test was used to assess the association between these parameters. A value of $P<0.05$ was taken as significant.

The collected data were analyzed with help of a software package (Statistical Package for the Social Sciences version 20.0). All the data enlisted in the investigation pro forma (name, age, sex, CR no, clinical diagnosis, and history) were collected. Frequency distribution and cross tabulation were used to create observation tables

and compare items within and across various categories. Association and correlation were assessed using the Chi-square test. P<0.05 was taken as statistically significant.

RESULTS

The present study was a descriptive study conducted on 100 cases of carcinoma breast. The diagnosis was made on modified radical mastectomy (MRM) specimens (Trucut and excisional biopsy were excluded) submitted to the Department of Pathology, Pt. B. D. Sharma, PGIMS, Rohtak.

The age ranged from 26 to 75 years with mean age of 50.98 years. The maximum number of cases (31 cases; 31%) were in the age group of 41–50 years (Figure 1).

The maximum number of cases (50 cases; 50%) were situated in the upper outer quadrant while the lower quadrant was least involved in 3% (3 cases) of the total cases. Only 10 cases (10%) had a positive family history. A negative family history was seen in 90 (90%) cases. Out of 100, 51 cases (51%) had tumor in the right breast while left laterality was seen in 49 cases (49%). Majority of cases, 93 (93%) were unifocal while multifocality was seen in 7 cases (7%) only.

Morphological evaluation

All the cases were divided according to tumor size into three subgroups (≤2 cm, 2–5 cm, and ≥5 cm). Maximum cases, 69 (69%) were in subgroup of tumor size 2–5 cm, followed by 18 cases (18%) in the group ≤2 cm, and the least cases, 13 (13%) belonged to the subgroup of ≥5 cm. The mean size of tumor was 3.67±2.32 cm while size range was 0.4–14.0 cm.

Infiltrating ductal carcinoma – NOS was the most common histologic subtype among all the cases accounting for 94 (94%) of the total cases. Rest of the cases were of histological subtypes invasive medullary 3 (3%), invasive lobular 2 (2%), and invasive mucinous 1 (1%). Lymph node metastasis was seen in 46 (46%) cases only. Lymphovascular invasion (LVI) was present in 15 (15%) cases. DCIS was seen in 12 (12%) cases.

MRM specimens were graded using Nottingham MBR grading system. Grade II comprised majority of the total cases 68 (68%). Twenty (20%) cases were under Grade I and the least number of cases were in Grade III 12 (12%). On the basis of NPI score, cases were categorized in three prognostic groups – good, moderate, and poor. Majority of the cases 66 (66%) belonged to the moderate prognostic group, followed by 21 (21%) cases in the good prognosis

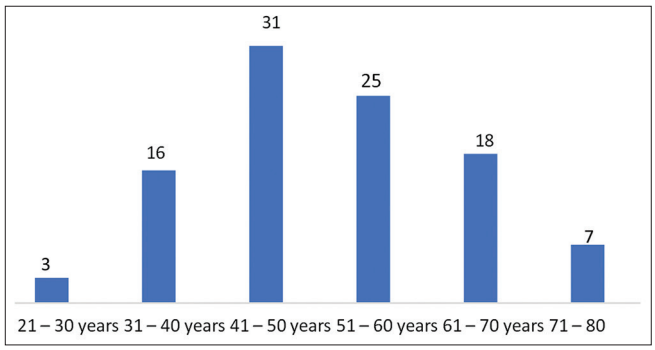

Figure 1: Distribution of cases according to age (n=100)

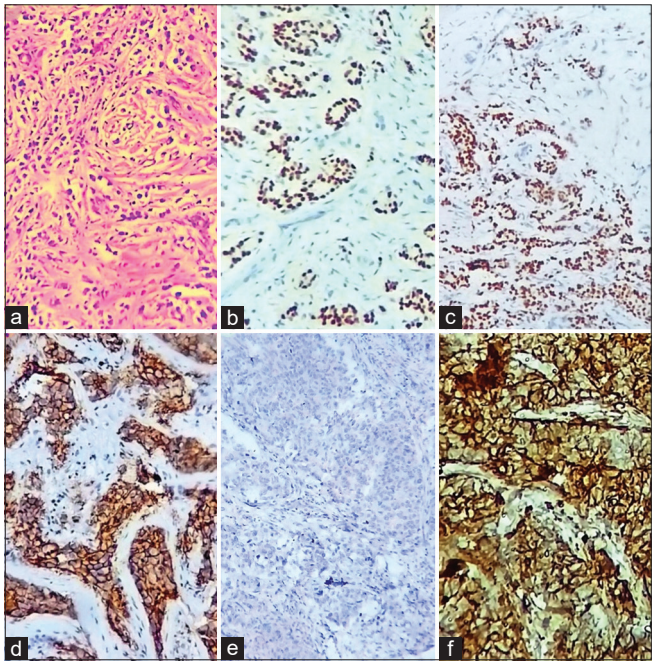

Figure 2: (a) Infiltrating ductal carcinoma breast-not otherwise specified (H and E, ×200) (b) nuclear positivity for estrogen receptor (Immunohistochemical, ×200) (c) nuclear positivity for PR (Immunohistochemical, ×100) (d) Membranous positivity for HER2neu (Immunohistochemical, ×400) (e) Negative Expression for CD44 (Immunohistochemical, ×100) (f) Membranous positivity for CD44 (Immunohistochemical, ×400)

group and 13 (13%) cases in the poor prognosis group. Mean NPI (±SD) was 4.15 (±1.17) while the range was 2.09–8.80.

IHC analysis (Figure 2)

ER and PR expression was assessed by Allred scoring. Only 39 (39%) of the total cases were ER positive while 61 (61%) cases were ER negative. Only 34 (34%) of the total cases were PR positive while 66 (66%) cases were PR negative.

HER2neu status was assessed by HER2neu scoring system. Complete and intense membranous staining in >10% of tumor cells was considered HER2neu overexpression. Positive HER2neu immunoexpression was seen in 22 (22%) cases, 3 (3%) cases were equivocal, and maximum cases 75 (75%) showed negative HER2neu immunoexpression.

Out of 100, 52 (52%) cases showed ≤15% Ki67 positivity and >15% Ki67 positivity was seen in 48 (48%) of the cases.

On the basis of molecular classification of breast carcinoma, 21 (21%) cases were Luminal A type, 16 (16%) cases were Luminal B type, 14 (14%) cases were HER2 enriched, and 43 (43%) cases were of basal type. Maximum number of cases 43 (43%) belonged to the basal type and the least number of cases 14 (14%) were of HER2 enriched type.

Membranous staining of the tumor component was taken as positive for CD44 staining. The adjacent breast tissue and fibrocystic disease were taken as positive control. Sixty four (64%) of the total cases were CD44 positive. Out of these 64 cases, 9 (9%) cases were 1+, 34 (34%) cases were 2+, and 21 (21%) cases showed 3+ positive expression of CD44 in tumor cells. Loss of CD44 expression was seen in 36 (36%) cases (Table 2).

Correlation of CD44 with clinicopathological parameters

The maximum number of cases that showed membranous positivity for CD44 fall in the age group 41–50 years (P=0.725). Out of CD44-positive cases, only 7 (7%) cases had a positive family history of breast cancer (P=0.380).

CD44-positive cases were more common in left-sided breast carcinoma cases. Out of a total of CD44-positive cases, 35 had left-sided breast cancer. Among these, six cases showed 1+, 18 cases showed 2+, and 11 cases showed 3+ expression of CD44. Twenty-nine out of the total CD44-positive cases had a right-sided tumor (P=0.407).

In 57 of total CD44-positive cases, the tumor was unifocal. Among these, seven had 1+, 32 had 2+, and 18 had 3+ expression of CD44. Seven multifocal tumors showed positive expression of CD44. A statistically significant association was seen between CD44 expression and focality of the tumor (P=0.011) (Table 3).

The maximum number of cases that showed membranous positivity for CD44 falls in the category with tumor size 2–5 cm. Among these, seven showed 1+, 22 showed 2+, and 15 showed 3+ expression of CD44 (P=0.252). Out of total CD44-positive cases, 24 had no nodal involvement, 23 cases showed 1–4 nodal involvement, and 17 cases were those in which the nodal involvement was 5 or more (P=0.666).

Out of total CD-44-positive cases, only eight cases showed LVI. Among these eight cases, one case showed 1+, four cases showed 2+, and three cases showed 3+ expression of CD44 (P=0.813). Out of total CD44-positive cases, only seven cases show DCIS, among these seven cases, two cases showed 1+, 3 cases showed 2+, and three cases showed 3+positivity (P=0.508). CD44 expression was more common in Grade II tumors. Out of the total CD44-positive cases, 41 cases had grade II tumors. Among these cases, seven showed 1+, 20 showed 2+, and 14 cases showed 3+ expression of CD44. Out of the total CD44-positive cases, 42 had a moderate prognosis. Among these cases, six showed 1+, 21 showed 2+, and 1 showed 3+ expression of CD44 (P=0.290) (Table 4).

There was a direct association of ER/PR expression with CD44-positive cases (P≤0.001). Thirteen out of 64 CD44-positive cases showed positivity for both ER and CD44, whereas 51 cases showed negativity for ER. 8 out of 64 CD44-positive cases showed positivity for both PR and CD44, whereas 56 cases showed negativity for PR. There was no significant association between CD44 and HER2neu expression (P=0.256). Out of total CD44-positive cases, 27 cases had Ki67 expression ≤15%, and 37 cases had >15% Ki67 expression (P=0.062).

Out of the total CD44-positive cases, the maximum number (40) cases belonged to the basal subtype followed by HER2 enriched (10) and the least number (6) belonged to Luminal B subtype. Seven cases belonged to Luminal A subtype. A significant association was seen with the CD44 expression and the molecular subtype of the carcinoma breast (P<0.001) (Table 5).

Table 2: Distribution of cases according to CD44 expression (n=100)

CD44 expression	Number of cases (%)
Negative	36 (36)
Positive	64 (64)
1+	9 (9)
2+	34 (14)
3+	21 (22)

DISCUSSION

Identification of biomarkers and gene expressions is needed to improve early diagnosis and prognosis of breast carcinoma, as well as to provide the most effective drug suitable with the molecular characteristics of the patients. Studies have indicated that prognostic and predictive biomarkers are molecules involved in the regulation of cellular mechanisms, including proliferation, apoptosis, angiogenesis, metastasis, and therapeutic resistance.

The present study was a descriptive study conducted in the Department of Pathology, Pt. B.D.S. PGIMS, Rohtak. Hundred cases of primary breast cancer were taken up for

Table 3: Association of CD44 expression with clinical parameters (n=100)

Clinical parameters	CD44				P-value
	Negative (%)	1+ (%)	2+ (%)	3+ (%)	
Age of the patient					
21–30 years	2 (5.55)	0 (0)	1 (2.94)	0 (0)	0.725
31–40 years	4 (11.11)	3 (33.33)	4 (11.76)	5 (23.8)	
41–50 years	12 (33.33)	3 (33.33)	8 (23.52)	8 (38.09)	
51–60 years	10 (27.77)	2 (22.22)	9 (26.47)	4 (19.04)	
61–70 years	7 (19.44)	0 (0)	8 (23.52)	3 (14.28)	
71–80 years	1 (2.77)	1 (11.11)	4 (11.76)	1 (4.76)	
Family history of the patient					
Positive	3 (8.33)	0 (0)	3 (8.82)	4 (19.04)	0.380
Negative	33 (91.66)	9 (100)	31 (91.17)	17 (80.95)	
Laterality of tumor					
Right	22 (61.11)	3 (33.33)	16 (47.05)	10 (47.61)	0.407
Left	14 (38.88)	6 (66.66)	18 (52.94)	11 (52.38)	
Focality of tumor					
Unifocal	36 (100)	7 (77.77)	32 (94.11)	18 (85.71)	0.011
Multifocal	0 (0)	2 (22.22)	2 (5.88)	3 (14.28)	

Table 4: Association of CD44 expression with pathological parameters (n=100)

Pathological parameters	CD44				P-value
	Negative (%)	1+ (%)	2+ (%)	3+ (%)	
Size of tumor					
≤2 cm	7 (19.44)	2 (22.22)	3 (8.82)	1 (4.76)	0.252
2–5 cm	25 (69.44)	7 (77.77)	22 (64.7)	15 (71.42)	
≥5 cm	4 (11.11)	0 (0)	9 (26.47)	5 (23.8)	
Lymph node involved					
None	22 (61.11)	4 (6.25)	12 (18.75)	8 (12.5)	0.666
1–4	8 (22.22)	4 (6.25)	11 (17.18)	8 (12.5)	
5 or more	6 (8.33)	1 (1.56)	6 (9.38)	10 (15.6)	
Lymphovascular invasion					
Present	7 (19.44)	1 (11.11)	4 (11.76)	3 (14.28)	0.813
Absent	29 (80.55)	8 (88.88)	30 (88.23)	18 (85.71)	
Ductal carcinoma *in situ*					
Present	5 (13.88)	2 (22.22)	2 (5.88)	3 (14.28)	0.508
Absent	31 (86.11)	7 (77.77)	32 (94.11)	18 (85.71)	
Grade of tumor					
Grade I	8 (22.22)	2 (22.22)	8 (23.52)	2 (9.52)	0.159
Grade II	27 (75)	7 (77.77)	20 (58.82)	14 (66.66)	
Grade III	1 (2.77)	0 (0)	6 (17.64)	5 (23.8)	
NPI					
≤3.4	10 (27.77)	2 (22.22)	7 (20.58)	1 (4.76)	0.290
3.4–5.4	21 (58.33)	6 (66.66)	21 (61.76)	15 (71.42)	
>5.4	2 (5.55)	1 (11.11)	4 (11.76)	5 (23.8)	

NPI: Nottingham prognostic index

study (MRM specimen only). Specimens were examined for tumor size, tumor grade (Nottingham MBR grading system), nodal involvement, NPI, hormonal status, and HER2neu expression. The expression of hormone receptors (ER/PR) using Allred scoring, HER2neu using HER2neu scoring system, and CD44 membranous expression were studied. The CD44 expression in the tumor area was then correlated with hormonal and other clinicopathological parameters.

Out of a total of 100 cases of primary breast carcinoma, the patient's age ranged from 26 to 75 years. The mean age was 50.98 years and the maximum number (31%) of the cases was in the age group 41–50 years. In our study, more than 50% of cases were diagnosed after 50 years of age. The mean age of various other studies (in Zou et al., 50% and in Rustamadji et al., 50.94%) was similar to our study.[9,10] The studies show that the risk for breast cancer increases significantly with age.

In this study, cases were divided according to tumor size into three subgroups. The subgroup of 2–5 cm, in size, formed the largest group with 69% of all the cases followed by 18% of the cases were in >5 cm and only 13% were in <2 cm. Tumor size >2 cm formed the largest group in many of the previous studies. The results of study done by Zou et al., Rustamadi et al. and Jang et al. were similar to our study, with majority of the cases in size category of 2-5 cm.[9-11] The presentation of the patient with a tumor

Table 5: Association between CD44 expression and immunohistochemical profile (n=100)

Immunohistochemical profile	CD44				P-value
	Negative (%)	1+ (%)	2+ (%)	3+ (%)	
ER					
Positive	26 (72.22)	5 (55.55)	5 (14.7)	3 (14.28)	<0.001
Negative	10 (27.77)	4 (44.44)	29 (85.29)	18 (85.71)	
PR					
Positive	26 (72.22)	2 (22.22)	4 (11.76)	2 (9.52)	<0.001
Negative	10 (27.77)	7 (77.77)	30 (88.23)	19 (90.47)	
HER-2 neu					
Positive	10 (27.77)	2 (22.22)	9 (26.47)	1 (4.76)	0.256
Equivocal	1 (2.77)	1 (11.11)	1 (2.94)	0 (0)	
Negative	25 (69.44)	6 (66.66)	24 (70.58)	20 (95.23)	
Ki67 (%)					
≤15	25 (69.44)	4 (44.44)	13 (38.23)	10 (47.61)	0.062
>15	11 (30.55)	5 (55.55)	21 (61.76)	11 (52.38)	
Molecular subtype					
Luminal A	14 (38.88)	2 (22.22)	3 (8.82)	2 (9.52)	<0.001
Luminal B	10 (27.77)	3 (33.33)	2 (5.88)	1 (4.76)	
HER-2 enriched	4 (11.11)	1 (11.11)	8 (23.52)	1 (4.76)	
Basal	3 (8.33)	2 (22.22)	21 (61.76)	17 (80.95)	

ER: Estrogen receptor, PR: Progesterone receptor, HER: Human epidermal growth factor receptor 2

size of more than 2 cm in the majority of cases may be attributed to low education levels, poor socioeconomic status, and also lack of awareness in the society.

IDC NOS constitutes the largest group in our study (94 cases), followed by other histological subtypes (6 cases) including invasive lobular, invasive medullary, and invasive mucinous. The results of Roosta et al., Rustamadji et al., and Looi et al., were in concordance with our study with the maximum number of cases of IDC NOS.[8,10,12]

The histological grading for cases of breast cancer was done using Nottingham MBR grading system. In our study, maximum number of cases were found to be of Grade II (68 cases). Grade I and Grade III tumors were found in 20–12 cases, respectively. Our results are concordant with results of Jang et al., (116 of 220 cases); Looi et al., (26 of 60 cases); and Syaifudin et al., (33 of 60 cases) having a maximum number of cases with histological Grade II.[11-13] Study by Rustamadji et al., (27 of 48 cases) showed a maximum number of cases in histological Grade III which was discordant with our study.[10]

Assessment of lymph node involvement was done in all the cases and staging was done based on a number of lymph node involvement. In 54 cases, lymph node involvement was not seen. The results of Chun et al., were similar to our study, showing maximum cases (133 of 262 cases) without lymph node metastasis.[14] In the study done by Jang et al., the maximum number of cases (29 of 46 cases) showed lymph node metastasis which was discordant to our study.[11]

Out of 100 cases, ER and PR-positive immunoexpression was found in 39% and 34% of cases, respectively. The results of study done by Chun et al., were concordant with our results for ER expression as there was less number of ER-positive cases then ER-negative cases.[14] Studies done by Jang et al., and McFarlane et al., were discordant to our study for ER expression.[11,15] For PR expression, studies done by Jang et al., Chun et al., and McFarlane et al., were concordant to our study showed that there were more number of PR-positive cases then negative cases.[11,14,15]

Majority of the cases (75%) showed negative immunoexpression for HER2neu. Only 22% of the total cases were positive, and 3% of cases had equivocal expression. Our results were in the concordance with the study by Jang et al., and McFarlane et al., with a maximum number of cases showing negative HER2neu immunoexpression.[11,15]

Immunoexpression of CD44 was reported using IHC and its membranous expression in >1% of tumor cells was taken as positive. Out of all the cases, 64 (64%) showed positivity for CD44 which is concordant with the study done by Jang et al., which showed CD44 expression in breast carcinoma being 63.7%.[11] Another study was done by Roosta et al., who reported 68% CD44 positivity in breast carcinoma patient.[8]

In the present study, the expression of CD44 was correlated with various clinicopathological parameters including tumor size, lymph node status, histological grade, NPI, histological type, and hormone receptor expression.

The maximum number of cases (19) were CD44-positive falling in the age group of 41–50 years. We did not find any statistically significant association between CD44

Table 6: Correlation of CD44 with ER and PR in other studies

Study (year)	CD44	ER		PR		P-value
		+	–	+	–	
Chun et al., (2013)[14]	+	57.1	42.9	61.9	38.1	Not significant
	–	40	60	60	40	
McFarlane et al., (2015)[15]	+	67.9	32.1	51.5	48.5	Significant
	–	57.3	73.2	66.4	71.2	
Jang et al., (2016)[11]	+	56.7	43.3	54.9	45.1	Not significant
	–	55.4	44.6	58.7	41.3	
Present study	+	20.3	79.7	12.5	87.5	Significant
	–	72.2	27.8	72.22	27.77	

ER: Estrogen receptor, PR: progesterone receptor

and age of the patient. The results of the studies done by Rustamadji et al., and Jang et al., were in concordance with our study.[10,11]

Tumor size >2 cm showed maximum positivity for CD44. Out of the total 64 positive cases, 58 cases had tumor size >2 cm. The results of studies by Zou et al., Rustamadi et al., and Jang et al., are in concordance with our study.[9-11] We did not find any statistically significant association between CD44 and tumor size. CD44 showed positivity in lymph nodes. Out of the total CD44-positive cases, 40 cases had nodal involvement. Our results are in accordance with the studies done by Jang et al., and McFarlane et al.,[11,15] We did not find any statistically significant association between CD44 and lymph node involvement.

CD44 showed higher positivity in Grade II tumors. There was no significant association seen in between histological grade and CD44 expression. The findings of studies by Jang et al., and Syaifudin et al., are in concordance with our study.[11,13]

Majority of ER and PR-positive cases show negativity for CD44. The results study done by of McFarlane et al., were concordant with our study.[15] Studies done by Jang et al., and Chun et al., were discordant with our study[11,14] (Table 6).

Out of total CD44-positive cases, 78.12% showed negative immunoexpression for HER2neu. Only 18.75% of the total cases were positive, and 3.12% of cases had equivocal expression. Our results were in the concordance with the study by Jang et al., and McFarlane et al., with the maximum number of cases showing negative HER2neu immunoexpression.[11,15]

In this study, maximum number of CD44-positive cases were Basal (40) followed by HER2 enriched (11). Seven cases were Luminal A and six cases were Luminal B indicating that a poor prognostic group of breast cancer was associated with positive CD44 expression.

Limitations of the study

The sample size was small to reach at a definitive conclusion. The maximum cases were in the histological Grade II. Grade I and Grade III cases were less. Hence, a statistically signification association could not be made out. A large sample size with an equal number of cases in all of the histological grades is desirable for a better conclusion.

CONCLUSION

A high CD44 expression in breast carcinoma correlates with aggressive tumor characteristics such as multifocality, negative ER/PR expression, and belonging to the basal subtype which is associated with poor prognosis. Conversely, low CD44 expression is linked to Luminal A and Luminal B subtypes which have good prognosis. These indicate the role of CD44 as a prognostic biomarker in the carcinoma breast.

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