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Research Article

Expression of the Hopeful Therapeutic Target CD15 in Women with Breast Cancer

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ABSTRACT

Background: CD15 is emerging as a recent prognostic marker with potential as a future therapeutic target in human malignancies. Its biological role in human cancer involves facilitating tumor cell adhesion through interaction with E-, L-, and P-selectins. This interaction allows adhesion to endothelial cells, enhancing metastatic potential.
Objective: to study CD15 expression and its relationship to some pathologic parameters to provide the first Iraqi data-driven analysis of this growing concept in women with breast cancer.
***Subjects and Methods*:** A retrospective study was carried out utilizing tissue samples obtained from 85 patients with invasive breast cancer. Materials included one formalin-fixed paraffin-embedded tissue block, one hematoxylin and eosin-stained histological section, and two immunohistochemical-stained ER and HER2/NEU sections for each patient. The samples were obtained through core needle biopsy under ultrasound guidance. Data review and immunohistochemical staining with CD15 were conducted, followed by statistical analysis using the chi-square test to explore correlations.
Results: CD15 expression was observed in 55% of breast cancer samples. There was a highly significant statistical relationship between CD15 expression and the presence of axillary lymph node metastasis (proven by fine needle aspiration cytology). However, there was no significant statistical relationship between CD15 expression and the histological grade, ER receptor, HER2-NEU status, and tissue calcification.
Conclusion: CD15 expression was detected in breast cancer cells, with a highly significant association observed between CD15 expression and axillary lymph node metastasis at presentation. CD15 could serve as a prognostic marker as association with axillary lymph nodes reflects negative impact on prognosis and at the same time a promising therapeutic target for patients with breast cancer as blockage of CD15 antigen's function will result in reduction of the metastatic potential and it could be an immunotherapeutic target.

Introduction

Breast cancer is the most prevalent malignant tumor in women; it is the main cause of cancer-related deaths (accounts for ~22%) in women globally (1-2). Breast cancer is not a single disease but rather a heterogeneous entity at both the histological and molecular levels, characterized by multiple subtypes with variable clinical outcomes (3). Although histological classification offers some insights, it has limited predictive value and clinical utility (4). Recently, molecular types, particularly those using microarrays for gene expression analysis, have gained significant attention. Despite their high prognostic and predictive value, molecular testing is expensive and not widely applied, especially in developing countries (5).
Immunohistochemistry-based classification, focusing on estrogen (ER)/ progesterone (PR) receptor and HER2 status, offers a less expensive alternative with good prognostic and therapeutic information (6). Hormone receptors on the surface of breast cancer

cells, such as ER and PR, facilitate tumor growth. However, the independent prognostic and therapeutic role of PR receptor status, irrespective of ER, remains highly debated in oncology. Consequently, the "Royal College of Pathologists" considers testing for Progesterone receptor status in breast cancer non manditory (7). Testing all invasive mammary cancers for ER and HER2 status, either on tru cut or excisional biopsy, has become standard care for patients with breast cancer (8).

CD15 (X-Hapten) is a clustered form of glycoproteins and glycolipids. Initially discovered on the cell surface of terminally differentiated myeloid cells but not in hematopoietic progenitors, CD15 is present in both sialylated and unsialylated forms (9). Its physiological roles include phagocytosis, bactericidal activity, and chemotaxis (10). Aberrant expression of CD15 in epithelial cells of the breast, kidney, lung, and intestinal tract has been noted, as well as its constant presence in astrocytes (10). the most common application of CD 15 immunohistochemical test is in the diagnosis of classical Hodgkin lymphoma.

Literature-based data suggest CD15's role in neoplasia involves facilitating tumor cell adhesion through interaction with E-, L-, and P-selectins, enhancing metastatic potential and promoting changes in cancer-associated membrane proteins (12,13). CD15 expression is often associated with lymphovascular invasion, lymph node metastasis, and distant metastasis in human malignancies (14).

Given CD15's crucial role in cancer metastasis, it is now considered an interesting target for cancer immunotherapy. Chemical preparations are under extensive investigation to modulate CD15 and E-selectin expression to block endothelial cell adhesion and, thereby, metastatic potential (13, 14). CD15 expression has been identified in various human malignancies, including thyroid papillary carcinoma, Hodgkin lymphoma, glial tumors, non-small cell lung cancer, invasive mammary carcinoma, and oral carcinoma (15). However, significant heterogeneity in antigen expression and prognostic value of CD15 among these cancer types has been reported, suggesting the need for individual tumor characterization (16).

This study investigates the expression of CD15 in breast cancer, mainly its association with axillary lymph node metastasis, histological grade, tissue calcification and ER and HER2 expression, to provide the first Iraqi data-driven analysis of this growing concept among females with breast cancer.

Subjects and Methods

This retrospective study collected histological materials of 85 patients with invasive breast cancer from the Pathology Department of Al Massa Center, a private breast center in Baghdad, from "January" 2023 to "December" 2023. Diagnostic histological materials were obtained through core needle biopsy under ultrasound guidance. For each patient with invasive breast cancer included in this study, the following were retrieved from the laboratory archive of the Pathology Department: one formalin-fixed paraffin-embedded tissue block, one hematoxylin and eosin-stained histological section, and two immunohistochemically stained sections of ER and HER2/NEU. Axillary lymph node metastasis (proven by fine needle aspiration cytology done for patients showing positive ultrasound finding) was reported. The only clinical information extracted from the patient data was age.

Sample selection

The inclusion criterion was all histological materials with complete radiological, histopathological, and cytological data in a single center within one year period. The exclusion criteria included histological materials with deficient relevant data and those with equivocal (2+) HER2/NEU immunohistochemically results.

Immunohistochemistry

A 4-micron thick tissue section was prepared from each paraffin block using "Leica Biosystems Microtome" , a charged slide "JSHD Jiangsu", China was used, after drying for 30 minutes at 62°C, the processing was performed by using an autostainer, "Agilent Link 48", "Dako A/S -Denmark-Glostrup" was performed , the samples underwent "Standard Heat Epitope Retrieval" at pH 8.0 for 30 minutes in "ethylene diamine tetraacetic acid" (Unilong Industry Co., Ltd.). Incubation with primary antibody monoclonal mouse CD15 "(clone Carb, code number M3631)" provided by "(Dako A/S -Denmark-Glostrup)". Followed by biotinylated anti-mouse immunoglobulin and peroxidase-labeled streptavidin "(LSAB Kit, Dako A/S -Denmark-Glostrup)", was conducted. Harris hematoxylin "PathnSitu Biotechnologies"; cat. no. PS021 was used for counterstaining. Optimal incubation times and concentrations for the primary antibody were determined via the instructions provided by the manufacturer of the products "(Dako A/S -Denmark-Glostrup)". The required dilution was 1:50 1:200 and the time for incubation

At room temperature was 30 60 min. Positive (renal tissue) and negative external controls were included in each run.

1. Samples obtained in our study included histological material obtained by true cut needle biopsy under ultrasound guidance, so, histological classification of the invasive mammary carcinoma may not be achieved on small tissue sample as it requires full histological evaluation of the entire tumor by excisional biopsy for definitive histological categorization. Reviewing the histological grade according to the "Nottingham modification of the Bloom-Richardson system" and scoring of ER according to "Allred score" (table 1).

"The final Allred scores are the summation of both intensity and proportion scores. Accordingly, "scores 0 and 2" are regarded negative for ER, while "scores of 3 to 8" are regarded as positive). Regarding HER2\NEU scoring on invasive cancer cells:

IHC 3+ was considered (strong positive) if strong complete, membrane staining pattern was observed in more than 10% of cancer cells.

IHC 2+ was considered (Equivocal) if weak to moderate complete membrane staining pattern was observed in more than 10% of the cancer cells.

IHC 1+ was considered (Negative) if weak incomplete membrane staining pattern was observed in more than 10% of the cancer cells.

IHC 0 was considered (Negative) if no stain or incomplete faint / barely perceptible membrane staining pattern was observed in less than or equal to 10% of the invasive tumor cells (17-19)

Table 1: Allred score "for estrogen and progestrone receptor evaluation".

ER Status (Positive Cells %)	Proportion Score	Intensity	Intensity Score
0	0	None	0
<1	1	Weak	1
1 to 10	2	Intermediate	2
11 to 33	3	Strong	3
34 to 66	4		
≥67	5		

2. Interpreting CD15 expressions.

The interpretation of staining results was done by immunohistochemecal evaluation of the intensity and proportion of the staining tumor cells (membranous or cytoplasmic patterns). The immunohistochemical staining intensity was regarded as 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The final score was calculated by multiplication of the proportion and intensity scores of the stained tumor cells, with results considered positive if the final score exceeded 10(19). A light microscope (Leica Microsystem GmbH) was used in interpretation of our results. Leica ICC 50E camera was used in photographing our results.

Ethical approval

The study was approved by the ethical committee of Gazi Al Hariri teaching hospital (reference number 0011-012-19 INT23) at 19\12\ 2023 and conducted by its institutional policy. As it was a retrospective study using data without violating patient privacy, consent to participate was deemed not applicable.

Statistical analysis:

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 25. The participant's age range was presented as means ± standard deviation (SD). Frequencies of various variables were tabulated for easy reference and comparison. The chi-square test was employed to compare between different variables. In this analysis, a p value of less than 0.05 was considered statistically significant.

Results

Current study analyzed 85 histological samples from female patients with invasive breast cancer (Figure 1).

The age range of these patients was from 30 to 89 years, with a mean age of 66.8235 +- 14.803SD. Among the 85 histological samples of invasive breast cancer, 47(55%) were CD15 positive (to (Figure 2).

Among the 85 histological samples of invasive breast cancer, 47(55%) were CD15 positive and 38 (45%) were negative (refer to Figure 2 and 3).

The correlation between CD15 expression and histological grade, ER status (Figure 4), HER2\NEU status (Figure 5), tissue calcification revealed a non-significant statistical relationship with p value > 0.05

However a highly significant statistical relationship was achieved between CD15 expression and the presence of axillary lymph node metastasis with p value < 0.01 (table 2).

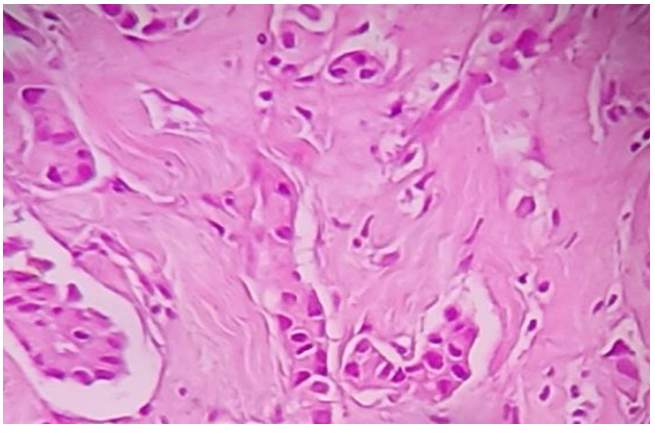

Figure 1: Histological section of invasive breast cancer with a desmoplastic stroma (Hematoxylin and eosin, X100**)**

The age range of these patients was from 30 to 89 years, with a mean age of 66.8235 +- 14.803SD. Among the 85 histological samples of invasive breast cancer, 47(55%) were CD15 positive (refer to Figure 2).

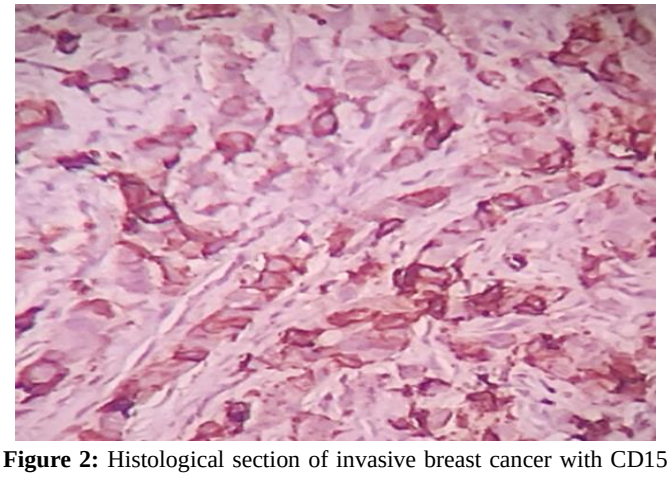

Figure 2: Histological section of invasive breast cancer with CD15 showed positive membranous and cytoplasmic staining pattern (arrow) (Immunohistochemically stained, X100**)**

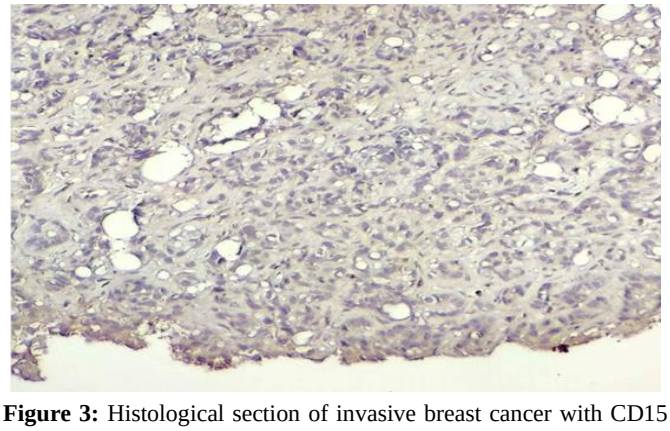

Figure 3: Histological section of invasive breast cancer with CD15 showed negative results (Immunohistochemically stained, X40)

The correlation between CD15 expression and histological grade, ER status (Figure 4), HER2\NEU status (Figure 5), tissue calcification revealed a non-significant statistical relationship with p value > 0.05.

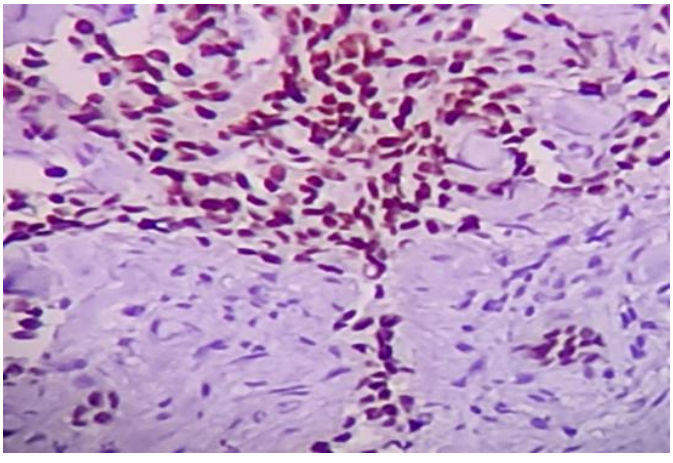

Figure 4: Histological section of invasive breast cancer with ER showed positive nuclear staining pattern (arrow) (Immunohistochemically stained, X100)

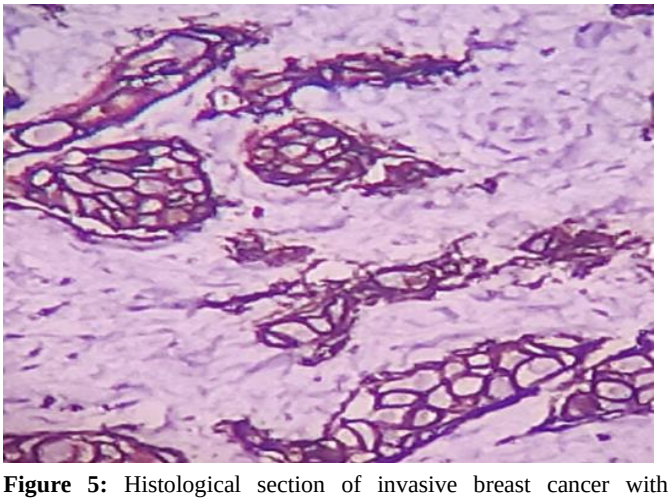

Figure 5: Histological section of invasive breast cancer with HER2\NEU showed positive cell membranous staining pattern (arrow) (Immunohistochemically stained, X100)

Discussion

CD15 is emerging as a recent prognostic marker with potential as a future therapeutic target in human malignancies (12,14). This study found that, out of 85 histological samples of invasive breast cancer, 47 (55.2%) were CD15 positive. There are conflicting data on CD15 expressions in the literature. For instance, a review by Wojciech Szlasa et al (14), which included two older studies, reported varied findings in this regard: 100% (expression in 30 out of 30 samples) and 34% (expression in 33 out of 98 samples) . More recently, Sozzani et al. (21) investigated the prognostic significance of CD15s in a prospective study with 127 primary mammary cancer patients and found CD15 antigen expression in 21% (37 out of 127 samples). The discrepancy with our results could be attributed to variations in sample sizes, differences in the manufacturers of immunohistochemical markers, and detection systems used in these studies.

Table 2: Association between CD15 expression and histological grade, ER receptor status, HER2/NEU status, tissue calcification, axillary lymph node status

Study variable	CD 15 status		Total	P value
	CD15 positive	CD15 negative		
Histological grade				
Grade I	**13 (15.3%)**	**13 (15.3%)**	**26 (30.6%)**	
Grade II	**25 (29.4%)**	**13 (15.3%)**	**38 (44.7%)**	
Grade III	**9 (10.5%)**	**12 (14.1%)**	**21 (24.6%)**	**0.192***
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	
ER receptor status				
ER-positive	**30 (35.2%)**	**22 (25.8%)**	**52 (61.2%)**	
ER-negative	**17 (20%)**	**16 (18.8%)**	**33 (38.8%)**	**0.577***
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	
HER2-NEU				
HER2-NEU positive	**20 (23.5%)**	**14 (16.5%)**	**34 (40%)**	
HER2-NEU negative	**27 (31.7%)**	**24 (28.2%)**	**51 (60%)**	**0.593***
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	
Tissue calcification				
Presence	**20 (23.5%)**	**12 (14.1%)**	**32 (37.6%)**	
Absence	**27 (31.7%)**	**26 (30.6%)**	**53 (62.3%)**	**0. 299***
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	
Axillary lymph node status				
Positive for metastasis	**32 (37.6%)**	**6 (7.1%)**	**38 (44.7%)**	
Negative for metastasis	**15 (17.6%)**	**32 (37.6%)**	**47 (55.2%)**	**0.000†**
Total	**47 (55.2%)**	**38 (44.7%)**	**85 (100%)**	

* Non-significant with p value > 0.05
† Highly significant with p value < 0.01

In current study, the correlation between CD15 expression and histological grade, ER and HER2/NEU receptor status, and calcification revealed a non-significant relationship. The only significant correlation was observed between positive CD15 expression and the presence of axillary lymph node metastasis (confirmed by ultrasound-guided fine needle aspiration cytology) at presentation. This finding aligns with CD15's well-documented biological effect in enhancing metastatic potential (12). Unfortunately, there are no similar studies discussing the correlation of CD15 expression in breast cancer with ER and HER2/NEU status, either Iraqi or worldwide, for further comparison and extensive discussion.

The expression CD15 in human malignancies was first described many years ago based on limited published data (14). Recently, there has been increasing interest in blocking CD15's function to mitigate metastatic potential, suggesting it may be a promising immunotherapeutic target. Several clinical trials have explored biological therapies targeting CD15 in various malignancy, including metastatic renal cell carcinoma (RCC), non-small cell lung cancer, leukaemia, melanoma, and colon cancer (14). Notably, two drugs, nivolumab and pembrolizumab, have been described; their therapeutic effects are either direct, targeting cancer cells, or indirect, monitoring CD15 expression on myeloid stem cells. This monitoring is crucial for understanding cancer initiation and progression (16, 22-24). Current research efforts should focus on enhancing the clinical application of anti-CD15 target therapy, particularly regarding its safety. This study aims to serve as a starting point for more extensive, prospective clinical trials, focusing on the significance of testing CD15 expression in breast cancer and providing reference data for future comparison and extensive discussion.

The main limitation of the present study was that it was carried in a single center with a limited small number of cases, many of them lacking relative clinical information

Conclusion

CD15 expression was detected in breast cancer cells. CD15 could serve as a prognostic marker as association with axillary lymph nodes reflects negative impact on prognosis and at the same time a promising therapeutic target for patients with breast cancer as blockage the antigen's function of CD15 will result in reduction of the metastatic potential and it could be an immunotherapeutic target. A prospective study, using a larger sample size is hopefully recommended with a special concern on enhancing the clinical application of anti-CD 15 target therapy.

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Conflict of Interest

The authors declare no conflict of interest.

Data availability

Data are available upon reasonable request

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