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Immunohistochemical Expression of CD47 in Breast Cancer in Iraqi Female Patients

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ABSTRACT

Background: Breast cancer remains the most prevalent malignancy and a leading cause of cancer-related death among women globally. In Iraq, it is the most commonly diagnosed female cancer. Cluster of differentiation 47 (CD47), a membrane glycoprotein found on most cells, binds to signal regulatory protein α (SIRP α) on macrophages to transmit a “do not eat me” signal, which inhibits phagocytosis. This study aimed to evaluate the immunohistochemical (IHC) expression of CD47 in malignant breast tumors compared with nonmalignant breast tissue from Iraqi women.

Methods: A total of 100 breast tissue samples were collected from various Iraqi hospitals and laboratories. This retrospective case-control study included 70 malignant cases and 30 control samples, the latter of which were obtained from the Forensic Medicine Institute in Baghdad with official approvals. Immunohistochemical analysis was performed to assess CD47 expression. The chi-square test and Fisher exact test were used with Bonferroni correction for multiple comparisons.

Results: CD47 expression showed a statistically significant difference between the malignant tumor group and the nonmalignant group. High CD47 expression was observed in 61 of 70 malignant cases (87.1%) compared with 0 of 30 controls (0.0%). No statistically significant association was observed between CD47 expression and pathologic stage after Bonferroni correction.

Conclusion: CD47 expression was significantly associated with breast malignancy. However, no significant associations between CD47 expression and clinicopathologic parameters were confirmed, suggesting that the finding represents an association with malignancy rather than having a diagnostic or prognostic value. These findings support further research on CD47 to validate these observations and clarify its expression pattern in breast cancer.

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INTRODUCTION

Breast cancer (BC) is the most common cancer and a leading cause of cancer-related death among women worldwide.¹ In Iraq, breast cancer is among the most common cancers in women, with a high incidence rate.^{2,3} One of the major challenges in cancer treatment is the ability of tumor cells to spread

from the primary site to distant organs and, most importantly, to evade destruction by the host immune system.⁴ Interactions between immune-mediated inflammation and epithelial tissues contribute to tumor-related histopathologic changes, including those seen in breast cancer.^{5,6}

Cluster of differentiation 47 (CD47) is a cell surface glycoprotein of the immunoglobulin superfamily that is widely expressed on hematopoietic and nonhematopoietic cells. CD47 interacts with its receptor, signal regulatory protein α

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(SIRP α), which is expressed on macrophages. This interaction delivers an inhibitory signal commonly known as the “don’t eat me” signal, which suppresses phagocytosis.⁷

In breast cancer, tumor cells often overexpress CD47 to evade detection and destruction by the host immune system. High CD47 expression has been reported in malignant tissues; however, its clinical significance remains unclear. Despite the observed significance of CD47 in breast cancer pathophysiology, research specifically examining its immunohistochemical (IHC) expression profile and association with clinicopathologic factors among Iraqi women remains limited. This study aims to address this knowledge gap by providing localized data on the differential expression of CD47 between malignant and nonmalignant tissues.⁸

The primary objective of this study was to evaluate the IHC expression of the CD47 protein in malignant breast tumors and to compare these expression levels with those observed in nonmalignant breast tissue controls obtained from Iraqi female patients. The secondary objective was to assess whether CD47 expression intensity correlates with key clinicopathologic characteristics, specifically tumor size, histologic grade, and pathologic stage.

METHODS

This retrospective case-control study included 100 breast tissue samples. The samples were obtained as formalin-fixed, paraffin-embedded (FFPE) blocks from several institutional sources in Iraq, including the Histopathology Department/Teaching Laboratories of Medical City and a private laboratory in Baghdad, as well as Al-Zahra Hospital and Al-Karama Teaching Hospital in Wasit Governorate. The samples were classified into a malignant group (70 samples from patients with confirmed malignant breast tumors) and a control group (30 samples from individuals with no discernible breast pathology). The nonmalignant control samples were obtained from the Forensic Medicine Institute in Baghdad following the acquisition of all necessary official approvals.

The inclusion criteria for the malignant group were primary invasive breast carcinoma confirmed by histopathology, no prior neoadjuvant chemotherapy, and well-preserved tissue. Cases of recurrent tumors, insufficient tissue samples, or prior immunotherapy were excluded. For the control group, samples consisted of histologically confirmed normal breast tissue with no evidence of malignancy, inflammation, or benign proliferative disease. Controls were age-matched as closely as possible to the cases, and a similar tissue fixation protocol was applied to both case and control tissues. Clinicopathologic data,

including patient age, tumor size, histologic grade (according to the Nottingham histologic score), and pathologic stage (according to the American Joint Committee on Cancer [AJCC] staging system), were collected for the malignant cases.

Immunohistochemistry staining protocol

Standard IHC procedures were performed on 4- μ m sections of the FFPE blocks. Slides were dewaxed, rehydrated, and subjected to heat-induced epitope retrieval. Endogenous peroxidase activity was blocked using a hydrogen peroxide solution. The sections were then incubated with the primary anti-CD47 antibody (E-AB-67354; Elabscience). Detection was carried out using a suitable secondary antibody and a chromogen (Detection System E-IR-R213), followed by counterstaining with hematoxylin. The IHC staining procedure was performed according to a previously described protocol.⁹

Scoring system and analysis

CD47 expression was evaluated by a pathologist blinded to clinical outcomes, following a previously established method.¹⁰ Scoring was based on the percentage of stained tumor cells and the intensity of membrane staining (since CD47 is a transmembrane protein). CD47 expression was semiquantitatively evaluated using the H-score method (percentage of positive cells multiplied by staining intensity [0–3], yielding a range of 0–300). High expression was defined as an H-score of 100 or greater, and low expression was defined as an H-score of less than 100.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics, version 26 (IBM Corp). Categorical variables were expressed as frequencies and percentages and compared using the chi-square test and the Fisher exact test (e.g., CD47 expression vs histologic grade, stage). Mean ages were compared between groups using the Student *t* test. Because of a zero cell in the control group, the odds ratio (OR) was not estimable; accordingly, the Fisher exact test was performed to evaluate the statistical significance of the association. Bonferroni correction was applied by multiplying the raw *P* values by the number of comparisons (8 comparisons). A 2-sided adjusted *P* value of less than .05 was considered statistically significant.

RESULTS

A total of 100 samples were subjected to histologic evaluation, of which 70 were malignant and 30 were nonmalignant controls. High CD47 expression (H-score $\geq$ 100) was observed in 61 of 70

malignant cases (87.1%), whereas none of the control tissues (0 of 30 [0.0%]) showed high expression. In contrast, low CD47 expression was detected in 9 malignant cases (12.9%) and in all 30 control samples (100.0%). The difference in CD47 expression between the malignant and control groups was statistically significant (Fisher exact test, $P < .001$) (Table 1). As noted, because of a zero cell in the control group, the OR was not estimable, but the

Fisher exact test confirmed the significance of this difference (Figures 1 and 2).

Table 1. CD47 Expression Among Study Groups

	Malignant (n = 70), No. (%)	Control (n = 30), No. (%)	P value ^a
CD47 expression			
High expression	61 (87.1)	0 (0.0)	<0.001
Low expression	9 (12.9)	30 (100.0)	

^a Determined using the Fisher exact test.

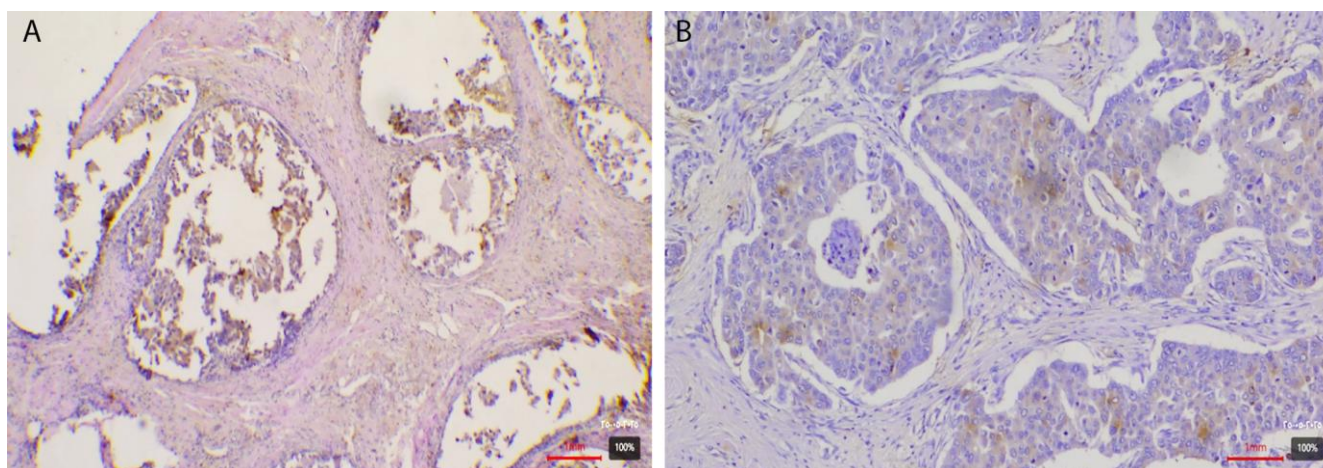


Figure 1. Immunohistochemical staining showing high expression of CD47 in breast cancer tissue. A, Ductal carcinoma, stage IIA, grade 2 (H-score, 210; original magnification $\times 4$). B, Lobular carcinoma, stage IIIC, grade 2 (H-score, 120; original magnification $\times 10$).

Table 2. Association Between CD47 Expression and Clinicopathologic Characteristics

Variable	Category	High CD47 expression (n = 61), No. (%) ^a	P value ^b	Adjusted P value (Bonferroni) ^c
Age, y	<40	4 (6.6)	0.50	>0.99
	40–49	21 (34.4)		
	50–59	20 (32.8)		
	≥ 60	16 (26.2)		
Tumor size	<2 cm	10 (16.4)	0.26	>0.99
	2–5 cm	34 (55.7)		
	>5 cm	17 (27.9)		
Histologic type	IDC	34 (55.7)	0.55	>0.99
	ILC	9 (14.8)		
	Mixed (IDC & ILC)	18 (29.5)		
Histologic grade	I	9 (14.8)	0.53	>0.99
	II	44 (72.1)		
	III	8 (13.1)		
Pathologic stage	Stage I or IA	6 (9.8)	0.08	0.60
	Stage II (IIA or IIB)	31 (50.8)		
	Stage III (IIIA, IIIB, or IIIC)	22 (36.1)		
Estrogen receptor	Positive	46 (75.4)	0.48	>0.99
	Negative	15 (24.6)		
Progesterone receptor	Positive	37 (60.7)	0.31	>0.99
	Negative	24 (39.3)		
HER2	Positive	19 (31.1)	0.47	>0.99
	Negative	42 (68.9)		

HER2, human epidermal growth factor receptor 2; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.

^a Subgroup analyses were performed on malignant cases with high CD47 expression and complete available data; cases with missing data were excluded. ^b Fisher exact test was applied when any expected cell count was < 5 . ^c Bonferroni correction was applied by multiplying the raw P value by the number of comparisons (8 comparisons). Adjusted P values > 0.99 are reported as > 0.99 .

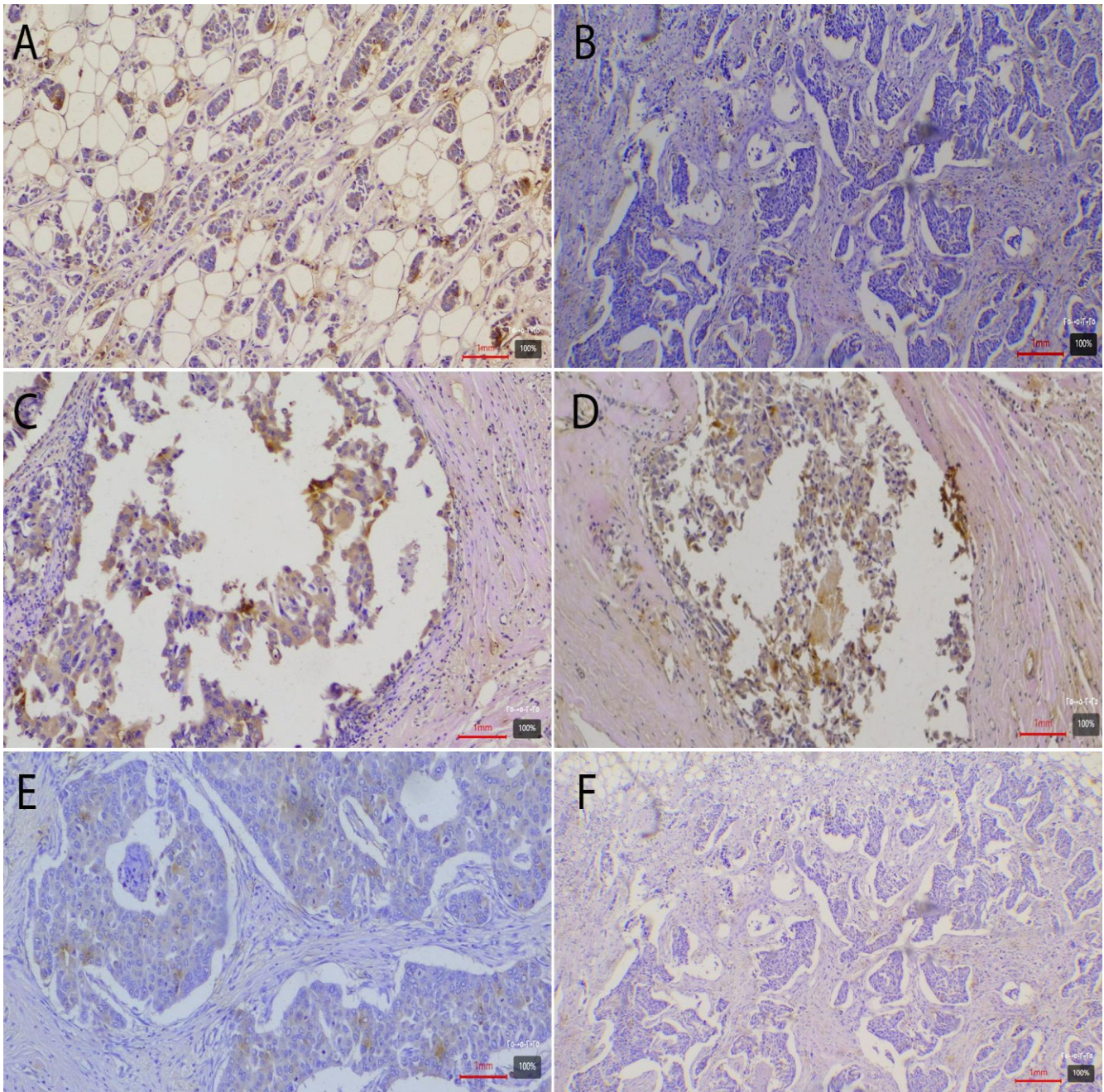


Figure 2. Immunohistochemical staining of CD47 in breast tissue sections. A, Strong CD47 expression in ductal carcinoma, stage IIIA, grade 2 (H-score, 210; original magnification $\times 10$). B, Low CD47 expression in invasive ductal carcinoma, stage IIA, grade 2 (original magnification $\times 10$). C, High CD47 expression with strong diffuse staining in ductal carcinoma in situ (H-score, 210; original magnification $\times 40$). D, Moderate CD47 intensity (score 2) with approximately 60% of malignant cells stained (H-score, 120; original magnification $\times 10$). E, Low CD47 expression in invasive ductal carcinoma, stage IIA, grade 2 (original magnification $\times 10$). F, Negative CD47 staining in invasive ductal carcinoma (H-score, 0; original magnification $\times 10$).

DISCUSSION

Recent advances in oncology have focused on immunotherapy and the inhibition of immune checkpoints to predict disease progression and develop novel treatments for breast cancer. Cluster of differentiation 47 (CD47) is a key macrophage checkpoint molecule that is consistently overexpressed in many malignancies associated with

a poor prognosis, including both hematological and solid tumors.¹¹ This overexpression is linked to tumor cells through the CD47–signal regulatory protein α (SIRP α) pathway.¹² Specifically, CD47 interacts with the extracellular domain of SIRP α on macrophages, inhibiting their phagocytic activity and preventing the innate immune response from attacking cancer cells.¹³



Recent evidence has demonstrated that CD47 is markedly upregulated in malignant tissues compared with normal tissues.¹⁴ The primary finding of this study was a highly significant difference in CD47 expression between the malignant group and the nonmalignant control group ($P < .001$), which is comparable to patterns previously reported in malignant tissues. Nonetheless, no clinical or prognostic interpretation can be made from this differential expression. While some studies^{15,16} have reported high CD47 expression in breast cancer and other solid tumors, our findings do not demonstrate a diagnostic or prognostic utility for this marker.

The second stage of our analysis examined the association between CD47 expression and clinicopathologic features. Although differences in CD47 expression across pathologic stages were observed, this association did not reach statistical significance after correction for multiple comparisons; therefore, no biological or clinical interpretation can be made. While several previous investigations have reported significant correlations between CD47 expression and clinical stage,^{17,18} our results did not achieve statistical significance following adjustment, and thus no clinical association could be established.

Our study also did not identify any significant association between CD47 expression and either clinical stage or histologic grade. This observation diverges from some previous studies on triple-negative breast cancer,¹⁹ colorectal adenocarcinoma,²⁰ and soft tissue sarcomas,²¹ while contradicting other breast cancer investigations²² that identified a significant association. These discrepancies may reflect variations in study populations or methodologies, which could differ across various tumor subtypes and cohorts.

Similarly, our findings revealed no association between CD47 expression and histologic type in the malignant group ($P = .55$). Comparable findings have been reported in some previous studies,²³ but not in others.²⁴ No significant association was observed between CD47 expression and tumor size within the malignant group ($P = .26$). This finding is consistent with previous reports that also failed to demonstrate a statistically significant association.²⁵ The relationship between age and CD47 expression was also examined, with no discernible association found in the malignant group. Similar findings have been reported in studies on osteosarcoma,²⁶ nasopharyngeal cancer,²⁷ and colorectal cancer,²⁸ as well as in general studies.²⁹ Although some studies have reported age-related differences in CD47 expression, such an association was not observed in this study.³⁰

Regarding hormone receptor status, no discernible association was found between CD47 expression and either estrogen receptor ($P = .48$) or progesterone receptor status ($P = .31$), which aligns with some previous studies.³¹ Conversely, other investigations have reported a strong association between high CD47 expression and estrogen receptor-negative breast cancer.¹⁵ Furthermore, CD47 expression did not differ significantly between HER2-positive and HER2-negative groups ($P = .47$), consistent with previous observations.³¹ However, elevated CD47 expression has been associated with triple-negative breast cancer in other studies.¹⁷

Although this study primarily investigated immunohistochemical expression, it was limited to phenotypic analysis and did not investigate underlying biological mechanisms.³² Furthermore, while recent studies have explored CD47 expression in various contexts, these implications were not examined in the present study, particularly regarding lung cancer and the wider applicability of CD47-targeted therapies in modern immunotherapy.³³

In the present study, we found that most malignant breast tumors overexpressed CD47. However, no statistically significant association was observed between CD47 expression and pathologic stage after Bonferroni correction. Nevertheless, because this is a retrospective, noninterventive study, the direction of a causal relationship cannot be inferred. Low CD47 expression in control tissues has been reported in previous studies, consistent with physiologic expression in normal breast epithelium. Thus, our data indicate an association between CD47 expression and malignancy only, without evidence of diagnostic, prognostic, or therapeutic significance. Further studies, particularly prospective and multicenter investigations, will be necessary to confirm these observations and to further evaluate CD47 expression patterns in breast cancer.

Limitations

Several limitations of this study should be recognized. First, the retrospective design and the use of controls from a forensic institute may have introduced selection bias. Second, although the sample size was adequate for the primary analyses, it lacked sufficient power for certain subgroup comparisons because of the limited number of cases. Additionally, the presence of zero cells precluded multivariable logistic regression analysis. Third, while dichotomizing CD47 expression into a binary variable aids clinical interpretation, it may lead to a loss of information compared with analyzing CD47 expression as a continuous variable.



CONCLUSION

The CD47 protein is significantly overexpressed in malignant breast tumors compared with nonmalignant breast tissue in Iraqi female patients. No statistically significant association was observed between CD47 expression and clinicopathologic parameters after adjustment for multiple comparisons. Consequently, these findings point to an association between CD47 expression and malignancy, though no diagnostic or prognostic value could be established. Larger prospective studies are required to further characterize CD47 expression patterns in breast cancer.

ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Scientific Research Committee of the College of Science, University of Baghdad (Ref CSEC/1124/0089, Date: November 8, 2024), signed by Prof Harith Jabbar Fahad Al-Mathkhury, Head of the College of Science Research Ethics Committee.

DATA AVAILABILITY

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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CONFLICT OF INTERESTS

None.

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None.

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AI DISCLOSURE

Artificial intelligence tools were used solely for language editing and stylistic improvement. All scientific content was produced by the authors, and all data analysis, and interpretations were also performed by them.

AUTHOR CONTRIBUTION

ISK: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. BJM: Conceptualization, Methodology, Writing – original draft, Writing – review & editing.



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