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Immunohistochemical Expression of Cyclin D1 in Invasive Breast Carcinoma

Tabish Hassan, Nadeem Zafar, Akhter Ali Bajwa, Rabia ahmed, Muhammad Umair, Mubina Qayyum

ABSTRACT

Objective: This study was conducted to determine the immunohistochemical expression of cyclin D1 in invasive breast carcinoma and its association with already established prognostic parameters like estrogen receptor (ER), progesterone receptor (PR), HER2/Neu, and Ki67 status.

Study Design & Setting: Cross sectional Observational. Department of Pathology, Armed Forces Institute of Pathology, Rawalpindi

Methodology: The study included 350 cases of invasive breast cancer diagnosed between January 2023 and December 2023. Data collected included patient age, histological subtype, molecular subtypes, tumor size, and the presence of estrogen (ER) and progesterone (PR) receptors, as well as HER2/Neu and Ki67 status. Patients who had undergone chemotherapy, received radiation to the breasts, or experienced relapse were excluded from the study. Immunohistochemistry was conducted using a Cyclin D1 antibody to assess Cyclin D1 expression in tissue samples. The expression levels were categorized as negative, weak, moderate, strong staining in tumor cells. Data analysis was performed using SPSS 29.0, and statistical comparisons were made between Cyclin D1 staining and ER, PR, HER2/Neu, and Ki67 status.

Results: Cyclin D1 moderate to strong staining was seen in 173/352 (49.14%) cases of invasive BC. Cyclin D1 expression was slightly statistically significantly associated with ER (x2 = 7.78, *P* value <0.051) and Ki67 positivity (÷2 = 7.27, *P* value <0.064).

Conclusion: Cyclin D1 has the potential to serve as a prognostic marker. Incorporating it into the routine IHC workup for breast cancer could enhance patient management, especially with the development of new targeted therapies that inhibit the Cyclin D-CDK4/6 axis.

Keywords: Breast carcinoma; cyclin D1; ER; PR; HER2; KI67

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INTRODUCTION:

Prevalence of breast cancer (BC) is 23.8% in women and 31.3% in Pakistan as of in year 2022.[1] A number of innovative therapeutic methods are currently being investigated in order to broaden the range of treatment choices available for breast cancer. An examination into the expression of genes has led to the discovery of a new classification system for breast cancer. This classification system includes luminal A, B, HER2-positive, and basal-like subtypes.[2]. A better understanding of the molecular alterations and genetics of breast cancer, as well as focused treatment, outcomes have improved even in patients who are in advanced stages.[3,4]

Oestrogen receptor (ER)-á positive breast tumours account for around 70 percent of all breast cancers, which means that endocrine therapy is the major treatment for these individuals. There is a relapse rate that is significantly greater in advanced breast cancer cases, even though tamoxifen reduces the incidence of recurrence by fifty percent. Approximately thirty percent of patients will experience a relapse either during or after treatment with tamoxifen. Consequently, both acquired and de novo resistance to tamoxifen present important obstacles in the therapy process

that must be addressed.[5] Standard endocrine treatments are completely ineffective against triple-negative breast cancer, which is another interesting fact. Consequently, the identification of biomarkers that can predict the response to endocrine therapy is critically important for the selection of different therapeutic options.

On 11q13, the CCND1 gene is responsible for regulating the cell cycle.[6] Cyclin D1, an essential G1 cell cycle regulator, is produced by this type of gene. It accomplishes this by binding to CDK4/6, which increases the rate at which the retinoblastoma protein (Rb) and other substrates are phosphorylated, so accelerating the process of cell proliferation.[7] For a comprehensive knowledge of the transitions from the G1 to S phase in tissue, it is vital to have a solid understanding of the intricate cell cycle mechanics. The activation of CDK4 during the G1 phase is a crucial function of cyclin D1. Cyclin-CDK complexes are activated as a result of this activation, which causes the cell cycle to progress into the S phase. Cyclin D1 mutations can hasten the proliferation of cells by disrupting essential processes, which can ultimately result in the development of cancer.[8] There are also actions that cytokine D1 can perform that are not related to CDK. These actions have the potential to trigger ER-mediated transcription regardless of oestrogen and to affect oestrogen and anti-estrogen responses.[9] The CCND1 gene is amplified in a significant number of breast tumours, and the majority of breast cancers that originated in the breast contain Cyclin D1 overexpression.

As per authors knowledge, there are no research conducted in Pakistan that investigate the expression of Cyclin D1 in breast cancer and its relationship with other factors that influence the prognosis. In the current investigation, the objective is to examine the expression of cyclin D1 in breast cancer patients using immunohistochemistry and to discover whether there is a probable association between this expression and other well-established prognostic criteria such as ER, PR, HER2/NEU, and Ki67% status.

METHODOLOGY:

This is a comparative cross-sectional study and was conducted in the Department of Pathology at Armed Forces Institute of Pathology, Rawalpindi between Jan 2023 and Dec 2023. Ethical approval was obtained from the institute's ethical committee. Based on the prevalence of breast carcinoma in Pakistan, which is around 35%, sample size of 350 BC was estimated using the formula $Z_{1-a/2}^2(p)(1-p)/d^2$, where $Z_{1-a/2}$=1.96, p=0.35 and d=0.05 with 95% confidence interval.[10] All patients having histological diagnosis of invasive breast carcinoma on breast core biopsy or lumpectomy were included in the study. Paraffin embedded tissue blocks received for ER, PR, HER2 and Ki67% were also included in the study. Patients having diagnosis of carcinoma in situ and post neoadjuvant chemotherapy samples were excluded from this study.

After fixation of tissue in 10% buffered formalin, specimen was processed in the TissueTek® tissue-processing equipment. After embedded in paraffin wax, 5µm-thick sections were prepared on a semi-automated rotary microtome. These sections were mounted on glass slides and stained with the conventional haematoxylin-and-eosin dyes. Mounted sections were viewed on the microscope for selection of cases having diagnosis of invasive breast carcinoma.

We selected optimal breast cancer tissue blocks for immunohistochemistry (IHC). Citrate buffer antigen retrieval was done in a pressure cooker. The main antibodies used were anti-human Cyclin D1, ER alpha, PR, HER2/neu, and Ki-67 (Clone EP12, EP1, PgR 636, MIB-1, Dako). This study's positive controls comprised tonsil sections for Cyclin D1 expression, endometrial tissue for ER and PR, previously established breast cancer tissue with high HER2/neu positivity, and skin for Ki-67. To eliminate observational bias, two independent pathologists used high power field (HPF) to examine the sections.[11]

Positive cyclin D1 staining was observed when at least 10% of tumor cells displayed nuclear expression with moderate to strong intensity. The intensity of Cyclin D1 was evaluated using a scale ranging from 0 to 3: Assigning numerical values to different levels of intensity: 0 represents a negative level, 1 indicates a weak level, 2 signify a moderate level and 3 represents a strong level. An evaluation of ER and PR was conducted using the Allred score, where positive scores range from 3 to 8.[12] The assessment of ER and PR immunoreactivity involved analysing the percentage of tumor cells displaying nuclear staining. A positivity threshold of more than 10% was used.[13] The HER2 staining was evaluated using the guidelines set by the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP). A positive result (3+) was determined if there was moderate to strong complete membrane staining in 10% or more of tumor cells.[14] A Ki-67 labeling index of 10% or higher was classified as positive.[15]

Data was analyzed using SPSS v 29.0. Cyclin D1 staining was used to tabulate age, histological type, tumor grade, ER, PR, HER 2, Ki67, and molecular classification for all patients. The correlation between cyclin D1 expression and histopathological characteristics was determined using Pearson's Chi-square test. The quantitative data was reported as mean ± SD and the qualitative data as f (% age). The results were statistically significant at $P < 0.05$ and highly significant at $P < 0.01$.

RESULTS:

The various histological and molecular subtypes of invasive breast carcinoma included in the study along with cyclin D1 staining spectrum are listed in Table 1 and Table 2. A total of 352 cases of breast carcinoma were included in the study. Age of the patient ranged from 35 to 67 years, and

all were females. Most common histological subtype reported was Invasive breast carcinoma of no special type (NST), 52% (n=184) of the total cases. Similarly, the most common molecular subtype of breast devised from ER, PR, HER2 and Ki67 status was Triple negative breast carcinoma, 22% (n=78).

Table 3 shows BC cyclin D1 expression and its connection with ER, PR, HER 2, and Ki67. Cyclin D1 moderate to strong staining was found in 173/352 (49.14%) invasive BC patients. Cyclin D1 expression was modestly linked to ER (÷2 = 7.78, P < 0.051) and Ki67 positive (÷2 = 7.27, P < 0.064). Our investigation found 78/352 (22%) triple-negative BC (TNBC) cases (Table 2). 43/78 (55%) TNBC cases had moderate to strong Cyclin D1 positive absent. In 35/78 (44%) TNBC instances, cyclin D1 expression was weak to negative.

Table 1: Cyclin D1 Staining in various histological types of breast carcinomas

Histological Classification	Negative N (%age)	Weak N (%age)	Moderate N (%age)	Strong N (%age)	Total N (%age)
Invasive Breast Carcinoma, NST	51.00(27.71)	49.00(26.63)	42.00(22.82)	42.00(22.82)	184.00(100)
Microinvasive Carcinoma	8.00(30.76)	6.00(23.07)	9.00(34.61)	3.00(11.53)	26.00(100)
Invasive Lobular Carcinoma	4.00(12.50)	10.00(31.25)	11.00(34.37)	7.00(21.87)	32.00(100)
Tubular Carcinoma	7.00(50.00)	3.00(21.42)	1.00(7.14)	3.00(21.42)	14.00(100)
Mucinous Carcinoma	3.00(14.28)	7.00(33.33)	5.00(23.81)	6.00(28.57)	21.00(100)
Invasive Micropapillary Carcinoma	3.00(23.07)	1.00(7.69)	5.00(38.46)	4.00(30.76)	13.00(100)
Carcinoma with Apocrine Differentiation	5.00(38.46)	3.00(23.07)	2.00(15.38)	3.00(23.07)	13.00(100)
Encapsulated Papillary Carcinoma	3.00(27.27)	2.00(18.18)	2.00(18.18)	4.00(36.36)	11.00(100)
Metaplastic Carcinoma	6.00(17.64)	7.00(20.58)	10.00(29.41)	11.00(32.35)	34.00(100)
Adenoid Cystic Carcinoma	0.00(0.00)	1.00(25.00)	0.00(0.00)	3.00(75.00)	4.00(100)
Total	**90.00(25.56)**	**89.00(25.28)**	**87.00(24.71)**	**86.00(24.43)**	352(100)

DISCUSSION

During our research, we investigated the immunohistochemical expression of Cyclin D1 in invasive breast cancer and its correlation with various prognostic factors, such as ER, PR, HER2/Neu, and Ki67 status. Within the range of moderate to strong Cyclin D1 positive, approximately 49.14% of invasive breast tumours met the criteria. There was a correlation that was statistically significant between the expression of Cyclin D1 and the presence of ER and Ki67 positive cells.

It is commonly accepted that cytokine D1 is associated with the development of breast cancer. This is because cytokine D1 stimulates cell proliferation and differentiation by speeding up the transition from G1 to S phase and interacting with nuclear receptors. Using an antibody that is specific to the cyclin D1 protein, it is possible to identify an increased expression of the protein, even in situations where there is no discernible increase in the number of copies that are present.[16] As a result of the findings of our ongoing inquiry, immunohistochemistry was able to identify moderate to strong expression of Cyclin D1 in 173 out of 352 samples, which is equivalent to 49% of the total. The expression of cyclin D1 has been found to be positive in around sixty to eighty-five percent of breast cancer cases, according to a large number of researches.[17-20] One possible explanation for the decrease in expression is that this particular centre does not have access to CCND1 gene amplification services, which are necessary for establishing true negative instances. According to the findings of the current inquiry, there is a significant statistical connection between the expression of Cyclin D1 and the existence of ER and Ki67 positive cells. The results of this study lend credence to the findings of other studies that have demonstrated the influence of cyclin D1 on the maturation and differentiating of cells.[21] Furthermore, the statistical analysis showed that there was no significant correlation between HER 2 positive and cyclin D1 expression (p=0.527). This was the conclusion reached by the researchers. In accordance with the findings of prior research carried out by Peurala et al[22] and Sarkar et al[18], this finding is consistent.

The expression of cyclin D1 was shown to be moderate to strong in 55% (43/78) of breast cancer cases; however, this has not yet been demonstrated to be a predictive factor.[23] In these patients, there was no research that demonstrated a substantial positive cyclin D1 level. Patients do not accept the traditional endocrine treatment offered. In many instances, the proliferation of breast cancer cells is driven by an excessive amount of activity along the cyclin D–CDK4/6 axis. In recent years, there has been a significant advancement in the treatment of cancer through the emergence of strong, selective, and orally accessible CDK4/6 inhibitors. ER-positive breast cancer is the subtype of breast cancer that is most likely to respond favourably to CDK4/6 inhibition. In addition, excess expression of CCND1, a gene that is directly influenced by the oestrogen receptor (ER), is frequently observed in breast tumours that are ER-positive.[24] In the

Table 2: Cyclin D1 Staining in various Molecular subtypes of breast carcinomas

Molecular Classification	Age Category (Years)	Negative N (%age)	Weak N (%age)	Moderate N (%age)	Strong N (%age)	Total N (%age)
Luminal A	35-55	12.00 (29.26)	8.00 (19.51)	8.00 (19.51)	13.00 (31.70)	**41.00 (100)**
	56-65	7.00 (36.84)	4.00 (21.05)	5.00 (26.31)	3.00 (15.78)	**19.00 (100)**
	>65	6.00 (50.00)	3.00 (25.00)	1.00 (8.33)	2.00 (16.66)	**12.00 (100)**
	Total	25.00 (34.72)	15.00 (20.83)	14.00 (19.44)	18.00 (25.00)	**72.00 (100)**
Luminal B Like (HER2 -ive)	35-55	10.00 (27.77)	8.00 (2.22)	11.00 (30.55)	7.00 (19.44)	**36.00 (100)**
	56-65	2.00 (11.11)	7.00 (38.88)	5.00 (27.78)	4.00 (22.22)	**18.00 (100)**
	>65	1.00 (11.11)	4.00 (44.44)	1.00 (11.11)	3.00 (33.33)	**9.00 (100)**
	Total	13.00 (20.63)	19.00 (30.15)	17.00 (26.98)	14.00 (22.22)	**63.00 (100)**
Luminal B Like (HER2 +ive)	35-55	12.00 (27.27)	15.00 (34.09)	8.00 (18.18)	9.00 (20.45)	**44.00 (100)**
	56-65	5.00 (20.00)	6.00 (24.00)	5.00 (20.00)	9.00 (36.00)	**25.00 (100)**
	>65	0.00 (0.00)	1.00 (33.33)	1.00 (33.33)	1.00 (33.33)	**3.00 (100)**
	Total	17.00 (23.61)	22.00 (30.55)	14.00 (19.44)	19.00 (26.38)	**72.00 (100)**
Non luminal (HER2 +ive)	35-55	13.00 (31.70)	7.00 (17.07)	12.00 (29.26)	9.00 (21.95)	**41.00 (100)**
	56-65	6.00 (33.33)	4.00 (22.22)	3.00 (16.67)	5.00 (27.78)	**18.00 (100)**
	>65	2.00 (25.00)	1.00 (12.50)	3.00 (37.50)	2.00 (25.00)	**8.00 (100)**
	Total	21.00 (31.34)	12.00 (17.91)	18.00 (26.86)	16.00 (23.88)	**67.00 (100)**
Triple Negative	35-55	6.00 (13.63)	11.00 (25.00)	16.00 (36.36)	11.00 (25.00)	**44.00 (100)**
	56-65	7.00 (36.84)	4.00 (21.05)	4.00 (21.05)	4.00 (21.05)	**19.00 (100)**
	>65	1.00 (6.66)	6.00 (40.00)	4.00 (26.67)	4.00 (26.67)	**15.00 (100)**
	Total	14.00 (17.94)	21.00 (26.9)	24.00 (30.76)	19.00 (24.35)	**78.00 (100)**

treatment of ER-positive metastatic breast cancer, the Food and Drug Administration has given its approval to three CDK4/6 inhibitors: palbociclib, ribociclib, and abemaciclib. The addition of these drugs to endocrine therapy has resulted in the highest improvement in progression-free survival in this kind of breast cancer; however, in order to clarify this, additional evidence from clinical trials is required.

There were a number of limitations to the study. The pricey technique of FISH was not successful in detecting the amplification of the CCDN1 gene. HER2 was evaluated solely by IHC. Without the use of fluorescent in-situ hybridisation, it was not possible to determine the presence of uncertain HER2 expression 2+ occurrences. It would be

Table 3: Association of ER, PR, HER2 and KI67 status with Cyclin D1 staining

		CyclinD1 Status				Total	P value
		Negative	Weak	Moderate	Strong		
ER	Positive	47.00(25.40)	56.00(30.27)	46.00(24.86)	36.00(19.45)	185.00(100)	0.051
	Negative	43.00(25.74)	33.00(19.76)	41.00(24.55)	50.00(29.94)	167.00(100)	
	Total	90.00(25.56)	89.00(25.28)	87.00(24.71)	86.00(24.43)	352.00(100)	
PR	Positive	39.00(23.92)	40.00(24.54)	43.00(26.38)	41.00(25.15)	163.00(100)	0.851
	Negative	51.00(26.98)	49.00(25.92)	44.00(23.28)	45.00(23.81)	189.00(100)	
	Total	90.00(25.56)	89.00(25.28)	87.00(24.71)	86.00(24.43)	352.00(100)	
HER2	Positive	48.00(26.66)	47.00(26.11)	47.00(26.11)	38.00(21.11)	180.00(100)	0.527
	Negative	42.00(24.41)	42.00(24.41)	40.00(23.25)	48.00(27.90)	172.00(100)	
	Total	90.00(25.56)	89.00(25.28)	87.00(24.71)	86.00(24.43)	352.00(100))	
KI67	Low	43.00(24.71)	35.00(20.11)	45.00(25.86)	51.00(29.31)	174.00(100)	0.062
	High	47.00(26.40)	54.00(30.33)	42.00(23.59)	35.00(19.63)	178.00(100)	
	Total	90.00(25.56)	89.00(25.28)	87.00(24.71)	86.00(24.43)	352.00(100)	

beneficial to conduct longitudinal research on cytokine D1 expression and clinical outcomes. This may improve the statistics regarding the development of Cyclin D1 disease and the prognosis. In breast cancer, it is important to investigate the clinical implications of Cyclin D1 expression. Patients who have a high level of Cyclin D1 expression may be candidates for the investigation of targeted Cyclin D-CDK4/6 axis inhibitors, which could serve to construct individualised therapy regimens.

CONCLUSION

Cyclin D1 has the potential to serve as a prognostic marker. Its inclusion in the standard immunohistochemistry (IHC) analysis of breast cancer cases can assist in the right management of patients, especially with the introduction of new targeted therapies that block the cyclin D-CDK4/6 axis. Cyclin D1`expression in TNBC patients could explore an additional treatment option like selective CDK4/6 inhibitors in these patients. However large scale placebo control, randomized trial are needed to determine prognostic significance in these TNBCs cases.

Authors Contribution:
Tabish Hassan: Data Collection and tabukation
Nadeem Zafar: Topic selection
Akhter Ali Bajwa: Abstract writing
Rabia Ahmed: Data interpretation
Muhammad Umair: Proof Reading
Mubina Qayyum: Analyzing data

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