International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 17s, 2025
https://theaspd.com/index.php

Immunohistochemical Analysis Of Cyclin D1 And Its Predictive Role In The Histopathological Evaluation Of Breast Cancer

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Abstract

Relevance: *Breast cancer ranks among the foremost causes of cancer-related mortality in women globally. Cyclin D1, an essential regulator of the cell cycle, has been associated with carcinogenesis and the advancement of breast cancer.*

The study aimed to *investigate the correlation between Cyclin D1 immunohistochemistry expression and histopathological characteristics in breast cancer patients, emphasizing age, tumor grade, histological type, and HER2 status.*

Methodology: *This study was conducted on a sample of 40 cases of breast cancer diagnosed in women from Najaf Governorate. This cross-sectional analytical investigation investigated the immunohistochemistry expression of Cyclin D1 in breast cancer tissues. Forty samples were obtained from Al-Sadr Teaching Hospital and private laboratories in Najaf (Iraq) from July 2024 to May 2025. Expression levels were assessed via immunohistochemistry and associated with clinicopathological characteristics.*

Results: *Cyclin D1 expression was positive in 72.5% of cases, with the highest expression noted in patients aged 50–59 years, homemakers, and individuals diagnosed with invasive ductal carcinoma and grade II malignancies. HER2-negative cancers exhibited an increased prevalence of Cyclin D1 expression. Significant correlations were identified between Cyclin D1 expression and age, tumor grade, histological type, and HER2 status ($P \leq 0.05$).*

Finally, Cyclin D1 shows potential as a key biomarker for predicting breast cancer, highlighting its clinical importance in detection, classification, and treatment choice.

Keywords: *Cyclin D1, breast carcinoma, immunohistochemical (IHC) analysis, tumor grading, HER2 status, invasive ductal carcinoma.*

INTRODUCTION

Breast cancer is among the most common malignancies impacting women worldwide, with over 2.3 million new cases and 585,000 fatalities each year [1, 2]. The research indicates that 5% to 10% of breast cancer cases are linked to genetic mutations and family history, while 20% to 30% are attributed to modifiable risk factors [3]. A breast cancer tumor is a group of cancerous cells that can invade nearby tissues, cause damage, and possibly spread to other parts of the body. However, changes in breast cells that are not cancerous can also cause conditions like atypical hyperplasia, cysts, or benign tumors like intraductal papillomas [4]. Recent studies highlight the importance of biomarkers that regulate cell division, including cyclins and cyclin-dependent kinases [5]. Cyclin D1 is notable for facilitating the G1/S phase transition, essential for cell proliferation [6]. Cyclin D1 overexpression is commonly observed in breast cancer, yet the literature presents contradictory findings concerning its prognostic significance [7]. Cyclin D1 aids in many processes that lead to the growth of cancerous cells, making it an oncogene. It encourages abnormal cell growth, improves angiogenesis, and helps cells fight apoptosis, all of which help tumors grow and spread [8]. Human epidermal growth factor receptor 2 (HER2) positive cells comprise 15 to 20 percent of all breast cancer cases. They are marked by high levels of HER2, rapid tumor growth, and a bad outlook [9].

This study aimed to investigate the correlation between Cyclin D1 immunohistochemistry (IHC) expression and histopathological characteristics in breast cancer patients, emphasizing age, tumor grade, histological type, and HER2 status.

Materials and Methods: Study Design: This study was conducted on a sample of 40 cases of breast cancer diagnosed in women from Najaf Governorate (Iraq). A cross-sectional analytical investigation. Collection of Samples: Forty pieces of formalin-fixed, paraffin-embedded breast cancer tissue were gathered from private laboratories and Al-Sadr Teaching Hospital in Najaf between July 2024 and May 2025. Inclusion criteria encompassed female patients aged 30 to 70 years with histopathologically verified breast cancer.

Histopathological Examination: Tissue sections were analyzed using a Human-type microscope at magnifications of 10x and 40x by pathologists from multiple centers in Al-Najaf province. Among the evaluated samples, 40 tissue blocks were confirmed to be cancerous. For each case, histological grading and tumor size were recorded.

Immunohistochemistry: IHC analysis was also performed on all 40 malignant tissue blocks. IHC is a basic diagnosis tool that finds certain proteins inside cells using interactions between antigens and antibodies. It is used extensively in cancer studies because it is accurate and can tell the difference between protein types [10]. The process is very careful and includes steps like deparaffinization, rehydration, and antigen retrieval using solutions like citrate buffer (pH 6.0) or Tris-EDTA (pH 9.0). Next, hydrogen peroxide and protein-blocking buffers stop nonspecific binding [11-12]. Next, primary antibodies are added. After washing, horseradish peroxidase (HRP)-conjugated secondary antibodies are added. Adding the chromogen 3,3'-diaminobenzidine (DAB) makes the target protein stand out as a dark solid [13, 14]. At the end of the process, hematoxylin counterstaining, dehydration, and mounting the slide for microscope examination are done. IHC is often used to find Estrogen receptor (ER), Progesterone receptor (PR), and HER2 hormonal receptors in breast cancer and CD3+, CD8+, and FoxP3+ markers of immune cell invasion [10, 15]. The Allred scoring system is applied to evaluate ER and PR staining, combining the proportion score (0–5) and intensity score (0–3), giving a total score from 0 to 8, categorized as negative (0–2) to strongly positive (7–8) [16]. If more than 30% of tumor cells show 3+ membrane staining, the test is positive for HER2. The test is not positive if more than 10% of cells show 2+ staining. Cancers that do not meet these requirements are called triple-negative [17].

Statistical Analysis: The links between Cyclin D1 levels and disease and clinical factors (age, histological type, tumor grade, HER2 status) were evaluated utilizing the chi-square test. It was decided that $P \leq 0.05$ was the level of statistical significance. The data was studied using SPSS version 26.

Results: Cyclin D1 positivity was detected in 72.5% (29 out of 40) of patients. Its expression exhibited considerable variation across diverse clinicopathological parameters:

• Age: The peak expression was observed in the 50–59 age bracket (40%).

• Occupation: Housewives demonstrated the highest Cyclin D1 positive at 40%, followed by employees at 25% and self-employed women at 7.5%. Histopathological Type: The most expression was seen in Invasive Ductal Carcinoma (IDC), at 60%. Invasive Lobular Carcinoma (ILC) and Ductal Carcinoma In Situ (DCIS) showed the least displayed lower levels.

• Tumor Grade: Grade II tumors exhibited the highest expression of Cyclin D1 at 37.5%, followed by grade III at 25% and grade I at 10%. HER2 Status: HER2-negative tumors exhibited Cyclin D1 expression more frequently (47.5%) than HER2-positive cancers (25%). All correlations exhibited statistical significance ($P \leq 0.05$).

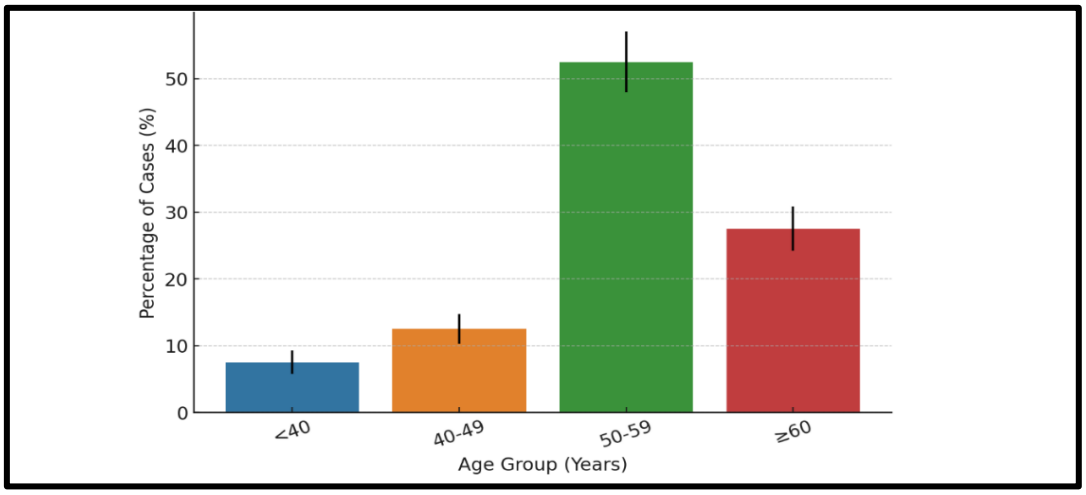
Table 1 – Participants' demographic characteristics and cyclin different age and histopathological groups of breast cancer have different expressions

Variables	**Expression of immunohistochemical biomarkers**	
	Cyclin D1 No (%)	
	+ve	**-ve**
Age (years)		
<40	2 (5) C, a	1 (2.5) D, b
40-49	2 (5) C, b	3 (7.5) B, a
50-59	16 (40) A, a	5 (12.5) A, b
>60	9 (22.5) B, a	2 (5) C, b
Total	29 (72.5) a	11 (27.5) b
Job (%)		
Self-employment	3 (7.5) C, a	3 (7.5) B, a
Employee	10 (25) B, a	3 (7.5) B, b
Housewife	16 (40) A, a	5 (12.5) A, b
Total	29 (72.5) a	11 (27.5) b
Histopathological type (%)		
Ductal Carcinoma In Situ - DCIS	2 (5) B, a	0 (0) C, b

Invasive ductal carcinoma (IDC)	24 (60) A, a	8 (20) A, b
Invasive lobular carcinoma (ILC)	2 (5) B, b	3 (7.5) B, a
Medullary Carcinoma	1 (2.5) C, a	0 (0) C, b
Total	29 (72.5) a	11 (27.5) b
Tumor grade		
I	4 (10) C, a	3 (7.5) B, b
II	15 (37.5) A, a	6 (15) A, b
III	10 (25) B, a	2 (5) C, b
Total	29 (72.5) a	11 (27.5) b
HER-2 (%)		
Positive	10 (25) B, a	3 (7.5) B, b
Negative	19 (47.5) A, a	8 (20) A, b
Total	29 (72.5) a	11 (27.5) b

Note: 1. Different letters in one line indicate a significant difference between the means (P ≤0.05). Similar letters indicate no significant difference between the means.

2. The numbers in parentheses in the table above represent the percentage of the sample size, while the numbers outside the parentheses represent the number of infected cases.



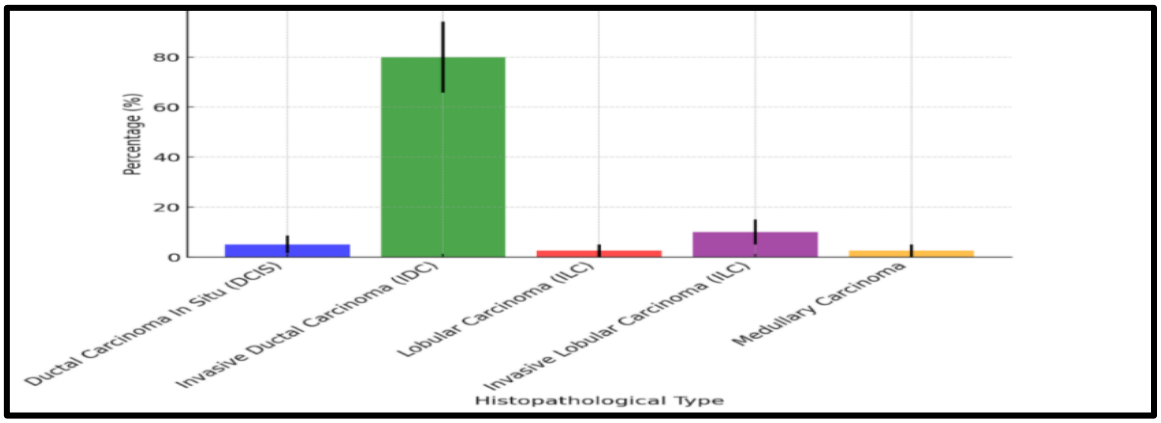


Fig. 1 : Breast Cancer Cases by Age Group (%).

Fig. 2 : Distribution of Breast Cancer Histopathological Types (%). Source: Output generated by IBM SPSS Statistics, version 26.0, based on study data collected in 2023.

Fig. 3: HER2 Status Distribution in Breast Cancer Cases (%). Source: Output generated by IBM SPSS Statistics, version 26.0, based on study data collected in 2023.

Comparative Analysis of Cyclin D1 IHC Expression in Breast Tissue. The **(fig 3)** represents a histological section of breast tissue analyzed using IHC for Cyclin D1 expression.
Cyclin D1 is a protein in the nucleus that controls how quickly cells divide. It is often overexpressed in several types of cancer, including breast carcinoma. However, in this particular image, there is no visible brown staining within the nuclei of the epithelial cells, indicating a negative result for Cyclin D1.
The labeled regions highlight:
- Ductal Structures: Epithelial-lined ducts that appear morphologically benign.
- Stroma: The surrounding fibrous connective tissue shows no atypical cellular activity.
- No Nuclear Staining: Demonstrating that Cyclin D1 is not expressed in the nuclei of these cells.

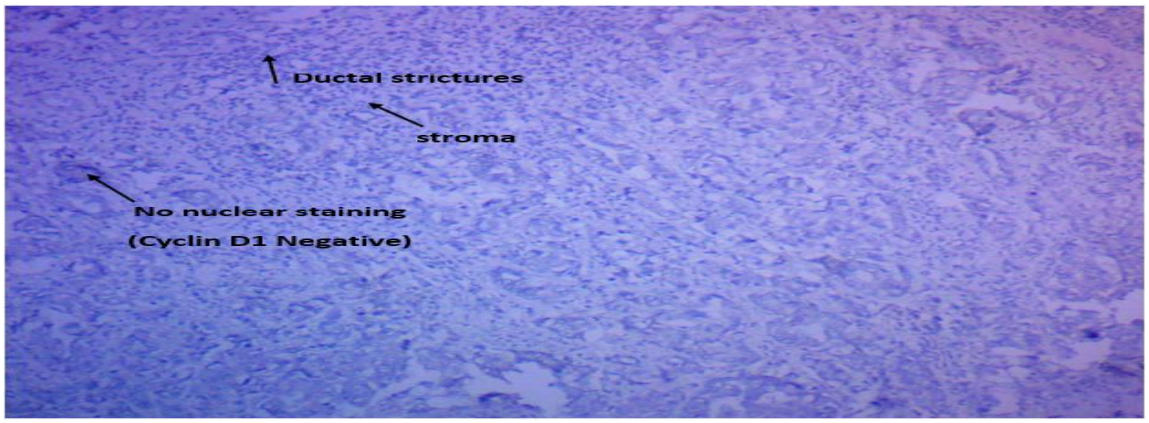


Fig. 4: Negative Cyclin D1 Expression in Breast Tissue (IHC Study)
This kind of pattern **(Fig 4)** can be found in either healthy breast tissue or some low-proliferation breast cancers. It is a useful way to compare it to Cyclin D1-positive cancerous tissues.

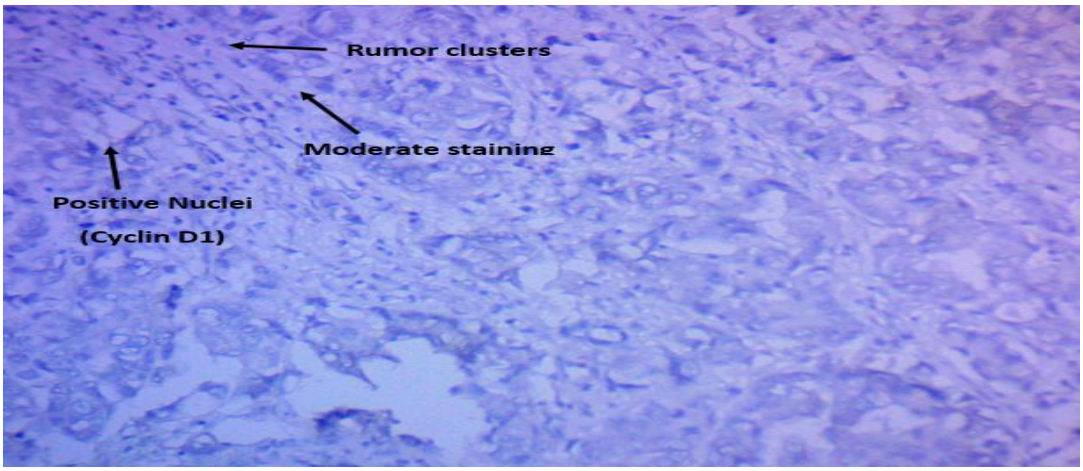


Fig .5: Positive Expression - Moderate Activity in Breast Carcinoma
This picture (Fig 5) shows a slice of breast cancer that was labeled with IHC for Cyclin D1.
Overexpression of cyclin D1, a central cell cycle regulator, is linked to more cell growth in some types of cancer. In this part, several nuclei inside tumor cell groups show brown staining, which shows that Cyclin D1 is expressed.
Some important features are:
tumor clusters are made up of cancerous epithelial cells that have abnormal nuclei.
- Positive Nuclei (Cyclin D1): nuclei that are dyed brown show that the protein is active.
- Moderate Staining: The staining is focal or patchy, which means the expression level is not widespread and is in the middle.

This expression pattern is typical of ER-positive, Luminal A or B-type breast tumors and may carry diagnostic and prognostic implications. It also assists treatment planning, especially when correlated with markers like Ki-67 and HER2.

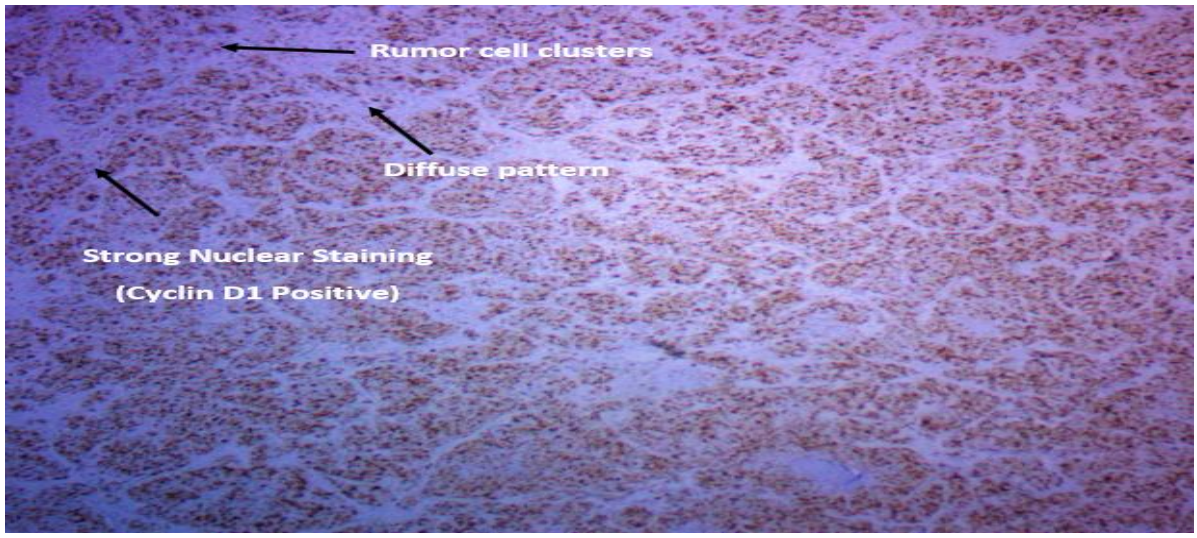


Fig. 6: Diffuse Strong Positive Expression - High Proliferative Tumor

This image reveals a breast carcinoma with diffuse and strong nuclear Cyclin D1 expression. Nearly all tumor cell nuclei show intense brown staining, indicating high proliferative activity. This pattern is often linked with aggressive tumor behavior and worse prognosis.

Discussion: This work underscores Cyclin D1's significance as a crucial biomarker in the development of breast cancer. The results correspond with earlier research, demonstrating elevated Cyclin D1 expression in IDC and intermediate-grade cancers [18, 19]. The prevalence of expression in the 50–59 age demographic and among homemakers may indicate lifestyle-related or hormonal factors.

In this work, we looked at how Cyclin D1 is expressed in breast cancer tissues and how that expression is linked to demographic, pathological, and molecular factors. The protein was positive in 72.5% of cases, and there was a statistically significant link between the two across all age groups, tissue types, tumor grades, and HER2 status. Cyclin D1 positivity was highest in people aged 50 to 59 (40%) and those over 60 (22.5%). This fits with evidence that Cyclin D1 excess is more common in women who have gone through menopause and rises with age due to genetic instability [20, 21]. Regarding occupational status, women had the highest positivity rate (40%), but this may be because of the demographics of the sample rather than a biological link. IDC had the highest positivity rate (60%), which aligns with earlier research that found Cyclin D1 overexpression to be a feature of IDC, especially those with luminal forms [22]. Interestingly, more cases of ILC were Cyclin D1-negative, which backs up studies that found lower Cyclin D1 expression in non-ductal forms [23]. According to the grade of the tumor, the expression was highest in grade II tumors (37.5% of all tumors tested). This suggests that Cyclin D1 may play a role in tumor development but not always aggressive transformation, which aligns with what has been seen before [24]. Cyclin D1 positivity was also strongly linked to HER2-negative tumors (47.5%), which could mean that Cyclin D1 and HER2-driven pathways are unrelated, as seen in earlier genetic studies [25]. These results show that Cyclin D1 could be useful as a prognostic biomarker, especially in ER+/HER2 − luminal breast cancers, and they support adding it to future diagnostic panels.

The inverse connection between Cyclin D1 and HER2 status substantiates the concept that Cyclin D1 functions as a compensating proliferative factor in HER2-negative malignancies. Cyclin D1's role in CDK4/6 activation and transcriptional control suggests that its profiling may inform customized therapy, especially in luminal subtypes where it has demonstrated both positive and negative prognostic significance [18, 26]. These results show that Cyclin D1 plays a complex role in the development of breast cancer and stress the need for future research that focuses on specific molecular subtypes.

Conclusion: Cyclin D1 expression is markedly correlated with age, tumor grade, histological type, and HER2 status in patients with breast cancer. The elevated occurrence in specific populations highlights its potential as a predictive biomarker and therapeutic target. Incorporating Cyclin D1 profiling into standard pathological assessments may improve diagnostic precision and guide therapeutic approaches.

Strengths of the study:

• Employment of advanced IHC methodologies.
Incorporation of various clinicopathological factors for correlation analysis.
Constraints of the study:
• Restricted sample size diminishes generalizability.
• Absence of molecular subtype categorization (e.g., Luminal A, Luminal B, Basal-like).
• Lack of subsequent data regarding patient outcomes.

Future research should encompass bigger, multicenter cohorts, incorporate molecular profiling, and entail longitudinal follow-up to evaluate the prognostic significance of Cyclin D1 over time.

REFERENCES:

1. Obeagu EI, Obeagu GU. Breast cancer: A review of risk factors and diagnosis. Medicine. 2024;103(3):e36905.
2. Pu, D., Zhang, C., Wang, Y., & Li, J. (2024). Efficacy of CDK4/6 inhibitors combined with endocrine therapy in HR+/HER2− breast cancer. Journal of Cancer Research and Clinical Oncology, 150(1), 16. https://doi.org/10.1007/s00432-023-05110-1
3. Cohen, S. Y., Stoll, C. R., Anandarajah, A., Doering, M., & Colditz, G. A. (2023). Modifiable risk factors in women at high risk of breast cancer: a systematic review. Breast Cancer Research, 25(1), 45.
4. Kashyap, R., Singh, S., & Verma, A. (2021). Cell cycle dysregulation in breast cancer: Mechanisms and therapeutic implications. Journal of Cancer Biology, 23(3), 345–359.
5. Chou, J., Quigley, D. A., Robinson, T. M., Feng, F. Y., & Ashworth, A. (2020). Transcription-associated cyclin-dependent kinases as targets and biomarkers for cancer therapy. Cancer Discovery, 10(3), 351–370. https://doi.org/10.1158/2159-8290.CD-19-0725
6. Vashchenko, L. N., Kosenok, V. K., & Semiglazov, V. F. (2018). Comparison of expression of proliferation markers Ki-67 and Cyclin D1 in triple-negative breast cancer. Voprosy Onkologii, 64(5), 611–616.
7. Abdalla, N., Ahmad, M., Al-Ali, B. M., & Al-Masri, A. (2020). Significance of targeting VEGFR-2 and Cyclin D1 in luminal-A breast cancer. Molecules, 25(20), 4606. https://doi.org/10.3390/molecules25204606
8. Saunders, C. M., Jassal, S., & Lim, E. (2018). Breast cancer: the facts. Oxford University Press.
9. Wang, S., Chen, Y., Li, W., Hao, C., Zhang, L., Zhao, W., ... & Tong, Z. (2024). Serum HER2 level predicts therapeutic efficacy and prognosis in advanced breast cancer patients. Breast Cancer: Targets and Therapy, 163-179.
10. Cho, J. (2022). Basic immunohistochemistry for lymphoma diagnosis. Blood research, 57(S1), 55-61.
11. Hassan Rahimi Koshkaki, S. Minasi, A. Ugolini, G. Trevisi, C. Napoletano, I. Zizzari Immunohistochemical Characterization of Immune Infiltrate in Tumor Microenvironment of Glioblastoma. 2020. Journal of Personalized Medicine.
12. Tsutsumi, Y. (2021). Pitfalls and caveats in applying chromogenic immunostaining to histopathological diagnosis. Cells, 10(6), 1501.
13. Khodeir, S. S., Elkerdany, T. A., Saad, A. A., El Sakhawy, Y. N., Hamed, G. M., & Hassan, N. B. (2018). Role of Immunostaining in Detecting Extra-Pattern and Subtle Lymphomatous Infiltration in Bone Marrow Biopsies of NHL Patients. Open Journal of Blood Diseases, 8(2), 27-36.
14. Zhaoyu Sun, Richard Nyberg, Yaping Wu, Brady Bernard, William L. Redmond. Developing an enhanced 7-color multiplex IHC protocol to dissect immune infiltration in human cancers. 2021. PLoS ONE
15. Rahman, M. T., & Karim, M. M. (2023). Techniques in Immunology. Immunology for Dentistry, 215-238. Bouzid, R. S., Bouzid, R., Labed, H., Serhani, I., Hellal, D., Oumeddour, L., ... & Belaaloui, G. (2025). Molecular subtyping and target identification in triple negative breast cancer through immunohistochemistry biomarkers. BMC cancer, 25(1), 1-16.
16. Bouzid, R. S., Bouzid, R., Labed, H., Serhani, I., Hellal, D., Oumeddour, L., ... & Belaaloui, G. (2025). Molecular subtyping and target identification in triple negative breast cancer through immunohistochemistry biomarkers. BMC cancer, 25(1), 1-16.
17. Jeffreys SA, Becker TM, Khan S, Soon P, Neubauer H, de Souza P, Powter B. Prognostic and Predictive Value of CCND1/Cyclin D1 Amplification in Breast Cancer With a Focus on Postmenopausal Patients: A Systematic Review and Meta-Analysis. Front Endocrinol (Lausanne). 2022 Jun 17;13:895729. doi:10.3389/fendo.2022.895729. PMID: 35784572; PMCID: PMC9249016.
18. Ortiz, B., Witek, E. M., Iyer, S., Hwang, M., & Koos, R. (2017). Prognostic significance of Cyclin D1 protein expression and gene amplification in invasive breast carcinoma. PLOS ONE, 12(6), e0179285. https://doi.org/10.1371/journal.pone.0179285
19. Mohammadizadeh, F., Mahmoudi, M., & Kadivar, M. (2013). Role of Cyclin D1 in breast carcinoma. Journal of Research in Medical Sciences, 18(12), 1021–1025.
20. Montalto, F. I., & De Amicis, F. (2020). Cyclin D1 in Cancer: A Molecular Connection for Cell Cycle Control, Adhesion and Invasion in Tumor and Stroma. Cells, 9(12), 2648. https://doi.org/10.3390/cells9122648
21. Siraj AK, Parvathareddy SK, Annaiyappanaidu P, Ahmed SO, Siraj N, Tulbah A, Al-Dayel F, Ajarim D, Al-Kuraya KS. High Expression of Cyclin D1 is an Independent Marker for Favorable Prognosis in Middle Eastern Breast Cancer. Onco Targets Ther. 2021 May 19;14:3309-3318. doi: 10.2147/OTT.S309091. PMID: 34040395; PMCID: PMC8141388.
22. Ortega MA, Fraile-Martinez O, García-Montero C, Borja-Vergel S, Torres-Carranza D, Pekarek L, Arribas CB, De León-Luis JA, Sánchez-Rojo C, Alvarez-Mon MA, García-Honduvilla N, Buján J, Coca S, Alvarez-Mon M, Saez MA, Guijarro LG. Patients with Invasive Lobular Carcinoma Show a Significant Increase in IRS-4 Expression Compared to Infiltrative Ductal Carcinoma-A Histopathological Study. Medicina (Kaunas). 2022 May 28;58(6):722. doi: 10.3390/medicina58060722. PMID: 35743985; PMCID: PMC9229273.
23. Lengare, Pranoti Vitthalrao; Sinai Khandeparkar, Siddhi Gaurish; Joshi, Avinash R.; Gogate, Bageshri P.; Solanke, Smita Ganeshrao; Gore, Sujit Hanumant. Immunohistochemical expression of cyclin D1 in invasive breast carcinoma and its correlation with clinicopathological parameters. Indian Journal of Pathology and Microbiology 63(3):p 376-381, Jul–Sep 2020. | DOI: 10.4103/IJPM.IJPM_106_20

24. Salvador, M. A., Wicik, Z., Brzoska, K., Barciszewska, M. Z., & Nowak, S. (2019). Cyclin D1 in breast cancer: A key player or a coincidental marker? Journal of Oncology, 2019, Article 4569643. https://doi.org/10.1155/2019/4569643
25. Tashiro, E., Tsuchiya, A., & Imoto, M. (2007). Functions of cyclin D1 as an oncogene and regulation of cyclin D1 expression. Cancer science, 98(5), 629-635.
26. Jayashree Bhawani, S. Shukla, S. Acharya, S. Vagha, M. Jagtap. To study the utility of COX-2 as immunohistochemical prognostic marker in comparison to various histopathological parameters and TNM staging in breast carcinoma: an observational, cross-sectional study protocol. 2023. F1000Research