

Immunohistochemical Expression of P21 In Invasive Ductal Carcinoma of Breast

Raquibul Hasan Kazal^{1*}, Sabiha Shabnam², Zillur Rahman³, Shantanu Biswas⁴, Purabi Sarkar⁵

ARTICLE INFO

Received: 17 September 2026

Accepted: 23 September 2026

Published Online: 3 October 2026

DOI: 10.5281/zenodo.23114210

Volume: 10, Number: 3, Page: 678-682

e-ISSN: 2789-5912

ISSN: 2617-0817

*Corresponding author



ABSTRACT

Background: Among women worldwide, breast cancer remains a major cause of cancer morbidity and mortality, while invasive ductal carcinoma is its most common histological type. Altered p21 expression may influence cell-cycle regulation and tumor behavior, but its clinicopathological significance remains inconsistent. **Aim of the study:** This study aimed to evaluate the immunohistochemical expression of p21 in invasive ductal carcinoma of the breast and assess its association with selected clinicopathological parameters. **Methods & Materials:** A descriptive cross-sectional study was conducted in the Department of Pathology, Chittagong Medical College, Chattogram, from March 2018 to February 2020, including 52 histopathologically confirmed cases of invasive ductal carcinoma of the breast. Immunohistochemical staining for p21 was performed at CARE Investigation in Chattogram using formalin-fixed, paraffin-embedded tissue sections. p21 expression was categorized as negative, weakly positive, or strongly positive and assessed in relation to selected clinicopathological parameters. **Results:** Among 52 patients, the mean age was 48.33 ± 10.81 years, and 98.1% were female. Grade II carcinoma predominated, at 75.0%. Overall, p21 immunoreactivity was observed in 73.1% of tumors, with strong expression in 51.9%, weak in 21.2%, and 26.9% negative. No significant association was found between p21 expression and age ($p=0.448$), estrogen exposure ($p=0.805$), or histopathological grade ($p=0.483$). **Conclusion:** Overall, p21 expression was common in invasive ductal carcinoma of the breast, with strong positivity in over half of cases. However, its expression was not significantly associated with age, estrogen exposure, or histological grade, suggesting limited utility as an isolated clinicopathological marker.

Keywords: p21; Immunohistochemistry; Breast cancer; Histopathological grade.

1. Assistant Professor, Department of pathology, Sir Salimullah Medical College Mitford Hospital, Dhaka, Bangladesh (ORCID: 0009-0002-7133-3067)
2. Assistant Professor, Department of Pathology, Shaheed Suhrawardy Medical College & Hospital, Dhaka, Bangladesh
3. Professor, Department of Pathology, Bangladesh Medical University, Dhaka, Bangladesh
4. Assistant Professor, Department of Pathology, Jashore Medical College, Jashore, Bangladesh
5. Associate Professor & Head, Department of Pathology, Ad-Din Akij Medical College & Hospital, khulna, Bangladesh

INTRODUCTION

Globally, breast cancer remains one of the most important malignancies affecting women and a major contributor to cancer-related morbidity and mortality. In 2020, more than 2.3 million new breast cancer cases and approximately 685,000 deaths were estimated worldwide, with projections indicating a further substantial increase as populations grow and age [1]. Across countries, marked disparities persist in incidence, stage at diagnosis, access to treatment, and survival, particularly between high-income settings and low- and middle-income countries [2]. Over recent decades, rising incidence has been observed across most age groups and world regions, reflecting demographic transition, reproductive and lifestyle changes, improved detection, and broader changes in cancer risk profiles [3,4]. Across Asia, the burden of breast cancer has increased rapidly and now accounts for a large proportion of global cases and deaths [5,6]. In 2022, approximately 985,000 new cases and 315,000 deaths were estimated in Asia, while Bangladesh alone was estimated to have about 13,000 new cases and 6,200 deaths [6]. Within South Asian populations, breast cancer often presents at a relatively younger age and may show clinically important differences in molecular subtype distribution compared with Western

populations [7,8]. Among Bangladeshi patients, available molecular data remain limited, although recent evidence has suggested substantial representation of aggressive subtypes, including triple-negative disease [7]. Consequently, locally generated pathological and biomarker data are needed to improve understanding of tumor biology in this population. Histologically, invasive ductal carcinoma represents the predominant form of invasive breast carcinoma encountered in routine practice. Clinically, its behavior is heterogeneous, with prognosis influenced by tumor size, lymph-node status, histological grade, hormone receptor status, HER2 expression, proliferative activity, and other molecular characteristics [4,9]. At the regional level, variation in molecular profiles has also been demonstrated among Asian populations, reinforcing the importance of population-specific biomarker research [9]. In South Asia, studies from India have similarly emphasized heterogeneity among tumor subtypes and the need for validated biomarkers to support risk stratification and treatment decisions [8,10]. At the molecular level, disruption of normal cell-cycle regulation is a fundamental event in breast carcinogenesis [11,12]. Among the key regulators, p21, also known as p21WAF1/CIP1 and encoded by CDKN1A,

is a cyclin-dependent kinase inhibitor that suppresses cell-cycle progression by inhibiting cyclin-CDK complexes [11]. Through both p53-dependent and p53-independent pathways, p21 participates in cell-cycle arrest, DNA-damage response, apoptosis, senescence, differentiation, and cellular stress responses [11,13]. Importantly, its biological role is context-dependent; nuclear p21 is generally associated with growth inhibition, whereas altered expression or localization may contribute to tumor progression, stemness, metastatic behavior, and treatment resistance [13]. In breast cancer specifically, reported associations between p21 expression and clinicopathological characteristics or prognosis have been inconsistent, suggesting that its significance may vary according to molecular subtype and cellular context [12,13]. Despite growing interest in p21 as a potential biomarker, relatively limited evidence is available from Bangladesh, particularly regarding its immunohistochemical expression in invasive ductal carcinoma and its relationship with routine clinicopathological features. Accordingly, this study aimed to evaluate the immunohistochemical expression of p21 in invasive ductal carcinoma of the breast and to assess its association with selected clinicopathological parameters, thereby

generating local evidence on the potential pathological relevance of p21 in breast cancer.

METHODS & MATERIALS

A descriptive cross-sectional study was conducted in the Department of Pathology, Chittagong Medical College, Chattogram, with immunohistochemical procedures performed at CARE Investigation, Chattogram. The study period extended from March 2018 to February 2020. A total of 52 histopathologically diagnosed cases of invasive ductal carcinoma of the breast were included in the study. Relevant demographic and clinicopathological information, including age, sex, occupation, family history of breast carcinoma, previous benign breast disease, tobacco use, history of estrogen exposure, age at menarche, and age at first live birth, was recorded using a structured data collection form. Histopathological diagnoses were confirmed by examination of hematoxylin and eosin-stained sections. Tumors were graded histologically and categorized as Grade I, Grade II, or Grade III according to

the grading system followed by the Department of Pathology. For immunohistochemical evaluation, representative formalin-fixed, paraffin-embedded tissue blocks containing adequate viable tumor were selected. Sections were prepared from the selected blocks and subjected to immunohistochemical staining for p21. Following routine deparaffinization, rehydration, antigen retrieval, and blocking procedures, the sections were incubated with the appropriate primary antibody against p21. Visualization was performed using a suitable detection system and chromogen, followed by hematoxylin counterstaining. Appropriate positive and negative controls were used during immunohistochemical staining. The immunohistochemical expression of p21 was assessed in tumor cells and categorized as negative, weakly positive, or strongly positive according to the staining criteria adopted in the study. For analytical comparison, p21 expression was further evaluated in relation to selected clinicopathological characteristics,

including age, history of estrogen exposure, and histopathological tumor grade. Data were compiled and analyzed using SPSS (V. 26.0). Continuous variables were summarized as mean ± standard deviation, while categorical variables were expressed as frequencies and percentages. Associations between p21 expression and clinicopathological parameters were assessed using appropriate categorical tests. A p-value of <0.05 was considered statistically significant.

RESULTS

Among the 52 patients, the mean age was 48.33 ± 10.81 years, with an age range of 21–73 years. Most frequently, patients belonged to the 40–49-year age group (38.5%), followed by those aged 50–59 years (25.0%). In terms of sex distribution, 98.1% were female, and 1.9% were male. Occupationally, 86.5% were housewives, whereas 13.5% were service holders (Table I).

Table I
Demographic characteristics of patients with invasive ductal carcinoma of breast (n=52).

Variables	Categories	Frequency (n)	Percentage (%)
Age (years)	20–29	2	3.8
	30–39	7	13.5
	40–49	20	38.5
	50–59	13	25
	60–69	8	15.4
	70–79	2	3.8
	Mean age ± SD	48.33 ± 10.81	
Sex	Male	1	1.9
	Female	51	98.1
Occupation	Housewife	45	86.5
	Service holder	7	13.5

With respect to risk factors, only 3.8% of patients had a positive family history of breast carcinoma, while 96.2% reported no family history. Additionally, 88.5% had no previous benign breast disease, although

9.6% had atypical ductal hyperplasia and 1.9% had fibrocystic disease. Tobacco use and estrogen exposure were reported in 21.2% and 38.5%, respectively. Among the female patients, menarche at 12–13 years

was most common, 47.1%, while 82.4% had their first live birth at ≤20 years (Table II).

Table II
Distribution of selected risk factors among patients with invasive ductal carcinoma (n=52).

Risk factors	Categories	n (%)
Family history of breast carcinoma	Positive	2 (3.8)
	Negative	50 (96.2)
History of benign breast disease	No history	46 (88.5)
	Atypical ductal hyperplasia	5 (9.6)
	Fibrocystic disease	1 (1.9)
Tobacco use	Positive	11 (21.2)
	Negative	41 (78.8)
History of estrogen exposure	Positive	20 (38.5)
	Negative	32 (61.5)
Age at menarche (n=51)	≤11 years	20 (39.2)
	12–13 years	24 (47.1)
	≥14 years	7 (13.7)
	≤20 years	42 (82.4)
Age at first live birth (n=51)	21–29 years	3 (5.9)
	≥30 years	6 (11.8)

Histopathologically, Grade II carcinoma was the most frequent grade, observed in 75.0% of cases. In comparison, 21.2% of tumors were Grade III, while only 3.8% were Grade I. Overall, intermediate-grade tumors constituted the predominant histopathological pattern in the studied patients (*Table III*).

Table III
Histopathological grade of invasive ductal carcinoma (n=52).

Tumor grade	Frequency (n)	Percentage (%)
Grade I	2	3.8
Grade II	39	75
Grade III	11	21.2
Total	52	100

Regarding immunohistochemical findings, strong p21 expression was detected in 51.9% of cases, making it the most frequent expression pattern. Weak p21 positivity was observed in 21.2%, whereas 26.9% of cases showed negative expression. Collectively, 73.1% of the tumors demonstrated either weak or strong p21 immunoreactivity (*Table IV*).

Table IV
Immunohistochemical expression of p21 in invasive ductal carcinoma (n=52).

p21 expression	Frequency (n)	Percentage (%)
Negative	14	26.9
Weakly positive	11	21.2
Strongly positive	27	51.9
Total	52	100

When clinicopathological characteristics were compared by p21 expression, no statistically significant associations were observed. Across age categories, the difference in p21 expression was not significant (p=0.448). Similarly, estrogen exposure showed no significant association with p21 expression (p=0.805). For tumor grade, Grade II lesions predominated in both p21-negative and p21-positive cases, accounting for 71.1% and 85.7%, respectively; however, this association was not statistically significant (p=0.483) (*Table V*).

Table V
Association between p21 expression and clinicopathological characteristics of invasive ductal carcinoma (n=52).

Variables	Categories	p21 negative	p21 positive	p-value
		n (%)	n (%)	
Age	≤50 years	26 (63.4)	8 (57.1)	0.448
	>50 years	12 (31.6)	6 (33.3)	
History of estrogen exposure	No	23 (60.5)	9 (64.3)	0.805
	Yes	15 (39.5)	5 (35.7)	
Tumor grade	Grade I	2 (5.3)	0 (0.0)	0.483
	Grade II	27 (71.1)	12 (85.7)	
	Grade III	9 (23.7)	2 (14.3)	

DISCUSSION

The present study evaluated immunohistochemical expression of p21 in 52 cases of invasive ductal carcinoma of the breast and explored its relationship with selected clinicopathological characteristics. The mean age was 48.33 ± 10.81 years; most tumors were Grade II (75.0%); and p21 immunoreactivity was common, with 51.9% showing strong and 21.2% weak expression. No significant association was detected between p21 expression and age, estrogen exposure, or histological grade. The relatively young mean age of 48.33 years is consistent with patterns reported from several Asian populations. The study by Pan et al., examining the molecular landscape of Asian breast cancers, demonstrated important population-specific differences in breast cancer presentation and biology [9]. Similarly, the Bangladeshi analysis by Islam et al. showed substantial heterogeneity in molecular subtypes and highlighted the relatively younger

presentation of breast cancer within this population [7]. An Indian systematic review by Jonnada et al. also demonstrated considerable variation in breast cancer phenotypes across regional populations [8]. In comparison, a Libyan series reported a mean age of 47.3 ± 10.7 years, remarkably close to the present value [14]. Such similarities may reflect demographic structure, reproductive patterns, genetic background, and differences in screening practices across low- and middle-income settings. Histologically, Grade II carcinoma predominated in the present series, accounting for 75.0%, while Grade III and Grade I tumors represented 21.2% and 3.8%, respectively. A recent Pakistani study reported Grade II disease in 65%, Grade III in 30%, and Grade I in 5%, showing a broadly comparable pattern [15]. In addition, the Kenyan study by Njihia found that 92.7% of breast carcinomas were moderate-to-high grade [16]. Studies examining other

immunohistochemical markers have similarly demonstrated that intermediate and high-grade invasive carcinomas constitute substantial proportions of clinical breast cancer cohorts [17-19]. The predominance of Grade II tumors in the present study may therefore reflect the usual biological spectrum of invasive ductal carcinoma, although referral patterns and the relatively small sample could influence the distribution. A particularly important observation was the frequent expression of p21: strong staining in 51.9%, weak in 21.2%, and negative in 26.9%. Biological evidence indicates that p21, encoded by CDKN1A, is a major cyclin-dependent kinase inhibitor involved in G1/S cell-cycle arrest, DNA-damage responses, senescence, and apoptosis [11,12]. Nevertheless, p21 does not behave exclusively as a tumor suppressor. The review by Manousakis et al. emphasized that its function in breast cancer depends substantially on cellular localization and molecular context; nuclear

p21 generally restrains proliferation, whereas cytoplasmic p21 may promote survival, migration, stemness, and therapeutic resistance^[13]. This dual function provides a plausible explanation for the substantial p21 expression observed in malignant tissue and for differences in expression frequencies among studies. No statistically significant association was found between p21 expression and age ($p=0.448$). Comparable uncertainty has been observed with several breast cancer biomarkers. For example, the study by Hussein et al. found no significant relationship between BRCA1 immunoexpression and patient age^[17], while the Pakistani study of 120 women also demonstrated no significant association between age and histological grade ($p=0.237$)^[15]. These findings suggest that chronological age alone may be insufficient to predict molecular alterations within breast tumors. Likewise, p21 expression was not significantly associated with histological grade ($p=0.483$), despite Grade II tumors comprising 71.1% of the p21-negative and 85.7% of the p21-positive groups. This differs from investigations of other cell-cycle markers. The study by Malik et al. reported p16 expression in 48% of invasive breast carcinomas and found significant associations with higher grade and Ki-67 proliferation^[18]. Similarly, Lashen et al. demonstrated that high nuclear CDK2 expression was strongly associated with high tumor grade, lymphovascular invasion, and adverse prognostic characteristics^[19]. Wu et al. also found that TOP2A overexpression was significantly correlated with higher histological grade ($r=0.59$, $p<0.001$)^[20]. Differences from the present results may arise from p21's context-dependent biology, variations in antibodies and scoring thresholds, molecular subtype composition, and, in particular, the smaller sample size of 52 cases. No significant relationship was observed between estrogen exposure and p21 expression ($p=0.805$). Breast carcinogenesis is multifactorial, and epidemiological estrogen exposure does not necessarily correspond directly with tumor-cell hormonal signaling or CDKN1A activity. Current molecular evidence indicates considerable interaction among ER signaling, HER2, p53, CDKs, and other proliferative pathways^[11-13,21]. Other immunohistochemical studies have likewise shown substantial marker-specific heterogeneity; TROP-2 expression, for example, was not prognostic in a cohort of 685 triple-negative breast cancers, whereas POC1A and NUF2 were associated with aggressive features in invasive ductal carcinoma^[22,23].

LIMITATIONS

The study was limited by its small sample size and single-center design, which may

restrict the generalizability of the findings. The absence of long-term follow-up and survival data also prevented assessment of the prognostic significance of p21 expression. In addition, p21 was not evaluated in relation to a broader panel of molecular markers or according to its nuclear and cytoplasmic localization.

CONCLUSION

In conclusion, p21 immunohistochemical expression was frequently observed in invasive ductal carcinoma of the breast, with strong positivity present in more than half of the cases. Although Grade II tumors predominated, p21 expression showed no statistically significant association with age, estrogen exposure, or histological grade. These findings suggest that p21 may reflect underlying tumor biology but, when assessed alone, has limited value for clinicopathological stratification.

RECOMMENDATIONS

Based on the present findings, larger multicenter studies with greater sample sizes are recommended to validate the pattern and clinical significance of p21 expression in invasive ductal carcinoma of the breast. To improve prognostic assessment, future research should evaluate p21 alongside established biomarkers such as ER, PR, HER2, Ki-67, and p53. In addition, standardized immunohistochemical scoring, separate assessment of nuclear and cytoplasmic expression, molecular subtype analysis, and long-term survival follow-up should be incorporated.

FUNDING

No funding sources

CONFLICT OF INTEREST

None declared

ETHICAL APPROVAL

The study was approved by the Institutional Ethics Committee.

REFERENCES

1. Arnold M, Morgan E, Rungay H, Mafra A, Singh D, Laversanne M, Vignat J, Gralow JR, Cardoso F, Siesling S, Soerjomataram I. Current and future burden of breast cancer: Global statistics for 2020 and 2040. *The breast*. 2022 Dec 1;66:15-23.
2. Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. *The British journal of radiology*. 2022 Feb 1;95(1130):20211033.
3. Lima SM, Kehm RD, Terry MB. Global breast cancer incidence and mortality trends by region, age-groups, and fertility patterns. *EClinicalMedicine*. 2021 Aug 1;38.
4. Łukasiewicz S, Czezelewski M, Forma A, Baj J, Sitarz R, Stanisławek A. Breast cancer—epidemiology, risk factors, classification, prognostic markers, and current treatment strategies—an updated

- review. *Cancers*. 2021 Aug 25;13(17):4287.
5. Youn HJ, Han W. A review of the epidemiology of breast cancer in Asia: Focus on risk factors. *Asian Pacific journal of cancer prevention: APJCP*. 2020 Apr;21(4):867.
6. Fu M, Peng Z, Wu M, Lv D, Li Y, Lyu S. Current and future burden of breast cancer in Asia: A GLOBOCAN data analysis for 2022 and 2050. *The Breast*. 2025 Feb 1;79:103835.
7. Islam D, Islam MS, Dorin SI, Jesmin. Prevalence of breast cancer subtypes among different ethnicities and bangladeshi women: demographic, clinicopathological, and integrated cancer informatics analysis. *Cancer Informatics*. 2023 Jan;22:11769351221148584.
8. Jonnada PK, Sushma C, Karyampudi M, Dharanikota A. Prevalence of molecular subtypes of breast cancer in India: a systematic review and meta-analysis. *Indian Journal of Surgical Oncology*. 2021 Apr;12(Suppl 1):152-63.
9. Pan JW, Zabidi MM, Ng PS, Meng MY, Hasan SN, Sandey B, Sammut SJ, Yip CH, Rajadurai P, Rueda OM, Caldas C. The molecular landscape of Asian breast cancers reveals clinically relevant population-specific differences. *Nature communications*. 2020 Dec 22;11(1):6433.
10. Bhattacharyya GS, Doval DC, Desai CJ, Chaturvedi H, Sharma S, Somashekhar SP. Overview of breast cancer and implications of overtreatment of early-stage breast cancer: an Indian perspective. *JCO global oncology*. 2020 Jun;6:789-98.
11. El-Deiry WS. p21 (WAF1) mediates cell-cycle inhibition, relevant to cancer suppression and therapy. *Cancer research*. 2016 Sep 15;76(18):5189-91.
12. Thu KL, Soria-Bretones I, Mak TW, Cescon DW. Targeting the cell cycle in breast cancer: towards the next phase. *Cell Cycle*. 2018 Aug 3;17(15):1871-85.
13. Manousakis E, Miralles CM, Esquerda MG, Wright RH. CDKN1A/p21 in Breast Cancer: Part of the Problem, or Part of the Solution?. *International Journal of Molecular Sciences*. 2023 Dec 14;24(24):17488.
14. Abousahmeem A, Saud MA, Elrais S, Kashbour M, Abuhlaiga M, Al-Aqmar DM, Al-Shareef JM. Molecular subtypes and clinicopathological features of breast cancer in Libya: a first glance. *Therapeutic Radiology and Oncology*. 2023 Dec 30;7.
15. Khan SN, Haider G, Rabail KZ, Zahoor S, Rehman A, Ramchand A, Anwer M, Jabeen M. Association Between Age of Patient and Grade of Breast Cancer. *J. BioMed Res. Reports*. 2025;7(1):1-8.
16. Njihia BN. Immunohistochemical p21 expression in Breast Cancer: Association with patient and tumour characteristics.
17. Hussein IA, Ahmed ST, Hameedi AD, Naji RZ, Alharbawi L, Alkhaytt M, Pity IS. Immunohistochemical expression of BRCA1 protein, ER, PR and Her2/neu in breast cancer: a clinicopathological study. *Asian Pacific journal of cancer prevention: APJCP*. 2020 Apr;21(4):1025.

18. Malik S, V S, Ahuja S, Ahluwalia C. Immunohistochemical expression of cyclin D1 and p16 in invasive breast carcinoma and its association with clinicopathological parameters. *Indian Journal of Surgical Oncology*. 2024 Dec;15(4):864-73.
19. Lashen A, Alqahtani S, Shoaqafi A, Algethami M, Jeyapalan JN, Mongan NP, Rakha EA, Madhusudan S. Clinicopathological significance of cyclin-dependent kinase 2 (CDK2) in ductal carcinoma in situ and early-stage invasive breast cancers. *International Journal of Molecular Sciences*. 2024 May 6;25(9):5053.
20. Wu Y, Han Y, Li Q, Zhang P, Yuan P, Luo Y, Fan Y, Chen S, Cai R, Li Q, Xu H. Predictive value of topoisomerase II alpha protein for clinicopathological characteristics and prognosis in early breast cancer. *Breast Cancer Research and Treatment*. 2022 Jun;193(2):381-92.
21. Cavalu S, Abdelhamid AM, Saber S, Elmorsy EA, Hamad RS, Abdel-Reheim MA, Yahya G, Salama MM. Cell cycle machinery in oncology: A comprehensive review of therapeutic targets. *The FASEB Journal*. 2024 Jun 15;38(11):e23734.
22. Izci H, Punie K, Waumans L, Laenen A, Wildiers H, Verdoodt F, Desmedt C, Ardui J, Smeets A, Han SN, Nevelsteen I. Correlation of TROP-2 expression with clinical–pathological characteristics and outcome in triple-negative breast cancer. *Scientific Reports*. 2022 Dec 28;12(1):22498.
23. Thabet DM, Thabit DM. Immunohistochemical expression of POC1A, NUF2, and Ki-67 in invasive ductal carcinoma of the breast: prognostic significance with insights into triple-negative breast cancer. *Am J Clin Pathol*. 2025 Dec;164(6):890-907.