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Immunohistochemical expression of cyclin-D1 in colorectal carcinoma with clinicopathological correlation

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Abstract
Background: Colorectal Cancer is the most common type of gastrointestinal cancer worldwide. In normal cells, cyclin D1 expression levels are strictly regulated, conversely, in cancer, its activity is intensified in various manners.
Aim: To investigate the expression of cyclin D1 in adenocarcinoma of colon. To correlate the expression of cyclin D1 with various clinicopathological parameters.
Method: A cross-sectional study was conducted in the Teaching Laboratories and Gastrointestinal Tract Hospital at the Medical City Teaching Complex. A convenient sampling method was adopted to enroll 50 who were diagnosed with colorectal cancer. The paraffin-embedded blocks of those patients were retrieved from the patient's records.
Results: High cyclin-D1 expression score was substantially greater in tumours with lympho-vascular invasion (62.5%) than those without (15.4%) (P-value=0.001). No significant correlation was seen between cyclin-D1 expression and tumour grade (P=0.510) or perineural invasion (P=0.895). The number of lymph nodes involved was associated with cyclin-D1 expression (P=0.016).
Conclusion: The cyclin D1 expression was significantly affected by lymphovascular involvement, and lymph node involvement implicating its potential prognostic role in CRC.

Keywords: Cyclin-D1, immunohistochemical, expression, colorectal carcinoma.

Introduction

Non-communicable diseases are the leading cause of death worldwide, with cancer anticipated to become one of the most critical causes in the 21st century, significantly impacting the quality of life [1]. Colorectal Cancer (CRC), encompassing cancer of the colon or rectum, has been a growing concern in developed countries for over 40 years and is increasingly problematic in low and middle-income countries due to urbanization and Western lifestyle risk factors [2]. Globally, CRC is the most common gastrointestinal cancer [3]. In Iraq, there has been a notable rise in CRC cases since 2007, affecting both sexes equally between 2000 and 2016 [4]. CRC is largely preventable as it usually arises from benign neoplasms, such as adenomas and serrated polyps, which can evolve into cancer over many years [5]. The most common form of CRC is adenocarcinoma, accounting for over 95% of cases, while rarer types include carcinoid tumors, sarcomas, and lymphomas, which present differently [6]. The etiology of colorectal neoplasms involves both genetic and environmental factors, although the precise causes remain unclear [2, 7]. The development of CRC is marked by the accumulation of genetic and epigenetic changes transforming normal glandular epithelial cells into invasive adenocarcinomas [5]. The slow progression from polyps to cancer allows for early detection and removal, preventing malignant transformation [5]. Screening guidelines for CRC vary globally based on population risk, healthcare resources, and societal values [8]. Effective screening programs use electronic health records to manage under-screening and over-screening by capturing key risk factors and determining screening eligibility [9]. Despite efforts to increase screening, about 33% of CRC patients present with symptoms requiring urgent or emergency surgical intervention, such as large bowel obstruction, perforation, and hemorrhage. These patients experience higher rates of morbidity, mortality, and stoma formation compared to those managed electively [10]. In cancer cells, cyclin D1 expression is often deregulated, in contrast to its strict regulation in normal cells.

Studies indicate that the cyclin D1 gene is amplified in several tumor types, serving as a negative prognostic marker [11]. The aim of study is to investigate the expression of cyclin D1 in adenocarcinoma of the colon and to correlate the expression of cyclin D1 with various clinicopathological parameters including (age of the patient, sex of the patient, grade of the tumor, depth of invasion, perineural invasion, lymphovascular invasion and lymph node involvement).

Methods

A cross-sectional study was conducted at the Teaching Laboratories and Gastrointestinal Tract Hospital, Medical City Teaching Complex, from May 2023 to February 2024. Ethical approval was obtained from the Scientific Council of Pathology of the Iraqi Board of Medical Specializations. Patient anonymity was maintained, and the data were used solely for scientific purposes.

Study Subject

Sampling Method: A convenient sampling method enrolled 50 patients diagnosed with colorectal cancer (CRC) from 2021 to 2023. Paraffin-embedded blocks from these patients were retrieved from their medical records.

Inclusion Criteria

1. Patients with colectomy specimens.
2. Adequate tumoral mass.
3. Presence of lymph node pathological slides.
4. Complete medical records.

Exclusion Criteria

1. Patients with a history of cancers other than CRC, or those who underwent chemotherapy or radiotherapy.
2. Endoscopic biopsy specimens of the colon.

Data Collection: Data were gathered using a checklist developed by the researcher after reviewing similar studies and revised by the supervisor. The data included patient age, sex, TNM staging (excluding metastasis due to unavailable data), tumor grade, perineural invasion, and lymphovascular invasion.

Immunohistochemistry Staining for Cyclin D1 Protein

Procedure: Five-micrometer paraffinized sections were soaked in a water-alcohol solution for five minutes and then placed in a microwave oven at 60°C for 30 minutes. Deparaffinization involved soaking slides in xylene followed by a series of alcohol baths (from 100% to 75% concentration) for 5 to 10 minutes each. Sections were rinsed with 10% phosphate-buffered saline (PBS), followed by H_2O_2/methanol (1:9), and 10% PBS for 10 minutes. Slides were then heated in a microwave oven for 10 minutes in ethylenediaminetetraacetic acid. After cooling to room temperature, sections were rinsed with PBS. Slides were incubated with 1 µg/mL diluted primary antibody against cyclin D1 for one hour at room temperature, followed by a 30-minute incubation with a biotinylated antibody and a 10-minute PBS soak. Sections were incubated with conjugated enzyme for 30 minutes and developed using 3,3′-diaminobenzidine hydrochloride. Hematoxylin was used for ground contrast staining. A mantle cell lymphoma sample was used as a positive control for staining accuracy, and PBS instead of specific antibodies was the negative control. Immunohistochemical slides were examined under a bright-field microscope at low (X40 and X100) and higher (X200 and X400) magnifications.

Interpretation of Immunohistochemistry Staining: The immunostaining for cyclin D1 was evaluated based on the percentage and intensity of positive epithelial cells. Intensity was scored as follows: [12]

- 0: Negative (no staining at high magnification).
- 1: Weak (only visible at high magnification).
- 2: Moderate (readily visible at low magnification).
- 3: Strong (strikingly positive at low magnification).

The percentage of stained nuclei was scored as: [13]

- 0: < 5% of cells
- 1: 5-25% of cells
- 2: 26-50% of cells
- 3: 51-75% of cells
- 4: 76-100% of cells

The final expression score was calculated for score 0, + for scores 1-3, ++ for scores 4-6, and +++ for scores >6. For statistical analysis, scores of and + were considered low, while ++ and +++ were considered high [14].

Statistical Analysis: Data were analyzed using Microsoft Excel and SPSS version 26. Categorical data were presented as frequencies and percentages, while continuous data were expressed as mean ± standard deviation (SD). The Chi-Square test and t-test were used to test the significance of differences between groups, with a p-value < 0.05 considered statistically significant.

Results

A total of 50 patients were enrolled in the current study. More than half of them (54%) aged ≥ 60 years. Males constituted 58% of the sample (Table 1).

Table 1: Distribution of the patients according to age and sex

Age and gender		N (%)
Age	<40 years	5 (10)
	40-59 years	18 (36.0)
	≥ 60 years	27 (54.0)
Sex	Male	29 (58.0)
	Female	21 (42.0)

As shown in table 3.2, 43 (86%) of the tumors were grade 2, 19 (38%) were associated with perineural invasion, and 24 (48%) were associated with lympho-vascular invasion.

Table 2: Distribution of the tumors according to the grade, perineural invasion, and lympho-vascular invasion

Tumor characteristics		N (%)
Grades	I	4 (8.0)
	II	43 (86.0)
	III	3 (6.0)
Perineural invasion	Yes	19 (38.0)
	No	31 (62.0)
Lympho-vascular invasion	Yes	24 (48.0)
	No	26 (52.0)

About 35 (70%) of the tumors were classified as T_3, and 44 (88%) were classified as N2, as shown in Table 3.

Table 3: TNM classification of the tumors in the current study

TNM classification	N (%)
Tumor	
T1	2 (4.0)
T2	7 (14.0)
T3	35 (70.0)
T4	6 (12.0)
Node	
N0	19 (38.0)
N1	9 (18.0)
N2	22 (44.0)

According to the results of the final cyclin-D1 expression score, 20 (40%) of the patients had negative cyclin-D1 expression, 11 (22%) of them had cyclin-D1 expression score of 1-3, 17 (34%) had cyclin-D1 expression score of 4-6, and 2 (4%) had cyclin-D1 expression score of > 6 as shown in Figure 1.

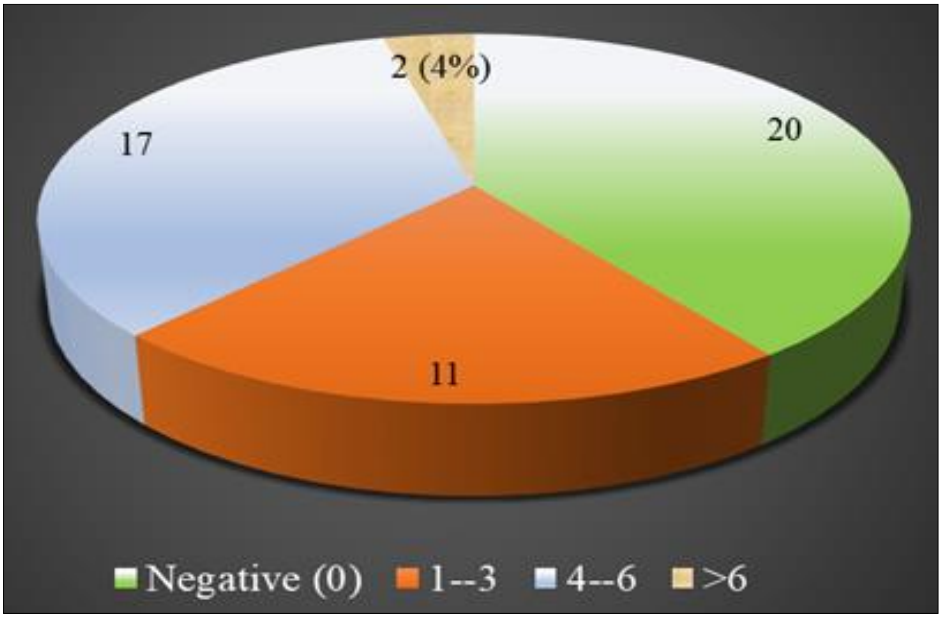

Fig 1: Distribution of the patients according to the results of the cyclin-D1 expression score.

For statistical analysis, the patients who had negative cyclin-D1 expression score and those who scored 1-3 were considered to have low cyclin-D1 expression while patients who scored > 3 were considered to have high scores Accordingly, 19 (38%) of the patients had high cyclin-D1 expression score as shown in figure 2.

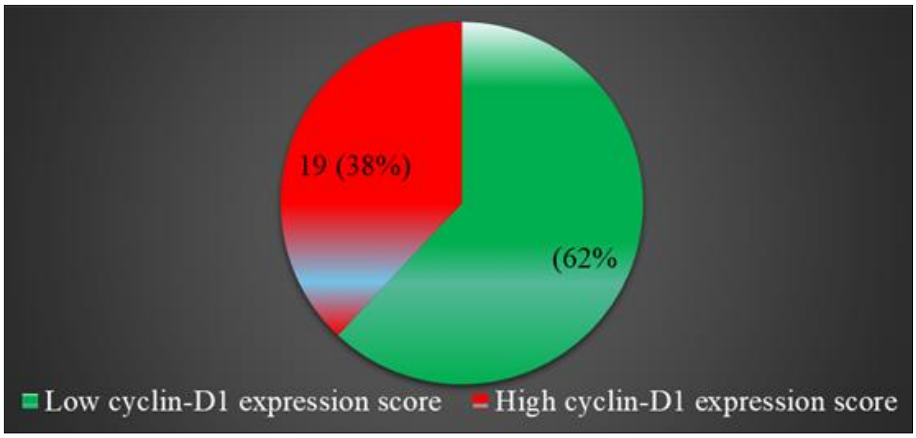

Fig 2: Distribution of the patients according to the results of immunohistochemistry staining.

There were no significant associations between age and gender and the cyclin-D1 expression (P-values were 0.091 and 0.99, respectively) as shown in Table 4.

The proportion of tumors with high cyclin-D1 expression score was significantly higher in those with lympho-vascular invasion (62.5%) compared to those without lympho-vascular invasion (15.4%) (P-value=0.001).

In contrast, there was no significant association between the cyclin-D1 expression and the grade of the tumors (P-value=0.510), and perineural invasion (P-value=0.895). As shown in Table 5.

Table 4: Association between the age and gender and cyclin-D1 expression

Age and gender		High score	Low score	P-Value
Age	<40 years	1 (20.0)	4 (80.0)	0.091
	40-59 years	4 (22.2)	14 (77.8)	
	≥ 60 years	14 (51.9)	13 (48.1)	
Gender	Male	11 (37.9)	18 (62.1)	0.991
	Female	8 (38.1)	13 (61.9)	

Table 5: Association between the cyclin-D1 expression and tumor characteristics

Tumor characteristics		High score	Low score	P-Value
Grade	I	1 (25.0)	3 (75.0)	0.510
	II	16 (37.2)	27 (62.8)	
	III	2 (66.7)	1 (33.3)	
Perineural invasion	Yes	7 (36.8)	12 (63.2)	0.895
	No	12 (38.7)	19 (61.3)	
Lymphovascular invasion	Yes	15 (62.5)	9 (37.5)	0.001
	No	4 (15.4)	22 (84.6)	

A significant association was obtained between the number of lymph node involvement and cyclin-D1 expression (P-Value=0.016), the proportion of tumors with high cyclin-D1 expression was higher among those with N2 compared to N1 and N0 as shown in Table 6.

Table 6: Association between cyclin-D1 expression and TNM classification

TNM classification		High score	Low score	P-Value
Tumor	T_1	0 (0.0)	2 (100.0)	0.168
	T_2	1 (14.3)	6 (85.7)	
	T_3	14 (40.0)	21 (60.0)	
	T_4	4 (66.7)	2 (33.3)	
Node	N0	3 (15.8)	16 (84.2)	0.016
	N1	3 (33.3)	6 (66.7)	
	N2	13 (59.1)	9 (40.9)	

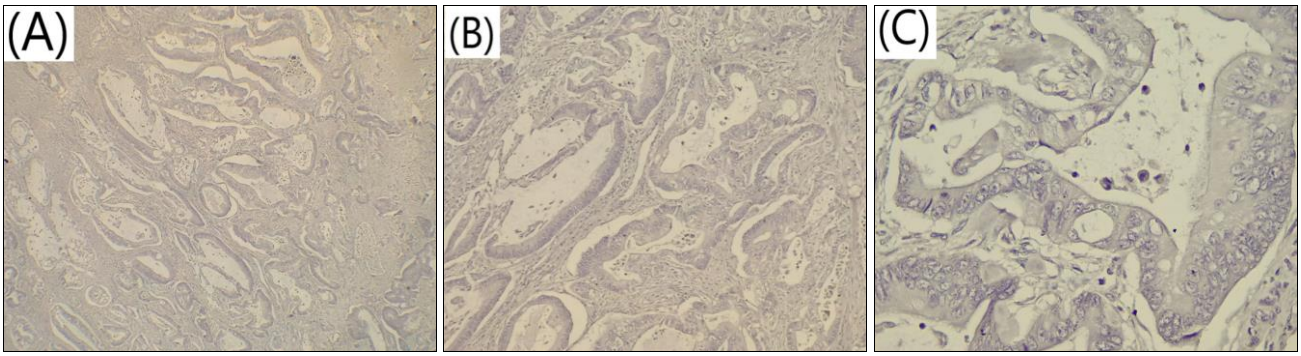

Fig 3: Immunohistochemistry staining of colonic adenocarcinoma (moderately differentiated, G2) with cyclin D1 showed negative expression (A) at 4X, (B) at 10X, (C) at 40X.

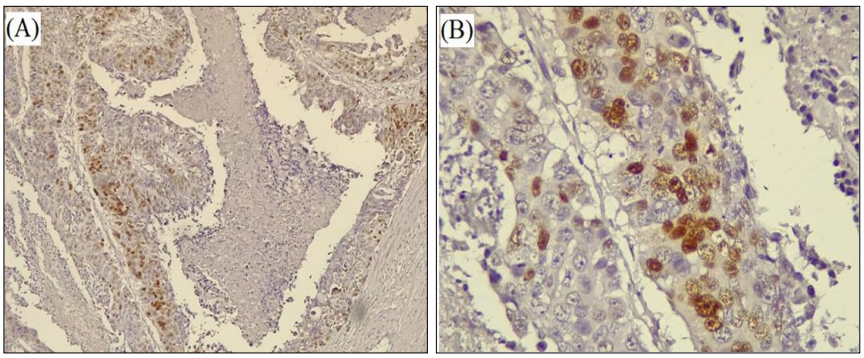

Fig 4: Immunohistochemistry staining of colonic adenocarcinoma, moderately differentiated, G2 with cyclin D1 showed high expression score (A) At 10X, (B) At 40X.

Discussion

Recent research has suggested potential roles for cyclin D1 overexpression in various cancers, including Colorectal Cancer (CRC), indicating an association with poor clinicopathological features and prognosis. Despite the established role of cyclin D1 in cell cycle progression,

studies on its impact on clinical outcomes in CRC have been inconsistent [15]. This study aimed to assess cyclin D1's role in CRC pathogenesis. The study sample predominantly comprised patients aged 60 years or older, aligning with findings from Maryam *et al*. in Iran and Abdulkader *et al*. in Saudi Arabia, but differing from a study by Sun *et al*. in Korea, which reported a majority of patients over 50 years old [12, 13, 16]. This contrasted with another Saudi study by Abdulkader *et al*., where most patients were 40 years or younger [16]. No significant association was found between age, sex, and cyclin D1 expression in this study, consistent with findings by Maryam *et al*. in Iran, which showed no significant relationship between cyclin D1 staining severity and age or sex [13]. Cyclin D1 expression was also not significantly associated with tumor grade, mirroring results from studies by Hanaa *et al*. in Sudan and Kyu *et al*., which found no significant link between cyclin D1 expression scores and tumor grade (17, 18). Maryam, *et al*. in Iran also reported no significant relationship between cyclin D1 staining and tumor grade (13). However, this contrasts with findings by Abdulkader *et al*. in Saudi Arabia, which reported a significant association between tumor grade and cyclin D1 expression, [16]. Lymphovascular invasion significantly affected cyclin D1 expression in this study, similar to results from Abdulkader *et al*. in Saudi Arabia and Rania *et al*. in Egypt, who found a significant correlation between lymphatic spread in CRC and high cyclin D1 expression scores [14, 16]. Conversely, other studies by Maryam *et al*. in Iran and Sun, *et al*. in Korea found no significant associations between cyclin D1 expression and lymphovascular or perineural invasion [12, 13]. The current study revealed no significant relationship between cyclin D1 expression and tumor invasion, aligning with findings from Maryam *et al*. in Iran and other studies from Korea, which concluded that tumor size did not affect cyclin D1 staining severity or extent [12, 13, 18].

Conclusion

The cyclin D1 expression was significantly affected by lymphovascular invasion, and lymph node involvement implicating its potential prognostic role in CRC.

Conflict of Interest: Not available

Financial Support: Not available

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