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Evaluation of the Immunohistochemical Expression of Cyclin D1 in Colorectal Carcinoma

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ABSTRACT:

BACKGROUND:

Cyclin D1, a main regulatory protein in the cell cycle, is encoded by the CCND1 gene, located on chromosome 11q13, and high-expression of this protein interrupts normal cell cycle control, hence enforcing the development and progression of cancer. The up-regulation of cyclin D1 plays a significant role in the pathogenesis and metastases of colorectal cancer, and it seems to be a useful prognostic marker for colorectal cancer.

OBJECTIVE:

To evaluate the immunohistochemical expression of Cyclin D1 in colorectal carcinoma cases. To assess its association with other clinicopathological features.

PATIENTS AND METHODS:

In this prospective and retrospective case series study, fifty cases of surgically excised colorectal carcinoma biopsies were included. The blocks of the cases were collected from Al-Jumhori teaching hospital and some private laboratories in Mosul city in a period from October 2023 to August 2024. Section slides from the blocks were stained by Cyclin D1 (immunostain).

RESULTS:

Age of 50 colorectal carcinoma cases ranged from 34 to 93 years (mean± SD= 60.44±12.73) with 52% of them ≥ 60 years, male to female ratio 2:3, 64% moderately differentiated, 66% with positive lymphovascular invasion, 68% were penetrating through the muscle layer into the pericolorectal tissue, 54% with negative lymph node metastasis and 46% diagnosed with stage III. Among the fifty cases, 68% were with high level of expression of cyclin D1 with 75% of them < 60 years old, 70% males, 72% moderately differentiated, 70% with positive lymphovascular invasion, 78% were invading the visceral peritoneum, 74% with negative lymph node metastasis and 81% of stage II disease.

CONCLUSION:

The high expression of cyclin D1 lies in colorectal carcinoma cases younger than 60 years old, males, moderately differentiated, with positive lymphovascular invasion and higher stages despite that there were no significant statistical association found between Cyclin D1 expression and the studied clinicopathological variables.

KEYWORDS: Colorectal carcinoma, Cyclin D1, prognosis.

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INTRODUCTION:

Colorecal Cancer (CRC) is the third most prevalent cancer. It is considered the second cause of death relating to cancer in the world [(1)]. Following the lung and prostate cancer, CRC is the third cancer more common in men. In women, CRC is the second of the most common cancers [(2)]. The mortality rate of this cancer in both sexes and all ages was 9.2% of all the cancer dead [(3)]. In Iraq, CRC classifies the third most common cancer [(4)]. Initial diagnosis of this cancer type leads to the highest mission of the appropriate treatment and lower its high metastatic potential, it will also be helpful to discover more novel predictive, prognostic and therapeutic targets for this malignancy in order to arrange more advanced therapeutic regimes [(5)]. Cyclin D1 (CNND1) is an oncogene regulates the cell cycle by promoting G1 phase progression towards S phase [(6)]. Its stimulation role in the cell cycle is inhibited by cyclin D1-dependent kinase (CDK) inhibitors as P27 and P21, so CNND1 is a cycle control factor [(5)]. A dysregulated transcription, accumulation and Ubiquitin 'of CNND1and uncontrolled activation of their associated CDKs lead to non-regulated cell growth. As a result, CNND1 is considered as oncogenic propeller in different types of cancer [(7)], including an endometrial and esophageal cancer [(8)] High CNND1 levels also seems to play a major role in the pathogenesis and metastases of CRC and they look like an important prognostic factor for this type of cancer [(9)]. Furthermore, CDK 4/6 inhibitor

therapy shows good results in the relief of patients affected with CRC and other Recent Students states that CDK therapeutic Potential amplified when associated with other medicines in CRC treatment [10]. The scarcity of research on CNND1 expression in CRC within Iraq presents a significant gap in the literature, making it crucial to investigate this relationship and this study seeks to provide valuable insights into the prognostic significance of CNND1 expression in CRC and potentially contribute to the development of region-specific diagnostic and therapeutic strategies.

PATIENTS AND METHOD:

In this prospective and retrospective case series study, fifty cases of surgically excised CRC biopsies were included. The blocks of the cases were collected from Al-Jumhori teaching hospital and some private laboratories in Mosul city in a period from October 2023 to August 2024. The most appropriate block of each case was chosen for immune-histochemical staining with CNND1 and other information regarding the clinicopathological variables were collected from surgical pathology reports. The inclusion criteria included cases of surgically excised CRC biopsies of any age and any sex while the exclusion criteria included the cases of colonoscopy obtained CRC biopsies and cases of undetermined stage, grade and lymphovascular invasion. This study was performed by using the primary antibody which is Cyclin D1, isotype IgG1, clone EP12, Rabbit Monoclonal Antibody. The staining done by autostainerLink 48, both supplied by Agilent company / USA.

Interpretation of immunohistochemistry staining: By using light microscope: Nuclear immunoreactivity for CNND1 was evaluated and scored manually. Both the quantity and quality of the staining of the tumor cells in an average of 10 microscopic fields (magnification x400) were demonstrated. The brown pigmentation of the nuclei was detected in the tumor cells and the CNND1 staining was evaluated as following: The Proportional Score (PS) of cyclin D1 graded as: Ps0: less than 5%, Ps1: 5–25%, Ps2: 26–50%, Ps3: 51–75%, Ps4: more than 75%. The Intensity Score (IS) scaled from 0 to 3, where: Is0: negative, Is1: weak, Is2: moderate, Is3: strong. The final expression score (Total Score = PS+IS) ranging from 0-7 calculated as follows: Ts 0 = - ve, Ts1= 1–3 (+), Ts2= 4–6 (++), Ts3= 7 (+++). For statistical analysis: negative and + results will be considered as low score, while ++ and +++ results will be considered as high score [9, 11].

Statistical analysis: Statistical analysis was done by using of Chi-square and Fisher exact test when indicated by using Statistical Package for the Social Sciences (SPSS) version 26.0. A "P value of ≤ 0.05" was considered statistically significant with confidence interval of 95%.

RESULTS:

This study included 50 cases of surgically resected CRC. Their ages ranged from 34 to 93 years (mean± SD= 60.44±12.73) with 26 cases (52%) were equal or older than 60 years old. Regarding the sex, 30 cases (60%) were females and 20 cases (40%) were males with male to female ratio was about 2:3. Other clinicopathological data are summarized in table (1). The result of CNND1 expression in general were 34 cases (68%) of high expression and 16 cases (32%) of low expression. Eighteen cases out of 24 cases with high level of expression (75%) were younger than 60 years old, 14 cases out of 20 (70%) were males, 23 cases out of 32 (72%) were G2 of differentiation, 23 cases out of 33 (70%) were with positive lymphovascular invasion, 23 cases out of 34 (68%) were of T3 level of invasion, 20 cases out of 27 (74%) were with negative lymph node metastasis and 17 cases out of 21 (81%) were within stage II. However, no significant statistical association found between CNND1 and these clinicopathological variables as p value was > 0.05. See table (2).

Table 1: The descriptive analysis of clinicopathological data of this study.

Parameters	Data	NO.	Percentage
Age in years	< 60	24	(48%)
	≥ 60	26	(52%)
Sex	Male	20	(40%)
	Female	30	(60%)
Grade	G1	15	(30%)
	G2	32	(64%)
	G3	3	(6%)
Lymphovascular invasion	Present	33	(66%)
	Absent	17	(34%)
Depth of invasion	T1	1	(2%)
	T2	6	(12%)
	T3	34	(68%)
	T4	9	(18%)
Lymph node metastasis	Positive	23	(46%)
	Negative	27	(54%)
AJCC stage	Stage I	6	(12%)
	Stage II	21	(42%)
	Stage III	23	(46%)

Table 2: The results of CNND1 expression and its association with the studied clinicopathological variables in this study.

Parameters	Data	NO.(%)	CNND1 expression		P value
			Low N0.(%)	High N0.(%)	
Age in years	< 60	24 (48%)	6 (25%)	18 (75%)	**0.318**
	≥ 60	26 (52%)	10 (38.5)	16 (61.5)	
Sex	Male	20 (40%)	6 (30%)	14 (70%)	**0.804**
	Female	30 (60%)	10 (33.3%)	20 (66.7%)	
Grade	G1	15 (30%)	5 (33.3%)	10 (66.7%)	**0.895**
	G2	32 (64%)	9 (28%)	23 (72%)	
	G3	3 (6%)	2 (66.7%)	1 (33.3%)	
Lymphovascular invasion	Present	33 (66%)	10 (30%)	23 (70%)	**0.7200**
	Absent	17 (34%)	6 (35%)	11 (65%)	
Depth of invasion	T1	1 (2%)	1 (100%)	0 (0%)	**0.507**
	T2	6 (12%)	2 (33.3%)	4 (66.7%)	
	T3	34 (68%)	11 (32%)	23 (68%)	
	T4	9 (18%)	2 (22%)	7 (78%)	
Lymph node metastasis	Positive	23 (46%)	9 (39%)	14 (61%)	**0.3184**
	Negative	27 (54%)	7 (26%)	20 (74%)	
AJCC stage	Stage I	6 (12%)	3 (50%)	3 (50%)	**0.218**
	Stage II	21 (42%)	4 (19%)	17 (81%)	
	Stage III	23 (46%)	9 (39%)	14 (61%)	

P value ≤ 0.05 is significant

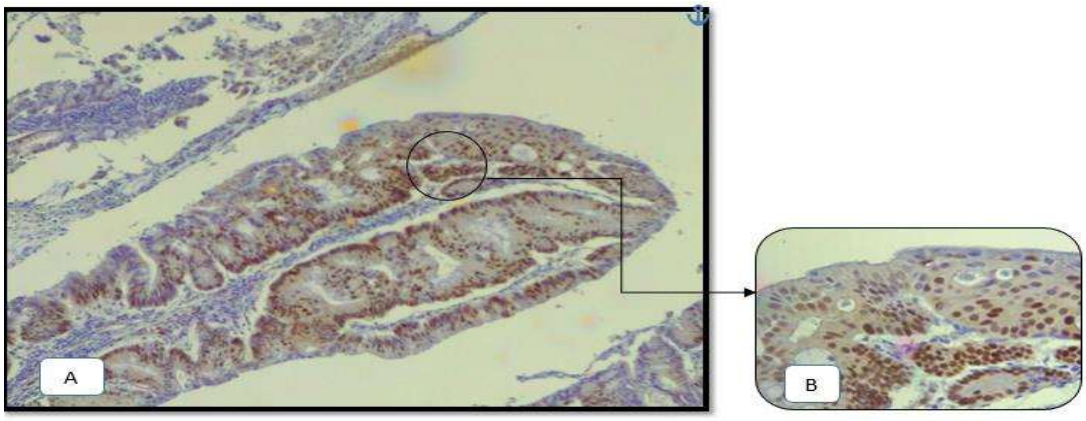

Figure 1: Colorectal carcinoma stained with cyclin D1,showing high expression (+++) (A)×100 (B)×400

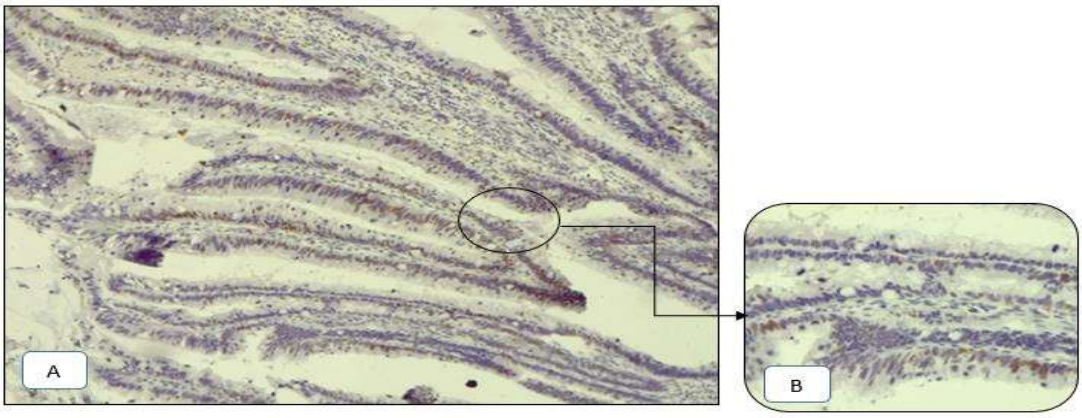

Figure 2: Colorectal carcinoma stained with cyclin D1, showing low expression (+) (A)×100 (B)×400

DISCUSSION:

Cyclin D1, a main regulator of the cell cycle, promoting development and cancer progression when being excessively expressed [9]. However, the role of the overly expressed CNND1 in CRC remains controversial. Some studies have linked CNND1 to bad outcomes, while others have linked it to more favorable results or do not have any significant association [9]. In this study, 68% of CRC cases shown a high expression of CNND1, in accordance with Sharma's. (India) [12] and Cheshori et al. (Iran) [8] (63% and 60% respectively). However, this was higher than et al. (Korea) [13] and Roshdy et al. (Egypt) [10] (78.6% and 80%) and lower than Almaghrabi et al. [14] and Albasri et al. (Saudi Arabia) [9] (23.1% and 24.1%). This discrepancy could be due to differences in anti-CNND1 antibody clones, immunostaining scoring thresholds, fixation times, number of cases or study techniques. [12,14] Regarding age, 75% of cases under 60 years showed high CNND1 expression, in consistent with studies obtained from Almaghrabi et al. [14], Sharma et al. [12], and Jun et al. [13] However, Albasri et al. [9] and Roshdy et al. [10] found higher expression in those patients aged over 60 with no clear explanation for this discrepancy in results. Sex-related differences in CNND1 expression were noted, with 70% of male cases exhibiting high levels, in agreement with studies by Jang KY et al. [15], Roshdy et al. [11], and Salem et al. [16] while in the other hand, Almaghrabi et al. [14] and Jun et al. [13] found higher expression in females. This could be due to sex hormone levels, or genetic and epigenetic modifications of steroid receptors. The highest CNND1 expression in G2 cases (72%) supports its potential as a poor clinicopathological factor in CRC, a conclusion reinforced by studies from Almaghrabi et al. [14], Sharma et al. [12], and Roshdy et al. [10] Similar results were observed in poorly differentiated

CRC, as stated by Albasri et al. [9] and Salem et al. [16]. In cases with positive lymphovascular invasion, 70% showed high CNND1 expression, in aligment with Albasri et al. [9] and Jun et al. [13], in contrast with Almaghrabi et al. [14] and Jang et al. [15] This discrepancy remains unexplained. CNND1 expression was highest in T4 cases (75%), in agreement with Salem et al. [16], but differing from Almaghrabi J et al. [14] and Jun et al. [13], who found higher expression in T3 cases. These findings suggest that CNND1 contributes to the progression of malignant cells, aiding in organ invasion. Regarding lymph node metastasis, this study found high CNND1 expression in 74% of cases without lymph node metastasis, in contrast to the findings from Sharma et al. [12] and Salem et al. [16], who linked high expression to positive metastasis. This discrepancy may arise from a collection bias in the study, where more cases were in the negative lymph node metastasis group. Finally, 81% of stage II CRC cases showed high CNND1 expression, in agreement with Jun et al. [13] and Belt et al. [17] However, this was inconsistent with Roshdy R et al. [11] and Salem et al. [16], who found higher expression in stage III and IV. This variation might also be due to collection bias, as most cases in this study were stage II.

CONCLUSION:

According to the results of the current study: There is high expression of CNND1 in the age group <60 years old of CRC patients, males with G2 of differentiation, positive lymphovascular invasion, T4 level of invasion and higher stage of CRC. No significant statistical association found between CNND1 expression and the studied clinicopathological variables.

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Author contributions:

Maab Najdat Hameed: Conceptualization; Data curation; Formal analysis; Investigation; Writing original draft.

Nadwa Subhi Alazzo: Writing-review & editing.

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