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Research Paper

Immunohistochemical Expression of Cyclin D1 in Colorectal Adenoma and Adenocarcinoma , A Comparative Study

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ABSTRACT:

BACKGROUND:

Colorectal cancer is the most common gastrointestinal tract cancer worldwide. Cyclin D1 shows increased expression both in advanced adenomas (Large size (> 1 cm), high dysplasia grade, and/or villous morphology) and colorectal adenocarcinoma with loss of expression in normal cells.

OBJECTIVE:

To evaluate the immunohistochemical expression of Cyclin D1 in colorectal adenoma and compare it to adenocarcinoma cases and to correlate it with different clinicopathological parameters.

PATIENTS AND METHOD:

This retrospective study was done on formalin fixed, paraffin embedded tissue blocks of (40) cases (20) of which are colorectal adenomatous polyps, (20) cases are colorectal adenocarcinoma collected from archived materials from the gastrointestinal and hepatology teaching hospital in Baghdad medical city and Baghdad medical city teaching laboratories (covering the period from November 2018 to March 2020).

RESULTS:

Cyclin D1 expression was lost in normal mucosa and showed variable staining, the adenoma staining percentage was score 0 in 20%(4), score I 20%(4), score II 40%(8) and score III 20%(4) of cases, none had score IV While the staining percentage of colorectal adenocarcinoma was score 0 10%(2), score I 35%(7), score II 35%(7), score III 5%(1) and score IV 15%(3) of cases.

The mean immunohistochemical staining score was **significantly** higher in distal colorectal han proximal adenoma (p=0.045).

When adenoma cases were evaluated separately for (histopathological type, grade, size) 2 cases (10%) had villous adenoma morphology and 9 (45%) for tubular and tubulovillous ,11 (55%) showed high grade dysplasia and 9 (45%) showed low grade dysplasia and colorectal adenoma sizes ranged between (0.3-2.5) cm 15 (75%) were less than 2 cm, 5 (25%) were equal to or larger than 2 cm.The mean score showed a **significant** difference according to histopathological type (P value =0.11), grade of dysplasia (P value <0.001) and size (P value =0.045) in colorectal adenoma.

CONCLUSION:

There was no significant difference in the Cyclin D1 score in colorectal adenoma and adenocarcinoma and showed a significant difference according to histopathological type, grade of dysplasia and size in colorectal adenoma.

KEYWORDS: Cyclin D1, colorectal adenoma , adenocarcinoma. Introduction.

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Iraqi Postgraduate Medical Journal, 2025; Vol. 24(2): 283-291
DOI: 10.52573/ipmj.2025.147389 *Received: 22 April, 2024* *Accepted: June 9, 2024*

INTRODUCTION:

Colon cancer is the most common malignancy of the gastrointestinal system and is one of the main causes of mortality and morbidity worldwide.

According to the Iraqi cancer registry data 2019 colorectal cancer was one of the top ten cancers in both sexes, in male patients it ranked third after (bronchus and lung) and urinary bladder (8.02%), in female patients it was fourth after breast, thyroid gland, brain and other central nervous system tumors (CNS). The multi-step genetic alteration in CRC is well studied: a series of events leads to an alteration of the normal epithelium, then to precancerous lesions and further to adenocarcinoma, and subsequently to the development of metastasis.

The cyclin D1 gene is localized at 11q23 and is a nuclear protein involved in the transition from G1 to S phase in the cell cycle. Overexpression of Cyclin D1, which occurs due to gene amplification, is encountered in many

malignancies.

PATIENTS AND METHODS:

This is a retrospective study including (40) cases Formalin fixed, paraffin embedded tissue blocks were collected from archived materials from the gastrointestinal and hepatology center in Baghdad medical city and Baghdad medical city teaching laboratories (covering the period from November 2018 to March 2020) (20 of which are colorectal adenomatous polyps,20 cases are colorectal adenocarcinomas).

Scoring system:

intense brown nuclear staining, evaluated by the percentage of positive tumor cells as to classify the results as [3]:

- Less than or equal to 5% was Negative
- 6-25% = 1+
- 26-50% = 2+
- 51-75% = 3+
- Over 76% = 4+.

Statistical Analysis:

Statistical analysis was performed with SPSS v18.88(Statistical package for social sciences) and also Excel 2010 programs. Data analysis was done using student's t-test, chi-square test, fisher exact test, ANOVA test for tables with frequencies, percentages, ranges, means and standard deviation. Values were considered Statistically significant when p-value is equal or less than 0.05.

RESULTS:

Forty patients (20 with colon adenoma and 20 with adenocarcinoma) were enrolled. Age distribution of studied groups showed no significant difference between colorectal adenoma and adenocarcinoma with a mean age of 54±12.2 years for colorectal adenoma and 55.3±14.5 for adenocarcinoma and median age of 57 as shown in table 1.

Table 1: Age distribution of the studied groups.

		Colorectal adenoma	adenocarcinoma	P value
Age inyears	<40	2(10%)	3(15%)	0.9*
	40-59	10(50%)	7(35%)	
	≥60	8(40%)	10(50%)	
	Mean±SD	54±12.2	55.3±14.5	
	Range	27-74	18-84	
	Median	57	57	

Sex distribution between the two groups shown in figure (1), There were no significant association noted (p=0.18).

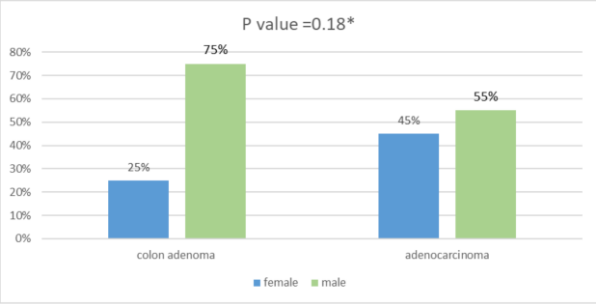

Figure 1: Sex distribution between the two groups.

The site of tumor was evaluated as proximal and distal (the rectum was included with distal location) in both groups (colorectal adenoma and adenocarcinoma) showed no significant correlation (p=0.5), as in table (2).

Table 2: Site of lesion in both groups.

Variable		AdenomaNo (%)	Adenocarcinoma No (%)	T	P
Site	Proximal	5(25%)	4(20%)	9(22.5%)	0.5*
	Distalcolon +rectum	15(75%)	16(80%)	31(77.5%)	
Total		20(100%)	20(100%)	40(100%)	

*fisher exact test, significant <0.05

It was observed that cyclin D1 showed varying degrees of nuclear staining, the percentage of adenoma group (evaluated quantitatively) was score 0 in 20%(4) of cases, score I in 20%(4) of cases, score II in 40%(8) of cases and score III in 20%(4) of cases with no cases had score IV, as in figures (2), (4) and (5).

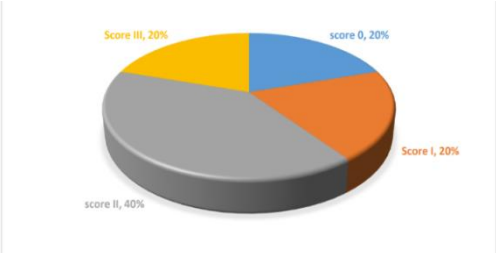

Figure 2: Cyclin D1 staining score in colorectal adenoma groups.

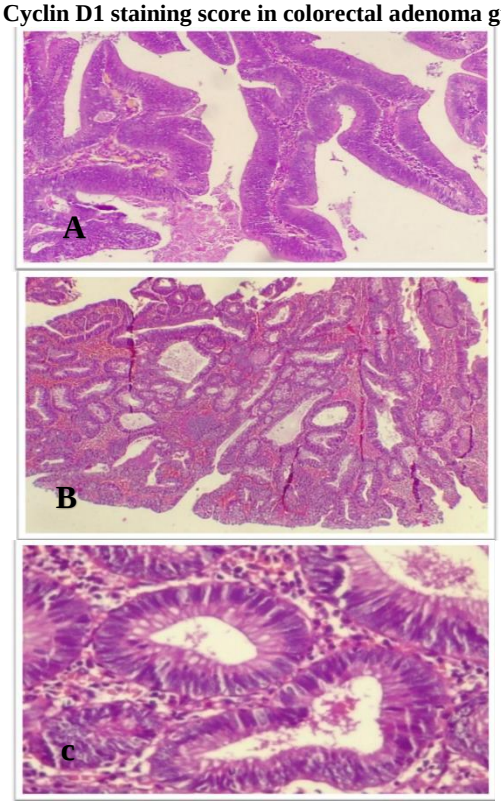

Figure 3: A. Villous adenoma: Epithelial finger-like projections formed by fibrovascular cores (frond-like) with high grade dysplasia hematoxyline and eosin stain (100X). B. Tubulovillous adenoma focal high grade dysplasia: Crowded pseudostratification of cells with elongated nuclei (100X). C. Tubular adenoma low grade dysplasia with mild cellular stratification and no evidence of complex architecture(400X).

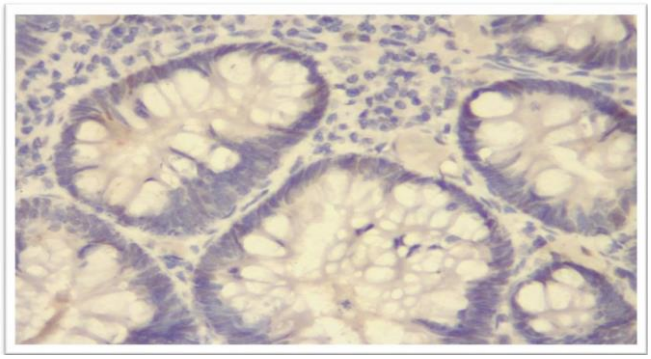

Figure 4: Tubular adenoma showing negative cyclin d1 immunohistochemical stain (400X).

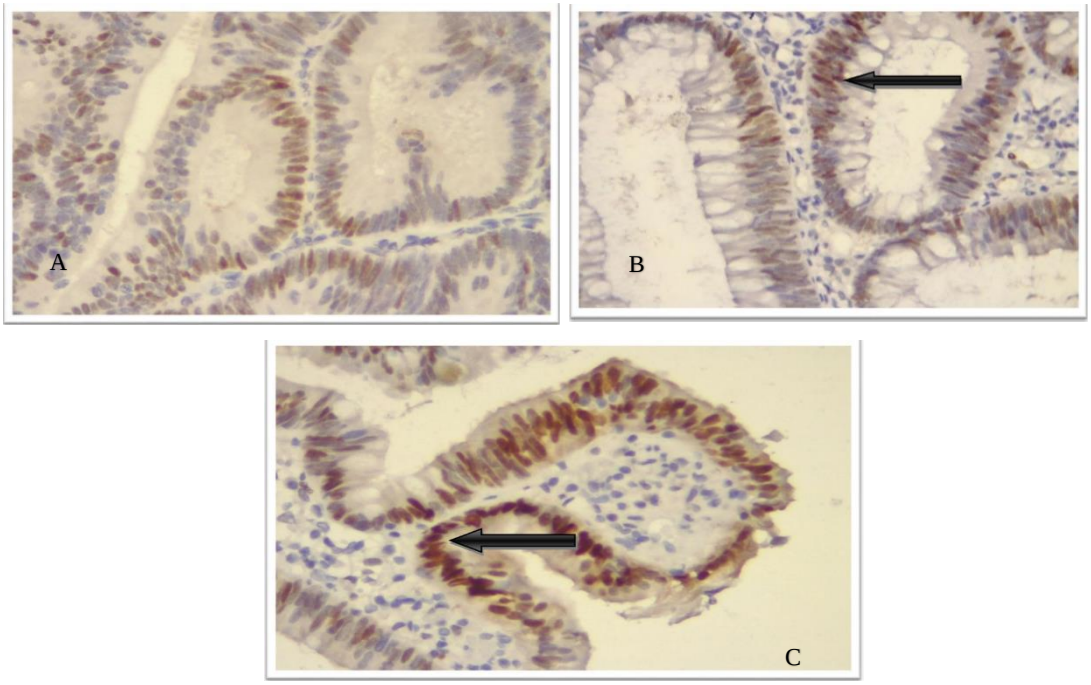

Figure 5: A. Tubular adenoma showing positive brown nuclear score +I B. score +II (100X), C. score +III Cyclin D1immunohistochemical staining in villous adenoma with high grade dysplasia (400X)(black arrow).

While the staining percentage of colorectal adenocarcinoma group was score 0 in 10%(2) of cases, score I in 35%(seven) of cases, score II in 35%(seven) of cases, score III in 5%(one) of cases and score IV in 15%(three) of cases, figures (6), (8) and (9).

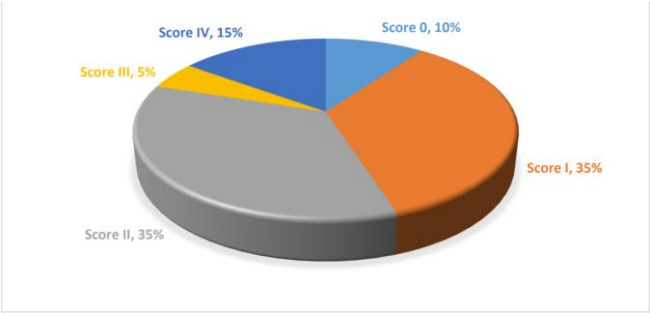

Figure 6: Cyclin D1 staining score in colorectal adenocarcinoma group.

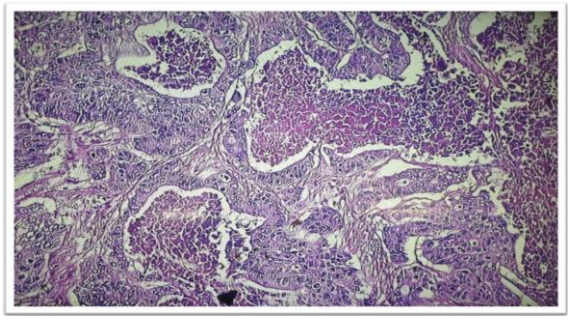

Figure 7: Colonic adenocarcinoma hematoxyline and eosin stain (400X).

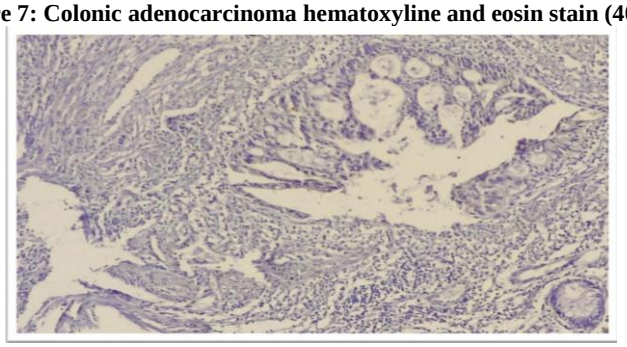

Figure 8: Colonic adenocarcinoma negative score 0 cyclin D1 immunohistochemical stain (100X).

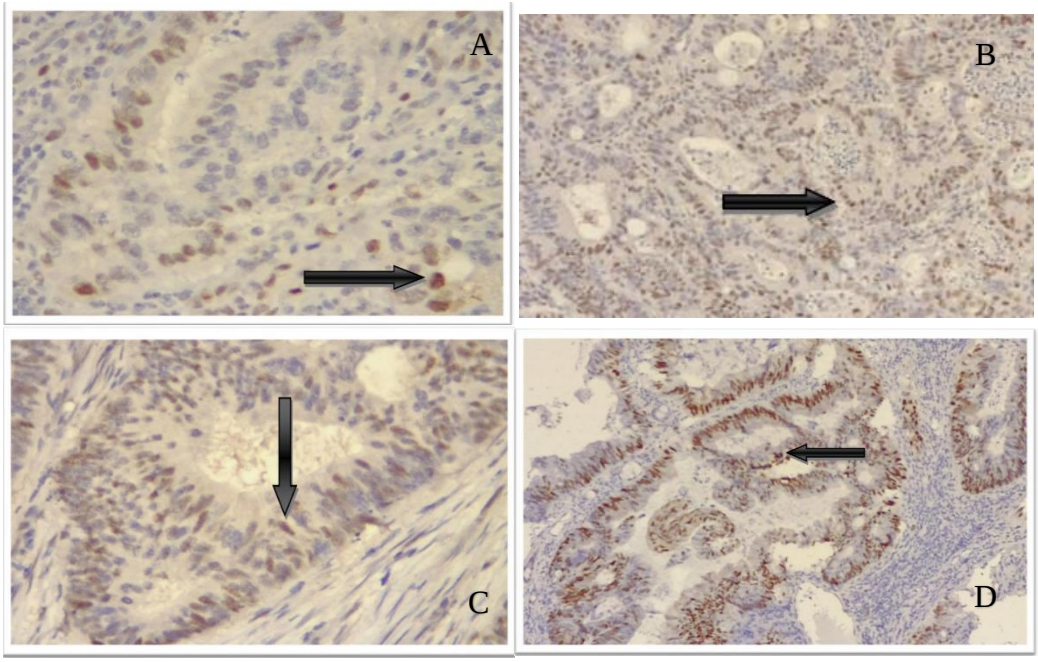

Figure 9: A. Colonic adenocarcinoma positive cyclin d1 immunohistochemical stain score +I (400X) (black arrow). B. Colonic adenocarcinoma positive score +II (100X) (black arrow). C. Colonic adenocarcinoma positive score +III (400X) (black arrow). D. Colonic adenocarcinoma positive brown nuclear cyclin d1 immunohistochemical stain score +IIII (100X) (black arrow).

The mean score of immunohistochemical expression of cyclin d1 protein in patients with colon adenoma was 1.6 and the mean score in patients with adenocarcinoma was 1.8, that difference was not significant statistically (p=0.57), table (3).

Table 3: Difference in the mean cyclin D1 expression score in both colon adenoma and adenocarcinoma.

Variable		Mean ±SD score	P value
Patients	Colon adenoma	1.6 ±1.04	0.57*
	Adenocarcinoma	1.8 ±1.1	

*student T test, significant <0.05

There was no significant difference in the mean immunohistochemical staining score of cyclin d1 between colorectal adenoma and adenocarcinoma cases in different age groupsand different sex (p>0.05), but was significantly higher in distal colorectal adenoma than proximal adenoma (p=0.045), as shown in table (4).

Table 4: Difference in the mean cyclin D1 expression score according to (age, sex, site) in both colorectal adenoma and adenocarcinoma.

Variable		Mean ±SD score	
		Colorectaladenoma	Colorectal Adenocarcinoma
Age in years	<40	2.5 ±0.7	3± 1.7
	40-59	1.8± 0.9	1.5± 0.97
	≥60	1.1± 1.1	1.6 ±1
	P value	0.178*	0.17*
Gender	Female	1.6 ±1.1	2.1 ±0.9
	Male	1.6± 1	1.5 ±1.3
	P value	0.99**	0.3 **
Site	Proximal	0.8 ±0.83	1.5 ±1.2
	Distal	1.86± 0.99	1.8 ±1.2
	P value	0.045**	0.58**

- Anova test, **student T test, significant <0.05.

Colorectal adenoma cases were evaluated separately for (histopathological type, grade, size) with 2 cases (10%) for villous adenoma and 9 cases(45%) for each tubular and tubulovillous colorectal adenoma , 11 cases (55%) showed high grade dysplasia and 9 cases (45%) showed low grade dysplasia and colorectal adenoma sizes ranged between (0.3-2.5)cm 15 cases (75%) were less than 2 cm , 5 cases (25%) were equal to or larger than 2 cm as shown in table(5).

Table 5: Number and frequency distribution of colorectal adenoma cases, studied separately for (histopathological type, grade, size).

Colorectal adenoma cases		Number	Percentage
Histopathologicaltype	Tubulovillous	9	45%
	Tubular	9	45%
	Villous	2	10%
Grade	High grade dysplasia	11	55%
	Low grade dysplasia	9	45%
size	<2cm	15	75%
	≥ 2 cm	5	25%

The mean cyclin d1 immunohistochemical expression score showed a significant difference according to histopathological type (p value =0.011), grade (p value <0.001) and size (p value =0.045) in colorectal adenoma, as shown in table (6) .

Table 6: Difference in the mean cyclin d1 expression score in colon adenoma cases according to (histopathological type, grade, size).

Histopathological study		Mean ±SD score	P value
Histopathologicaltype	Tubulovillous	2.1 ±0.78	0.011*
	Tubular	0.88 ±0.92	
	Villous	2.5 ±0.7	
Grade	High grade dysplasia	2.2 ±0.6	<0.001**
	Low grade dysplasia	0.7 ±0.8	
Size	< 2 cm	1.04 ±0.27	0.045**
	≥2 cm	2.4 ±0.5	

*Anova test, **student T test, significant < 0.05.

DISCUSSION:

age between colorectal adeonoma and adenocarcinoma:

the age distribution of studied cases showed no significant difference between adenoma and adenocarcinoma group (p value 0.9) and most patients (90%) in adenoma group aged 40 years and older. Meanwhile, the peak age for adenocarcinoma where equal to and more than 60 years (50%). This is in accordance with studies conducted by **A Rosidah et al (2020)[1] and Sheref M. El-taher et al (2016)[2]** who did a case-control study on 25 cases of colorectal adenocarcinoma and 100 cases of control subjects which revealed that CRC cases were predominant in patients over 50 years and no significant association between them regarding age (P-value =0.09) and to **Albasri et al (2019)[3]** (p value = 0.091).

Sex between adenoma and adenocarcinoma:

There were 75% males and 25% females in colorectal adenomas. This is consistent with the previous studies of **A Rosidah et al (2020)**[1] who found that adenoma cases were (53.3%) men and (46.7%) women.While in adenocarcinoma were 55% male versus 45% females, and no significant correlation between sex in the two groups (p=0.18). this in contrast to the study of **A Rosidah et al (2020) [1]** where (40%) male and (60%) female found , this could be explained by different sample size, But consistent with **Sheref M. El-Taher et al (2016)** [2]who reported slightly higher risk of association in men (16/25) (64%) than women (9/25) (36%) (P value 0.92), **Murphy et al (2010) [4]** who stated that colorectal carcinoma are about 35% to 40% higher in men than in women, Also with **Gao et al(2008) [5] and to Sazan Abdulwahab et al (2016) [6]** who found higher incidence in male (63.3%) than female (36.7%).

D Ayerden (2017) et al [7] found nearly similar results 8/40 (20%) women and 32/40 (80%) men were diagnosed with adenoma; Of those with adenocarcinoma, 21/40 (52.5%) were female and 19/40 (47.5%) were male, also consistent with **Albasri et al (2019)** [3] who found no significant association (p value= 0.59).

Site correlation between colorectal adenoma and adenocarcinoma: The site of lesion showed no significant association (p=0.5). In both groups, the distal colon and rectum were the most frequent location representing 15/ 20 (75%) and 16/ 20 (80%) respectively, whereas, the proximal colon represented 5/20 (25%) in adenoma and 4/20 (20%) in adenocarcinoma. This is also in accordance with the study by **Albasri et al (2019) [3]** who showed no significant correlation (p value = 0.897), distal and right-sided (proximal) tumors were seen in 63.5% and 36.5% of cases, respectively. This is in agreement with **A Rosidah et al (2020) [1]** regarding the adenoma group and in contrast with it regarding the adenocarcinoma. where the most frequent location was the rectosigimoid in (56.7 %) of colorectal adenoma and (46.7%) of adenocarcinoma were found in the ascending colon region. This is probably due to random selection of cases. Also, this is consistent with **D Ayerden (2017) et al [7]** who found the majority of adenoma cases (77,5%), (50%) of adenocarcinoma cases in the distal colon and rectum. **Sara Saad Bonyan et al (2020) [8],Bahnassy et al (2004) [9]; Jiang et al. (2006) [10] ; Li et al. 2014 [11] and N Arber et al (1996) [12]** also found similar results (p value = 0.897).The study was in contrast with **Ogino S et al(2009) [13]** who found Cyclin D1 overexpression was more common in proximal (34%=117/343) than distal tumors (excluding rectum) (24%=62/252, p=0.009) and rectal tumors (21%=36/169, p=0.003).

Correlation of Immunohistochemical expression of cyclin D1 score between colorectal adenoma and adenocarcinoma. Positive expression was 80% in colorectal adenoma and 90% in adenocarcinoma, and the correlation was not significant (p=0.57). This is consistent with **Albasri (2019) et al** [3], who compared the results of the tissue microarray between normal colonic mucosa, adenoma and adenocarcinoma and to **N Arber et al (1996)**[12] were Cyclin D1 staining occurred in 30% of adenocarcinomas and 34% of adenomatous polyps and to **Hikaru Izawa et al (2002)** [14] who found that cyclin D1 expression was 2.3-times and 2.5-times higher in adenomatous tissues and neoplastic foci, respectively. Meanwhile, **A Rosidah et al (2020)** [1], **Ayhan et al (2010)**[15] **and D Ayerden (2017) et al** [7] found a significant difference in cyclin D1 expression (p value <0.05), perhaps due to different antibody clone.

Colorectal adenocarcinoma in relation to the studied parameters: Jang et al (2012) [16] found similar results with expression of cyclin D1 in 59.4% of colorectal adenocarcinoma and no significant correlation between it and age, gender, location of lesion (p value> 0.05) also **Ogino S et al (2009)** [13]. found expression in 99 /326 (30%) in ages between 60-69 years and (28.4%) of adenocarcinoma cases. **Marcolino TF et al (2020)** [17]**, Utsunomiya T et al (2001)** [18] found staining positivity in 55%, 47% cases respectively.

Colorectal adenoma in relation to the studied parameters: 55% of polyps had a villous histology (villous and tubulovillous), were <2cm in size 75%,located in distal colon and rectum, had high grade dysplasia 55%.

Histological type of colorectal adenoma and cyclin d1 expression: Our study showed a significant difference regarding the type of colorectal adenomas with a higher cyclin D1 score in both villous and tubulovillous types as opposed to tubular p value = 0.011,This is consistent with study by **FL Nassrat et al (2016)** [19] who detected a significant correlation with villous type (P=0.003) and with **T Hunter et al 1994** [20] **and D Ayerden (2017) et al** [7] (p: 0.010) suggest that Cyclin D1 plays a role in the early stage of colorectal carcinogenesis.

Degree of dysplasia of colorectal adenoma and cyclin D1 expression: The mean cyclin D1 expression score in high grade dysplasia was 2.2 ±0.6 while in low grade dysplasia it was 0.7 ±0.8 and there was a significant correlation between colorectal adenoma grade and mean cyclin D1 expression (P value <0.001), This is consistent with **FL Nassrat et al (2016)** [19] who found that there was a significant correlation (P=0.021). **Zhang et al (1997)** [21] **and Tenya T. Abdulhameed et al (2021)**[22] also noted significant association, While in contrast to the study by **A Rosidah et al (2020)** [1] (P value of 0.502) and to **N Arber et al (1996)** [12] who found no significant relationship.

Correlation of size of adenomatous polyp with cyclin d1 expression: we found a significant statistical correlation between the size of polyps and cyclin d1 staining (p value =0.045), This is in agreement with **Timothy su et al (2016)**[23] where larger adenoma (>1cm) had higher level of cyclin d1 expression than small adenoma (p value<0.001),while in contrast to the studies of **N Arber et al (1996)** [12] and **FL Nassrat et al (2016)** [19] (p value >0.05). However, this could be explained due to the methodology of case selection.

CONCLUSION:

1-There was no significant difference in the mean immunohistochemical expression score of cyclin D1 between colorectal adenoma and adenocarcinoma and between them in different age groups, gender and site.

2- The mean cyclin D expression score showed a significant difference according to histopathological type, grade of dysplasia and size in colorectal adenoma cases.

REFERENCES:

1. Rosidah a, norahmawati e, anita kw, al rasyid h. perbedaan ekspresi protein cyclin d1 antara adenoma dan adenokarsinoma kolorektal, serta korelasinya dengan grading dan stadium tumor. majalah kesehatan fkub. the differences in cyclin d1 protein expression between adenoma and colorectal adenocarsinoma, and its correlation with grading and tumor stadium. 2020 oct 5;7(3):149-58.
2. Sheref M. El Taher , Risk factors of colorectal cancer;a case-control study, Egypt. J. Community Med. 2016; 34 (1): 1-15.
3. Albasri A, Elkablawy M, Ansari I, Alhujaily A. Prognostic Significance of Cyclin D1 Over-expression in Colorectal Cancer: An Experience from Madinah, Saudi Arabia. Asian Pacific Journal of Cancer Prevention. 2019;20(8):2471- 76.
4. Murphy G, Devesa S, Cross A, Inskip P, McGlynn K, Cook M. Sex disparities in colorectal cancer incidence by anatomic subsite, race and age. International Journal of Cancer. 2010;128(7):1668-75.
5. Gao R, Neutel C, Wai E. Gender differences in colorectal cancer incidence, mortality,

hospitalizations and surgical procedures in Canada. Journal of Public Health. 2008;30(2):194-201.
6. Abdulwahab Mirza S, Hassan AL-Tai TH, Shaker HH. Immunohitochemical expression of p53 in human colocrectal carcinoma. Journal of the Faculty of Medicine Baghdad. 2016 Oct 2;58(3):289–92.
7. Ayerden d. kolon adenom ve adenokarsinomlarinda histopatolojik bulgular ile siklin d1 ve ki-67 ekspresyonunun değerlendirilmesi evaluation of histopathological findings and cyclin d1 and ki-67 expression in adenoma and adenocarcinomas 2017.
8. Assessment of immunohistochemical expression of her2\neu and BRCA1 in colorectal tumors and it's correlation with clinicopathological variables. University of Thi-Qar Journal of Medicine. 2020.
9. Bahnassy AA, Zekri AR, El-Houssini S, El-Shehaby AM, Mahmoud MR, Abdallah S, El-Serafi M. Cyclin A and cyclin D1 as significant prognostic markers in colorectal cancer patients. BMC gastroenterology. 2004 ;4(1):22.
10. Jiang J, Wang J, Suzuki S, Gajalakshmi V, Kuriki K, Zhao Y et al. Elevated risk of colorectal cancer associated with the AA genotype of the cyclin D1 A870G polymorphism in an Indian population. Journal of Cancer Research and Clinical Oncology. 2005;132(3):193-99.
11. Li Y, Wei J, Xu C, Zhao Z, You T. Prognostic Significance of Cyclin D1 Expression in Colorectal Cancer: A Meta-Analysis of Observational Studies. PLoS ONE. 2014;9(4):e94508.
12. Arber N, Hibshoosh H, Moss S, Sutter T, Zhang Y, Begg M et al. Increased expression of cyclin D1 is an early event in multistage colorectal carcinogenesis. Gastroenterology. 1996;110(3):669-74.
13. Ogino S, Nosho K, Irahara N, Kure S, Shima K, Baba Y et al. A Cohort Study of Cyclin D1 Expression and Prognosis in 602 Colon Cancer Cases. Clinical Cancer Research. 2009;15(13):4431-38.
14. Izawa H, Yamamoto H, Ikeda M, Ikeda K, Fukunaga H, Yasui M et al. Analysis of cyclin D1 and CDK expression in colonic polyps containing neoplastic foci: A study of proteins extracted from paraffin sections. Oncology Reports. 2002.
15. Ayhan S, Isisag A, Saruc M, Nese N, Demir MA, Kucukmetin NT. The role of pRB, p16 and cyclin D1 in colonic carcinogenesis. Hepatogastroenterology. 2010;57(98):251-56. PMID: 20583423.
16. Jang K, Kim Y, Bae J, Chung M, Moon W, Kang M et al. Expression of Cyclin D1 Is Associated with β-Catenin Expression and Correlates with Good Prognosis in Colorectal Adenocarcinoma. Translational Oncology. 2012;5(5):370-78.
17. Marcolino T, Pimenta C, Artigiani Neto R, Castelo P, Silva M, Forones N et al. p53, Cyclin-D1, β-catenin, APC and c-myc in Tumor Tissue from Colorectal and Gastric Cancer Patients with Suspected Lynch Syndrome by the Bethesda Criteria. Asian Pacific Journal of Cancer Prevention. 2020;21(2):343-48.
18. Utsunomiya T, Doki Y, Takemoto H, Shiozaki H, Yano M, Sekimoto M et al. Correlation of Beta-Catenin and Cyclin D1 Expression in Colon Cancers. Oncology. 2001;61(3):226-33.
19. Nassrat F, Qasim B ,Ali H. Immunohistochemical expression of cyclin D1 in colorectal adenomas: a clinicopathological study. Kasr Al Ainy Medical Journal. 2016;22(3):115.
20. Hunter T, Pines J. Cyclins and cancer. II: cyclin D and CDK inhibitors come of age. Cell 1994;79:573–82.
21. Zhang T, Nanney LB, Luongo C, Lamps L, Heppner KJ, DuBois RN et al. Concurrent overexpression of cyclin D1 and cyclin-dependent kinase 4 (Cdk4) in intestinal adenomas from multiple intestinal neoplasia (Min) mice and human familial adenomatous polyposis patients. Cancer Res 1997;57:169–75.
22. Abdulhameed T, Jaff Z, Maaruf N. Clinicopathological study and immunohistochemical evaluation of cyclin D1 in adenomatous polyps. Zanco Journal of Medical Sciences. 2021;25(1):456–63.
23. Su T, Washington M, Ness R, Rex D, Smalley W, Ulbright T et al. Comparison of biomarker expression between proximal and distal colorectal adenomas: The References 85 Tennessee-Indiana Adenoma Recurrence Study. Molecular Carcinogenesis. 2016;56(2):761-73.