

# Correlation Between Cyclin D1 Expression With Different Pathological Changes in Colorectum Patients

Hanaa Ibrahim Salih Mohammed

University of Science and Technology

Mohammed Abdelgader Elsheikh

Shendi University Faculty of Medicine and Health Sciences: University of Shendi Faculty of Medicine

Alkhair Idris (✉ [alkhair20@hotmail.com](mailto:alkhair20@hotmail.com))

Ahfad University for Women School of Medicine <https://orcid.org/0000-0002-9278-5591>

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## Research Article

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# Abstract

## Background:

Cyclin D1 plays a vital role in cancer cell cycle progression and is overexpressed in many human cancers, including colorectal cancer.

## Objectives:

This study was aimed to detect cyclin D1 in colorectal cancer patients and to correlate cyclin D1 expression with different pathological changes in colorectum.

## Methods:

Tissues microarray paraffin block with 48 colorectal cancer samples were retrieved from the archives of Elrahma Medical Center. The cyclin D1 was analyzed.

## Results:

Cyclin D1 did not correlate with pathological alterations and with tumor grade.

## Conclusion:

Results indicated that; cyclin D1 not correlates with pathological alteration of colorectal cancer.

# Background

Colorectal cancer (CRC) malignancy begins as uncontrollably (1). CRC is one of the most common cancers in the world.

This study conducted on Sudanese patients with CRC, aimed to detect cyclin D1 biomarker and to correlate its expression with tumor grade and patients age, also this study performed to determine of tumor prognosis.

# Methods

This was a retrospective cross sectional study.

The study samples were collected and processed in Elrahama Medical Center- Khartoum North (Sudan).

Forty-eight formalin fixed paraffin embedded blocks (FFPE), previously diagnosed with colorectal carcinoma were selected in this study.

FFPE tissue blocks with CRC from Sudanese patients were included in this study as a case group. Other tissues with a colon and rectum diseases and benign lesions were excluded from this study. Race, tribe,

age and residence were not considered in this study.

## **Sample processing**

The collected samples were used tissue microarray (TMA) block using conventional mechanical pencil tips. Then the TMA block was sectioned (3 microns), using rotary microtome (MR22150-K2258-1124 Histoline – Italy). Section was floated in 70% ethanol, and then floated in water bath (LAB TECK, 009222 - India) at 45c<sup>0</sup>, after floatation the slide was dried in dry oven at 50c<sup>0</sup> for 12-24 hours. After flotation TMA sections were contained in forested end positive charge slide.

## **Method of staining**

Immunohistochemistry staining was done as the protocol of (Dako-USA).

## **Interpretation of result**

We scored the intensity of nuclear cyclin D1 expression as negative (scored zero) when no brown nuclear stain was observed in (0.00 cells or 1% of cells), weak positive expression was scored as score one when pale brown nuclear stain was observed (2-25% of cells). Moderate expression was scored 2 when 26-50% of cells were with brown nuclear stain while strong expression was scored when 75% or more of cells showed brown nuclear stain (9).

## **Results**

The age of patients between 30 to 75 years old with mean 50 years old. The age of patient's sub grouped into 2 groups, group one included patients with less than 50 years old, the second age group included those with age equal or more than 50 years old.

The age group one included 20 (42%) patient samples, while the second age group included 28(58%) patient samples.

Concerning tumor grade, the tumor grade1 (low grade) comprised one tissue block (2.1%), grade 2 (moderate) comprised 23 samples (47.9%) while grade 3 (high) comprised 24 samples (50%), as summarized in figure (4).

The frequency of cyclin D1 positive expression among study populations were as followed; the negative expression was detected in 7(14.6%) samples, while positive expression was detected in 41(85.4).

The cyclin D1 positive expression scored as followed; score 1 (weak expression) was detected in 18 samples (43.9%), score 2(moderate expression) was detected in 17 samples (41.5%), score 3 (strong expression) was detected in 6 samples (14.6).

Regarding correlation of cyclin D1 immunoexpression with tumor grades, our results revealed that; the negative expression score (0.00) was detected in (0 out of 1=0.00%) samples in grade1, (3 out of

23=13%) samples in grade 2, and (4out of 24=16.7%) samples in grade 3. The positive expression was detected (1 out of 1=100%) samples in grade1, (20 out of 23=87%) samples in grade 2, and (20 out of 24=83.3%) samples in grade 3, the p value was 0.999.

Regarding correlation of cyclin D1 immunoexpression with age our results revealed that; the negative expression was detected in 1 sample out of 20 samples (5%) from patients with age group below 50 years old, while the positive expression in the same age group was 19 =95%. The negative expression in the second age group of 50 years old and above was 6 out of 28 samples (21.4%), while the positive expression in the same age group was 22 =78.6%, the p value was 0.214.

Regarding cyclin D1 immunoexpression with the gender of patients, our results revealed that; the positive expression was detected in 29 out of 36 males (80.6%), the remainder 7 males (19.4%). Regarding immunoexpression of cyclin D1 among females our results showed that; all samples (100%) were present with positive expression.

## Discussion

This is study aimed to detect immunoexpression of cyclin D1 in CRC. To achieve these aim 48 FFPE tissues with CRC were involved in this study. Our results showed that; the CRC in this study more frequently observed in advanced ages. This finding consistent with those studies conducted by Motaz *et al.*, who concluded that; the mean age of the patients was 50.5 ( $\pm$ 11.7) (10), and Ahmed *et al.*, (11). On the other hand, our results regarding correlation of age with the occurrence of CRC inconsistent with those studies conducted by Alsanae., *et al.*, who summarized that; CRC presents at a younger age (12), and Mohamed *et al.*, (13) and Abdalla*et al.*,(14).

Regarding the association of gender with CRC, our results revealed that; the CRC among males were predominantly (3/4) than females (1/4), this result agree with Alsanea*et al.*, (12) and Saeed *et al.*, (14). But our result regarding gender and CRC disagree with that study conducted by Ahmed *et al.*, who concluded that; fifty-six percent were females (11), and Mohamed *et al* (13). Concerning the frequency of tumor grades in samples with CRC, our results showed that; usually patients presented with advanced stages of disease, because we observed more than 90% of patients with advanced grades, and this finding usually due to lack of systemic health care system, lack of screening program, lack of sufficient early detection method, and also due to bad culture in health and protection methods. This finding regarding tumor grade similar to results achieved by Motaz *et al.*, and Alsanea (10).

Regarding the relationship between cyclin D1 and CRC, our results indicated that; near to 90% of stained sections showed positive expression, this results matched with other studies done by Abeer *et al.*, (15) and Sakariaset *al.*, (16), Blaserczak *et al.*, (9), also agree with shujiet *al.*, (17), and von stockmar *et al.*, (18). And disagree with Other studies done by Jaudah *et al.*, (19).

According to the obtained findings we revealed that; Cyclin D1 immunoexpression was higher among high tumor grades, but with no significant correlations, higher in females than males, frequently observed

in elderly patients.

## Abbreviations

**APC:** Adenomatous polyposis coli

**BCL1:** B cell lymphoma

**CCND1:** Cyclin D1

**CDK:** Cyclin-dependent kinase

**CRC:** Colorectal cancer

**D.W:** Distilled water

**DAB:** Di amino benzidine

**DPX:** Dixerena Plasticizer and Xylene

**FFPE:** Formalin fixed paraffin embedded blocks

**PBS:** Phosphate buffer saline

**TMA:** Tissue microarray

**UMST:** University of Medical Sciences and Technology

## Declarations

### **Ethics approval and consent to participate**

Written Approval from UMST Ethical Research Committee in accordance with the Declaration of Helsinki Principles, similar approval took from Khartoum Ministry of Health and Elrahma medical center was taken. Research purpose benefits and objectives were explained to hospital and laboratory administration in clear simple words with assurance on confidentiality.

Cyclin D1 Ab was applied in formalin fixed paraffin embedded tissue section already diagnosed as colorectal cancer sample and after got permission from hospital administration.

**Approval reference number:** UMST-REC/04-020./02

**Approval date:** 26/4/2020

**Consent for publication:**

Not applicable

### **Conflict of interest**

Authors declare no conflict of interest

### **Availability of data and materials**

The data sets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests.

### **Source of funding**

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### **Authors' contributions**

HISM and MAE conceived the design and carried out the experiments. AAI obtained, analyzed and interpreted the data. MAE and AAI wrote and revised the manuscript. HISM provides financial support for all experiments. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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## Tables

**Table (1): Association between cyclin D1 expression with tumor grades.**

Tumor grade	Cyclin D1 expression		p value
	Positive	Negative	
Grade I	1 (2.10%)	0.00 (0.00%)	0.999
Grade II	20 (41.70%)	3 (6.30%)	
Grade III	20 (41.7%)	4 (8.3%)	
Total	41(85.4%)	7 (14.6%)	
<b>N=48</b>			

- p value <0.05 that's considered as statistically significant.

**Table (2): Correlation between cyclin D1 expression and patients age.**

Patient age	Cyclin D1 expression		p value
	Positive	Negative	
Less than 50 years	19 (39.60%)	1 (2.1%)	0.214
50 years and above	22(45.80%)	6 (12.50%)	
Total	41(85.4%)	7 (14.6%)	
<b>N=48</b>			

**Table (3): Comparison between cyclin D1 expression and patient gender**

Patient gender	Cyclin D1 expression	p value	Patient gender	Cyclin D1 expression
	Positive	Negative		Positive
Males	29 (60.40%)	7 (14.60%)	Males	29 (60.40%)
Females	12 (25%)	0.00 (0.00%)	Females	12 (25%)
Total	41(85.4%)	7 (14.6%)	Total	41(85.4%)
N=48			N=48	

- p value <0.05 that's considered as statistically significant.

## Figures

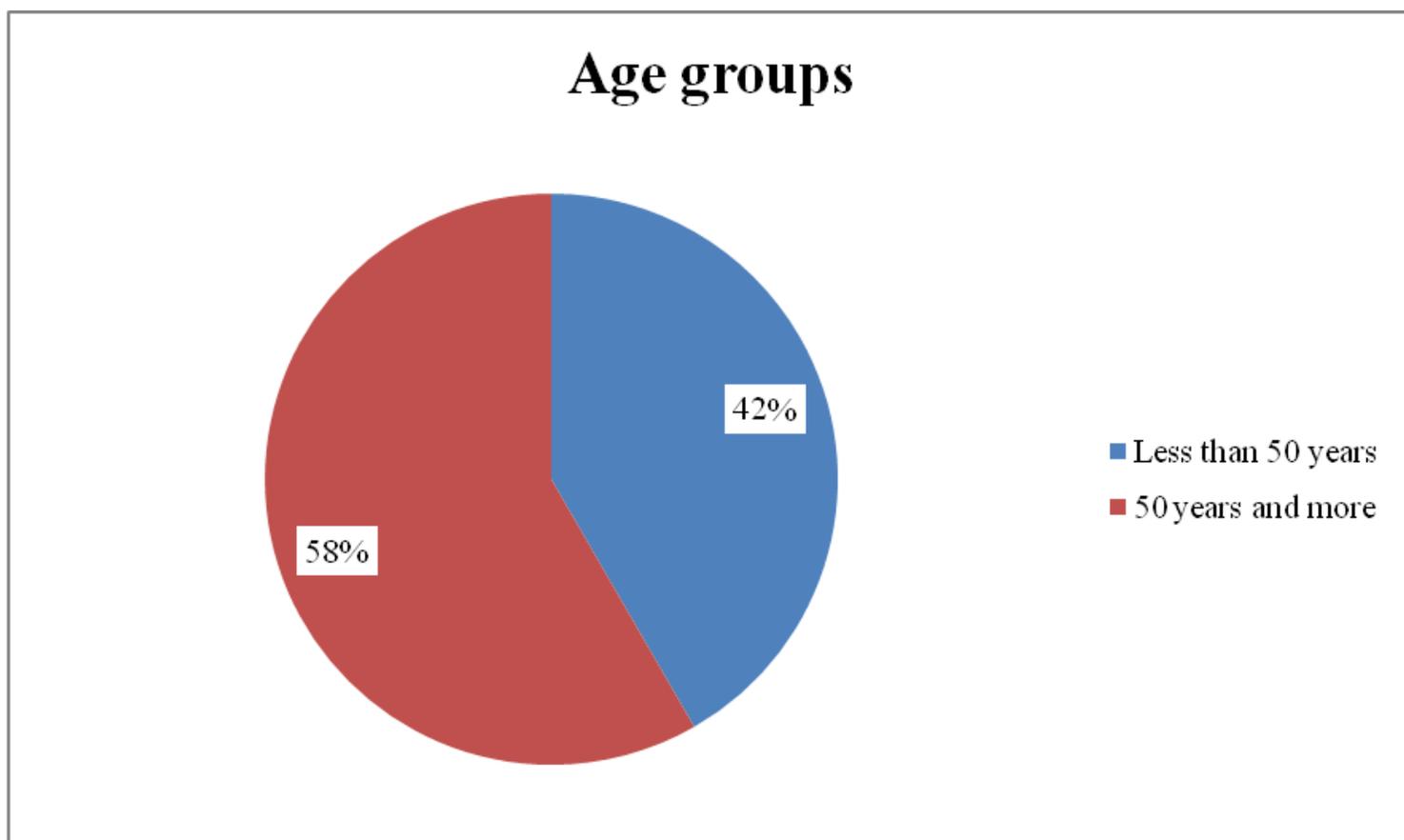


Figure 1

Summarizes the age sub groups.

# Gender

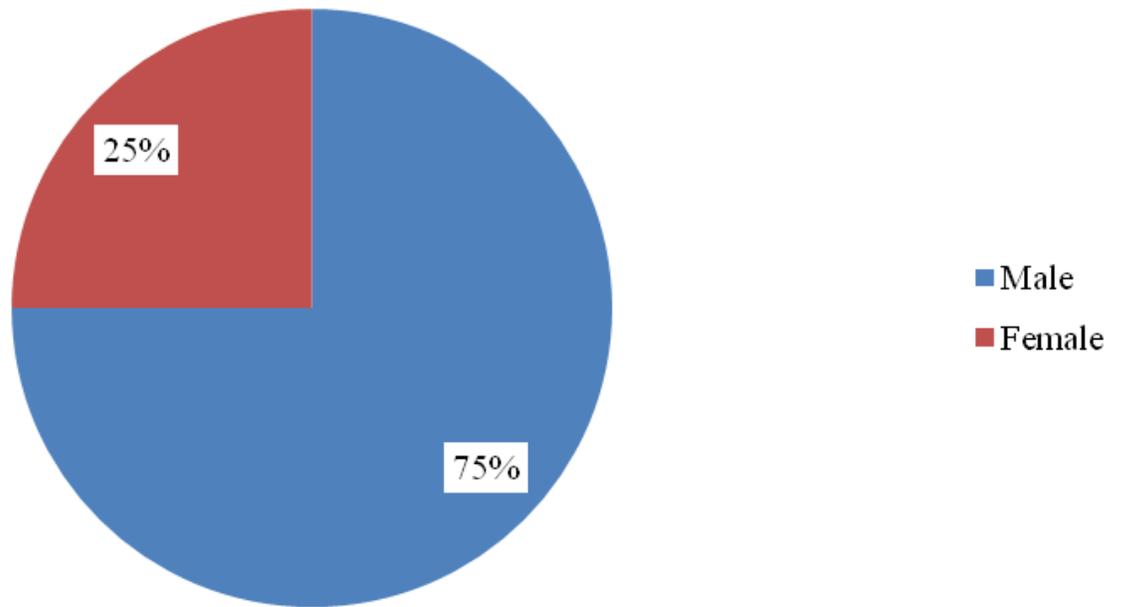


Figure 2

Sex of study populations.

## Histological grade

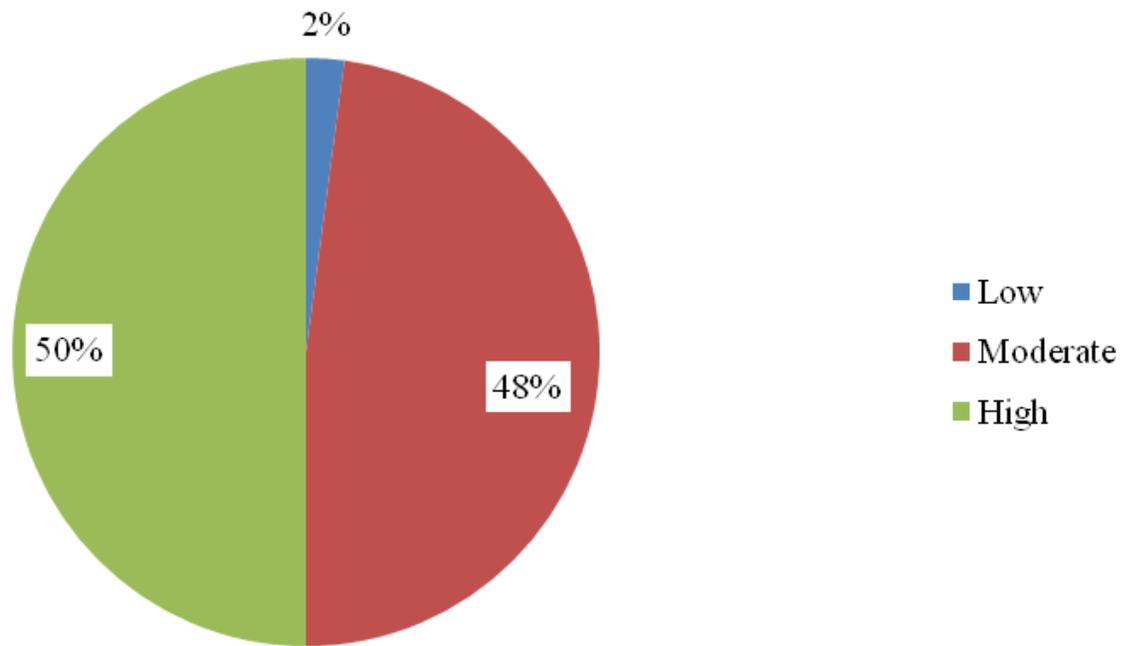


Figure 3

The histological grade among studied samples.

# Cyclin D1 Expression

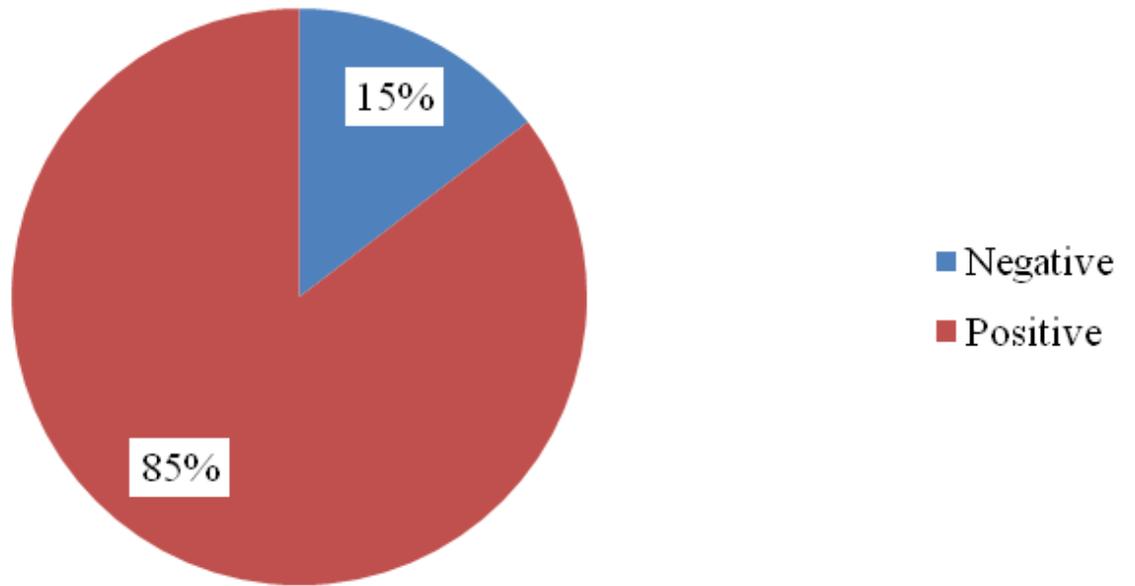


Figure 4

Represents the result of cyclin D1 immune reaction.