

# Evaluation of the Role of miRNA-21 Levels as a Potential Diagnostic Biomarker for Colorectal Cancer Associated with Prognosis

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## Abstract

Colorectal cancer one of the most common and aggressive tumor diagnosed in humans. The potential role has been suggested that circulating microRNAs assuring diagnostic markers for early detection of colorectal cancer. Hereabouts, this investigation intends to study the performance of miRNA-21 in CRC. Fifty blood samples were collected from individuals with colon cancer who enrolled in the Teaching Laboratories of the Medical City/Baghdad from June 2018 to September 2019. Taq Man microRNA Real-time PCR was utilized to recognize the expression of miRNA-21 in the patient's plasma. Representative in the expression of miRNA-21 by gender and pathological grading were not statistically important ( $P>0.05$ ). Moreover, alterations in the expression of miRNA-21 amongst patients and control were significances statistically ( $P<0.05$ ). While a higher risk of the disease was associated with high miRNA-21 and correlated with poor prognosis. The AUC for miRNA-21 was 0.657. The optimal cut-off value was 1.961-fold with sensitivity and specificity 64%, 68% respectively. In Conclusion, the high miRNA-21 expression might be a prognostic marker of colon cancer patient consequently; high expression of miRNA-21 was associated with poor prognosis of patients with colorectal cancer.

**Keywords:** Colorectal cancer, MiRNA-21, biomarker.

## Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy in both genders with approximately 1.2 million individual investigations and above 50% of deaths globally each year<sup>1</sup>. The majority of CRC related fatalities can be prevented through early diagnosis and surgical removal of early-stage cancer<sup>2</sup>. In addition, the available methodologies for early detection are based on traditional screening method, such as the fecal occult blood test (FOBT) as the primary screening tool, followed by colonoscopy for FOBT positive patients<sup>3</sup>. Colonoscopy, despite its specificity and sensitivity, is not suitable for the general population due to its high cost, invasiveness, requirement for bowel preparation and sedation, and association with medical complications. Therefore, the development of new markers is urgently required for the rapid, noninvasive, and highly sensitive screening of CRC patients<sup>4</sup>. MicroRNAs are near to 20 to 25 nucleotide non-coding RNAs that post-transcriptionally regulate gene expression and control

various cellular mechanisms<sup>5</sup>. There was increasing evidence that microRNAs were widely dysregulated in cancer and may have potential applications for cancer diagnosis, prognosis, and treatment<sup>6</sup>. The diagnostic value of circulating microRNAs for the early detection of cancer has been successfully investigated in numerous malignancies, including CRC. MicroRNA 21 is over expressed in various human tumors, particularly in the serum and tissue of CRC patients<sup>8</sup>. Diverse researches have investigated the diagnostic value of miRNA-21 in CRC and have raised interest concerns regarding the biomarker potential of miRNA-21<sup>(9, 10)</sup>. However, the findings of these studies were inconsistent. Therefore, this study is conveyed to assess the diagnostic value of miRNA-21 in CRC.

## Materials and Method

**Subjects:** This study was performed during a period extended from March 2018 to February 2019 at the Teaching Laboratories of the Medical City/

Baghdad. Clinical data were collected from the hospital reports including age, sex, and grade of the tumor. The histological grading which was based on reviewing H & E stained representative slides was labeled as grade (G1) for well differentiated, (G2) for moderately differentiated, and (G3) was for poorly differentiated tumors. Ninety subjects participated in this study where included fifty subjects (40 males, 10 females) with age range from (31-85) years with histologically confirmed of CRC. Fourteen subjects (30 males and 10 females) with age range from (39-70) years who had no documented for cancer attended the same hospital. About five milliliters of venous blood samples were collected from patients and healthy persons in sterile tubes for serum and plasma isolation then stored at  $-70^{\circ}\text{C}$  until use.

**Molecular detection of miRNA-21:** Molecular detection of miRNA-21 was carried out according to (Taq Man™ MicroRNA Assay, inventoried, SM, Applied Biosystems, USA) which occurred in three steps:

1. RNA extraction.
2. Reverse transcription step.
3. Real-time PCR for the detection of mature miRNA-21.

**Statistical Analysis:** Analysis of data was carried out using the available statistical package of SPSS-25 (Statistical Packages for Social Sciences- version 25). Data are presented as mean  $\pm$  SD, median, percentage and standard error. Qualitative relations were evaluated using the Chi-square test. A p-value of  $\leq 0.05$  was considered statistically significant. Cut-off values were estimated according to ROC<sup>12</sup>.

## Results

**Demographic characteristics of the studied groups:** The results of this study were based on the investigation of fifteen patients with CRC, compared with 40 apparently healthy persons considered as controls. Colorectal cancer patients whom ages were ranged from thirty one years to eighty five years. The mean age of these patients was  $(52.7 \pm 14.2)$  years, whereas the mean age of their counterpart's apparently healthy control was  $(46.1 \pm 11.9)$  years). There were no significant statistical differences ( $p < 0.05$ ) between different groups according to age. It was found that 31 (62.3 %) of CRC were males, while the rest 19 cases (47.7%) were females. While the sex distribution in apparently healthy control was found that 20 (50%) were males and 20(50%) were females. The statistical analysis showed no significant difference ( $P < 0.05$ ) between CRC and control groups according to gender.

**Table 1: Distribution of patients and controls according to age intervals**

Age Interval	Patients Group		Healthy Women Group		P-value
	No.	%	No.	%	
$\leq 40$ yrs.	10	20	20	50	
$> 40$ yrs.	40	80	20	50	
Total	50	100.0	40	100.0	
Mean age $\pm$ SD	52.7 $\pm$ 14.2		46.1 $\pm$ 11.9		0.720 NS
Age range	(31-85) year		(30-70) year		
Gender	Male No. (%)	31 (62.3%)		20(50%)	P=0.655 NS
	Female No. (%)	19 (47.7%)		20(50%)	
Total No.	50		40		

NS: Non-Significant at  $P > 0.05$ .

**Levels of miRNA-21 in the studied groups:** The mean log fold change values of gene expression of miRNA-21 in plasma of CRC patient was higher as in

control group (1.306 vs 0.125 respectively), and revealed a statistically significant difference between them; the P-value was 0.012 as mentioned in table 2.

**Table 2: MiRNA-21 plasma levels in studied groups.**

MiRNA-21	Plasma CRC	Control
Mean	1.306	0.125
Standard Error of Mean	0.106	0.082
Median	0.872	0.098
CRC vs Control	0.012	

**Relationship study between CRC grading and miRNA-21:** In this investigation uncovered that well-

differentiated carcinomas were seen in 16 cases (32%) of the CRC group, while 31 cases (62%) have moderately differentiated grade. Poorly differentiated carcinomas were seen in 3 cases just as appeared table (3). There were no measurably critical contrasts ( $P > 0.05$ ) among the CRC group according to the grade. The highly Folding 221 concentration reached ( $1.74 \pm 0.621$ ) in poor differentiation CRC patients comparison with 0.287 and 0.394 in Well and Moderate differentiation CRC grad respectively ( $P = 0.38$ ).

**Table 3 :Relationship study between CRC grading and miRNA-21**

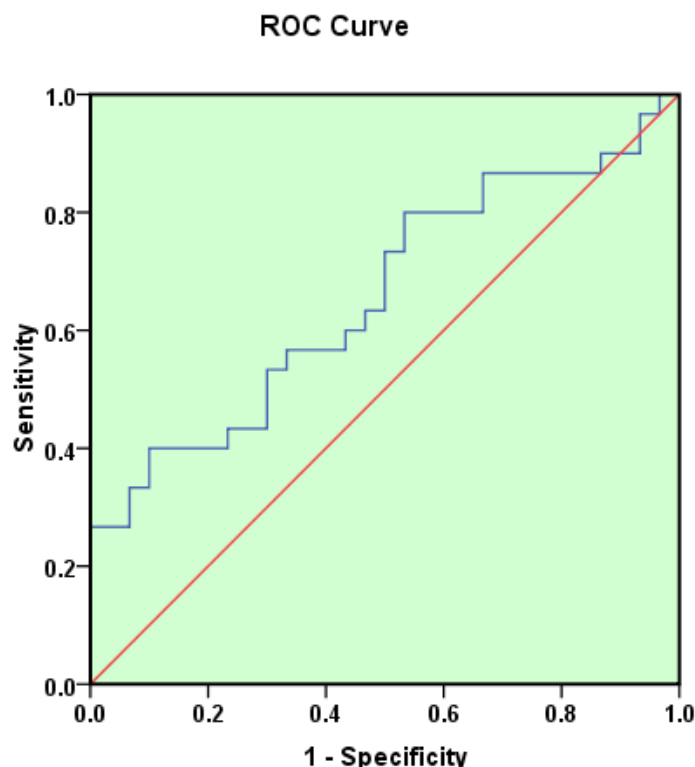
Marker		Well differentiation 16 (32%)	Moderate differentiation 31 (62%)	Poor differentiation 3(6)%	p- value
Folding MiRNA-21	Mean folding $\pm$ SD	0.287 $\pm$ 0.142	0.394 $\pm$ 0.263	1.74 $\pm$ 0.621	0.38

**Estimation of cutoff value, sensitivity, specificity and AUROC of the miRNA-21 in CRC patient:** The area under the curve (AUC) for miRNA-21 was 0.657, and P- value equal to 0.037. The optimal cut-off value

was 1.961-fold with sensitivity and specificity 64%, 68% respectively with 95% confidence interval as in table (4) figure (1).

**Table 4: Estimation of cutoff points, sensitivity, specificity and AUROC of the miRNA-21 in CRC patient.**

Parameters	Cut -off points	Sensitivity (%)	Specificity (%)	CI	AURO	P-values
MiRNA-21 Log fold change	1.961	64	68	95%	0.657	0.037



**Figure (1): Receiver Operating Characteristic curve for miRNA-21 as a marker for CRC**

## Discussion

Colorectal cancer (CRC) was a major cause of cancer-related fatalities worldwide<sup>13</sup>. The reasonableness of colorectal cancer diagnosis rises after the age of 40 years, increases progressively from the age of 40 years, growing clearly after age 50 years<sup>14</sup>. Larger than 90% of colorectal cancer events occur in people aged fifty years or older<sup>15</sup>. According to, age match as exhibited in the present results harmonized with other studies from Iraq prepared by Al-Hummadi, 2009<sup>16</sup> and Tahir, 2011<sup>17</sup> found that the mean age of CRC was 50 years. A prior study by Abdul Ghafour, 2014 found that the mean age was about 53 years<sup>18</sup>. The investigation of miRNA-21 as a diagnostic biomarker contributed to colon cancer pathogenesis in current study revealed that the log folded of miRNA-21 in patients was significantly higher compared to healthy control similar to this result reported by<sup>(19,20)</sup>. A meta-analysis investigation conducted by Yu *et al.*, 2016 this study enlisted in the systemic review was carried in diverse countries such as China, Japan, Iran, Germany, and the USA. Amongst the nine studies involved, six of them were carried in Asian populations and three in Caucasian populations. Nine of these studies were written within 2012 and 2014, reviewed that the diagnostic value of miRNA-21 for CRC<sup>21</sup>. Earlier studies by Pan *et al.*, 2010 and Wang *et al.*, 2014 described that miRNA-21 is one of the most leading oncomiRNAs in CRC, and becomes expressed pro-tumorigenic features in various another hard tumor types<sup>(22,23)</sup>. In mouse design study through Shi *et al.*, 2015<sup>24</sup> summarized that MiRNA-21 seems to be fundamental to the inflammation recognized in colitis-associated colon cancer in a carcinogen-induced mouse model of CRC applying the mutagen azoxy methane (AOM) plus DSS, genetic inactivation of miRNA-21 decreased tumor burden and decreased levels of pro-inflammatory cytokines. Various studies were recording in miRNA-21 was raised in CRC tumors, with a step-wise increase in its expression as tumors progression to later grades<sup>(25, 26)</sup>. Nemours investigations illustrate that miRNA-21 in serum and stool indicates its levels in CRC tumors; this consequently might assist as both a diagnostic and prognostic biomarker via predicting the TNM stage, possible metastasis, and responsiveness to chemotherapy. Notwithstanding, enhanced miRNA-21 serum levels have also been described for pancreatic, lung and breast cancers recommending that stool analysis should be combined to enhance the specificity of CRC screening<sup>(27,28,29,30)</sup>. Besides, Mima *et al.*, 2016 noticed a close association between raised tumor

miRNA-21 expression and CRC is correlated with the poorer clinical issue and this association is stronger in carcinomas<sup>31</sup>. The meta-analysis published by Zhang *et al.*, 2014 and Du *et al.*, 2014 conducted on the diagnostic value of miRNA-21 for CRC. Zhang *et al.*, 2014<sup>32</sup> reported a collected sensitivity of 76% and a collected specificity of 81% while; Du *et al.*, 2014<sup>33</sup> reported a pooled sensitivity of 76% and a pooled specificity of 82%; those events were comparable to the present result, but not identical, the AUC was 0.657 with sensitivity and specificity 64%, 68% respectively with 95% CI, indicating that the CRC patients have higher than a nine-fold possibility to express miRNA-21 in comparison to healthy individuals indicating that miRNA-21 can be used as a good marker for CRC diagnosis. Overall, miRNA-21 was not a special biomarker in CRC; it requires to be combined with another tool for enhanced specificity. However, current evidence indicates that circulating miRNA-21 has moderate sensitivity and good specificity as a diagnostic marker for CRC diagnosis. Large-scale prospective studies must be conducted in the future for verification. In addition, improving the diagnostic accuracy of circulating miRNA-21 and exploring new biomarkers with high diagnostic accuracy in CRC should still be considered in the future.

## Conclusion

MicroRNA-21 is overexpressed in the serum of patients with colorectal cancer, submitting that miRNA-21 is a hopeful diagnostic biomarker for CRC. In distinction, a patient with a high expression of the miRNA-21 level was connected with a poorer prognosis.

## Conclusion

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Collage of Health & Medical Technology and all experiments were carried out in accordance with approved guidelines.

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