Role of miR-7 as Potential Biomarker for Colorectal Cancer

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Abstract
Two-thirds of colorectal cancers (CRCs) occur in more developed parts of the world, making it the third most frequent cancer globally after lung and breast cancers. All racial and ethnic groups are affected by CRC, which primarily affects those 50 years of age and older. This condition may arise as a result of a variety of circumstances, including environmental and hereditary ones. The majority of cases are discovered until after symptoms appear, despite the fact that colorectal cancer screening lowers the frequency of diagnosis at advanced stages. MicroRNAs (miRNA), which are frequently dysregulated in cancer, are crucial for regulating gene expression. Since miR-7 affects the expression of several oncogenes, revealing how miR-7 activity is regulated will probably help us better understand the causes of different types of cancer. The purpose of this study was to look at how miR-7 affects colorectal cancer. We discovered a strong correlation between CRC and miR-7.
miRNA-7 is circulating as a possible non-invasive biomarker for several illnesses and disorders, including colorectal cancer. This study sought to determine whether microRNA-7 may be used as a diagnostic tool for colorectal cancer.

Keywords: Colorectal cancer and microRNA-7.

1.Introduction

Globally, colorectal cancer (CRC) ranks fourth in terms of cancer-related mortality and is the third most frequent kind of cancer [1]. Individual risk for colorectal cancer (CRC) is influenced by both lifestyle and hereditary variables, making it a complicated illness.[٢]

Colorectal cancer has been linked to a wide variety of disorders. A increased risk of acquiring colorectal cancer (CRC) is associated with a history of cancer, colon polyps, inflammatory bowel disease (IBD), diabetes mellitus, or cholecystectomy, according to research. The etiology of colorectal cancer can be significantly influenced by lifestyle variables [3]. Colorectal cancer risk factors include being overweight or obese, not exercising, smoking, drinking alcohol, and eating an unhealthy diet heavy in red and processed meat and low in fiber, fruits, vegetables, calcium, and other nutrients. Factors known to influence colorectal cancer risk include the gut microbiota, gender, age, race, and socioeconomic status [4].

It has been demonstrated that a number of screening instruments have the ability to significantly lower the incidence and death of colorectal cancer during the last three decades. These include flexible sigmoidoscopy, colonoscopy, and fecal occult blood tests (faecal immunochemical tests [FITs], which are now the most popular tests because to their superior diagnostic performance and acceptability over the conventional guaiac-based faecal occult blood tests [gFOBTs]).[٥]

Single-stranded RNAs with a length of 19–24 nucleotides that do not code are known as endogenous microRNAs (miRNAs) [6]. When these posttranscriptional regulators bind to the target gene's 3' untranslated region (UTR), they destabilize the mRNA and suppress translation, which in turn inhibits protein production and translation [7]. The best molecules to use as biomarkers are miRNAs. Recent data indicates that miRNAs may act as tumor suppressors or oncogenes by influencing carcinogenesis, proliferation, and apoptosis .[٨]

A potential tumor suppressor in hepatocellular carcinoma, gastric cancer, and colorectal cancer has been found by concentrating on several oncogenic signaling pathways; miR-7 is one of these pathways. A number of fundamental biological processes in cancer cells are also controlled by it.[٩]

It has been demonstrated in vitro that miR-7 targets the EGFR (10). One downstream gene that miR-7 targets is RAF-1, or v-raf-1 murine leukemia viral oncogene homolog 1, as found by Rai et al. Consequently, tumor resistance to EGFR inhibition therapy is already a problem in clinical practice; miR-7 modification might help.[١٠]

The fact that miR-7 inhibits the activity of several cancer-related signaling pathways—including those involving the EGF receptor, IRS-142, Raf122, and Kruppel-like factor 4-mediated stem cell production—strongly suggests that it has a tumor-suppressing role .[١١]

It has been demonstrated that MiR-7 is downregulated in colorectal cancer (CRC) and that it regulates multiple oncogenic signal transduction pathways, including the RAF/MEK/ERK and PI3K/AKT pathways, suggesting that it may serve as a tumor suppressor.[١٢]

2.Subject and Methods

Following approval by the Benha Faculty of Medicine's Research Ethical Committee and written informed consent from each participant, 90 participants—45 from the General Surgery Department at Benha University Hospital and 45 from a healthy control group—participated in the study. In addition to 45 patients with colorectal cancer (CRC), there were 45 volunteers who were healthy and matched by age and sex.

The blood Blood was drawn in an EDTA tube, separated into many parts, and then moved to a tube devoid of RNase. Until the RNA was isolated, the whole blood and plasma samples were stored at -80°C.

Estimation of miRNAs expression levels according to the following steps:

1.   Total RNA including microRNA extraction by total RNA Purification Kit & reverse transcription of RNA into cDNA.

2.   Relative quantitation of microRNA-7 by two-step quantitative Real time PCR: Relative Quantitation using comparative Cycle Threshold (CT) describes the change in expression of the nucleic acid sequence (target gene) in a test sample relative to the same sequence in a calibrator sample..

3.   Data analysis: Fold expression changes are calculated using the equation $2^{-\Delta\Delta}$ CT and expressed as relative units (RU).

3.Analytical synthesis

Version 20.0 of IBM's statistical programme for social science (SPSS) was used for data analysis (IBM Corp., 2017). With Version 25.0, IBM Corp. of Armonk, New York, released IBM SPSS Statistics for Windows. Quantitative and qualitative data were characterised by percentages and counts. To ensure the distribution was normal, the Shapiro-Wilk test was used. Range (including minimum and maximum), mean, standard deviation, median, and interquartile range were used to represent quantitative data (IQR). A significance threshold of 5% was used to evaluate the findings.

4.Results

The age range of patients with CRC was 44–66 years, with a mean of 57 ±13 years. The control group had an average age of 50.90± 8.64 years, with a mean of 54 ±8 years. In the patient group, there were 30 men and 15 females, making up 66.6 percent and 33.3 percent, respectively. In the control group, men made up 57.7% (n=26) and females 42.2% (n=19), The age distribution and sex distribution of the groups under study did not vary statistically significantly (p value > 0.05). Table (1).

Table (1) Groups' demographic information.

Variable		Patients group (N=45)		Control group (N=45)		Test value	P-value
		No.	%	No.	%		
Gender	**Male**	30	66.6%	26	57.7%	X^2= 0.187	0.662
	Female	15	33.3%	19	42.2%		
Age (years)	**Mean± SD**	57 ±13		54 ±8		F= 2.418	0.272
	Median (IQR)	56.0 (52.5-60.0)		52.5 (43.5-56.0)			
	Range	44.0 - 66.0		36.0 - 69.0			

A P value less than 0.05 is considered significant, and a P value less than 0.01 is considered extremely significant, according to the standard deviation (SD), F (one-way ANOVA test), and X2 (chi-square test).

Regarding smoking, diabetes, and hypertension, there were no appreciable differences between the groups under study. Furthermore, with a P-value of 0.04, patients were substantially more likely than controls to have a positive family history of the illness. Table.(٢)

Table (2) Clinical history of the two groups that were investigated.

Variable		CRC group (N=45)		Control group (N=45)		Test value	P-value
		No.	%	No.	%		
Smoking	**Positive**	29	64.4%	26	57.7%	X^2= 0.413	**0.520**
	Negative	16	35.5%	19	42.2%	X^2= 0.406	**0.523**
DM	**Positive**	22	48.8%	19	42.2%		
	Negative	23	51.1%	26	57.7%		
HTN	**Positive**	23	51.1%	21	46.6%	X^2= 0.833	**0.833**
	Negative	22	48.8%	24	53.3%		
Family History	**Positive**	15	33.3%	6	13.3%	X^2= 4.442	**0.04***
	Negative	30	66.6%	39	86.6%		

An very significant P value is less than 0.01, while a p-value less than 0.05 is also considered significant. SD stands for standard deviation.

X2= Chi- Square test

Table (3) Laboratory profile in the studied groups

Variable		CRC group (N=45)	Control group (N=45)	Test value	P-value
Albumin (g/dl)	Mean ±SD	3.1 ±0.74	4.0 ±0.62	t = 9.921	<0.001*
ALT (U/L)	Median (range)	30 (17 - 66)	23 (12 43)	Z = -1.832	0.067
AST (U/L)	Median (range)	28 (15 - 98)	26 (11-48)	Z = -0.787	0.43

The results are considered significant when the p-value is less than 0.05 and highly significant when the p-value is less than 0.01.

Table (4) miR-7 in the three groups that were investigated

		CRC group (N=45)	Control group (N=45)	Test value	P-value
miR-7	Median (range)	0.51 (0.4 - 0.7)	0.8 (0.4 - 2.9)	Z =-5.921	<0.001

Statistical significance is defined as a p-value of less than or equal to 0.05, with a p-value of less than or equal to 0.01 being highly significant. The analysis was conducted using the F-ANOVA test.

5.Discussion

In terms of mortality, colorectal cancer (CRC) is the second most frequent cancer worldwide [13]. In industrialized nations, CRC primarily affects individuals 50 years of age or older and affects men and women of all racial and ethnic backgrounds [15]. High-income nations have greater incidence rates of colorectal cancer (CRC) than low-to-middle-income countries (LMICs); nevertheless, LMICs have higher death rates [1]. This might be due to disparities in health care structure, financial resources, and screening infrastructure, as well as the fact that incidence is underreported in LMICs due to a lack of early diagnosis and access to cancer registries [14]. Environmental and/or genetic factors can raise the risk of getting colorectal cancer. Age over 50, poor socioeconomic status, overweight and obesity, a sedentary lifestyle, tobacco use, and excessive alcohol use are some of the risk factors for colorectal cancer [16].

Chromosome 15 contains miR-7, a member of the miRNA family. Different tumor types express miR-7 differently [17]. In the human A529 non-small cell lung cancer cell line, Xiong et al. found that elevated expression of miR-7 suppresses cell growth and invasion [18].

In this study, the control group's mean age was 54 ±8 years, whereas the CRC patients' mean age was 57 ±13 years. The CRC patients ranged in age from 44.0 to 66.0, whereas the control group was between 36.0 and 69.0 years old.

According to Zeng et al., the average age of CRC patients was 26 years under 60 and 32 years over 60. This suggests that the risk of CRC is increased over 60 with no discernible changes.[19]

According to Bader El Din et al., all of the participants were between the ages of 25 and 65. The CRC patients were 48.7 ± 9.3 years old on average.[20]

With a median age of 73 years (IQR 66, 80), the age range was 40–97 years. Of the 106 patients, 106 (73%), had left-sided tumors, with 37 (26%) having sigmoid tumors and 44 (33% of the total) having rectal tumors. The caecum was the most frequent location, accounting for 17 of the 39 right-sided tumors (27%).[21]

For every stage of diagnosis, patients with colorectal cancer (CRC) under 50 years of age have higher 5-year relative survival rates than their older counterparts. However, due to a later stage at diagnosis, overall survival for patients under 50 years of age (68%) is comparable to that of patients between 50 and 64 years of age (69%.[1]

Given that there were 30 men and 15 women in the patient group and 26 men and 19 women in the control group in our study, there were no appreciable variations in the prevalence of CRC between the sexes.

We found that the sick group's albumin levels were lower than the control group's (P < 0.001). This is consistent with a research conducted by Walts et al. to look into the relationships between low albumin, a biomarker of inflammation, and the risk of colorectal cancer. The scientists discovered that a higher albumin standard deviation was linked to a lower risk of colorectal cancer.[22]

The findings indicated that there were no appreciable differences between the groups under study in terms of smoking, diabetes, or hypertension.

Walts et al. discovered no changes in smoking, hypertension, or diabetes mellitus between the CRC group and the control groups, which is in line with the current findings.[22]

Positive family history was found to be independently linked to colorectal cancer (CRC) (P = 0.04).

This result is consistent with a research conducted by Far et al. to measure the risk of colorectal cancer in those with a positive family history. According to the scientists, the risk of developing colorectal cancer is 1.87 times higher for those with a positive family history of the disease.[٢٣]

According to our findings, miR-7 expression was much decreased in CRC (p<0.001).

In line with other studies that found that CC tissues had lower levels of miR-7 expression than the matched neighboring normal tissues, and that this was connected with TNM stages and lymph node metastases in CC. In vitro research on miR-7 expression showed that it prevented CC cell lines from proliferating and invading. After transfection with miR-7, it was discovered that FAK's protein expression levels were considerably lower [19]. The findings aligned with a prior study that demonstrated miR-7 suppressed glioma development and metastasis by targeting a negative regulator of FAK [24]. By focusing on FAK protein expression, miR-7 suppressed the development and spread of breast cancer by blocking the epithelial-mesenchymal transition.[٢٥]

Conclusion

Based on earlier discussions, we came to the conclusion that miR-7 can be employed as an early diagnostic marker for colorectal cancer (CRC) patients.

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