RESEARCH ARTICLE

Evaluation of Circulating MicroRNA-221 and Il-10 Levels as Potential Diagnostic Markers for Colorectal Cancer in Correlation with Prognosis

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ABSTRACT

Background: Because of the capacity of MicroRNAs to down-regulate tumor suppressor expression and enhance tumorigenesis, they are of great importance in the knowledge of cancer.

Aim of the study: To study the performance of miR-221 and IL-10 in colorectal carcinoma.

Patients and methods: In the present study, venous blood specimens were taken from (55) patient suffering from colorectal cancer who were attending the Medical City, Baghdad (Teaching laboratories), during the period from 1st Jun. 2018 to 30th Sep. 2019. For detection of miRNA-221 expression in patient's sera, the Real-time polymerase chain reaction (PCR) was used.

Results: Descriptive analysis of miRNA-221 expression according to pathological grading and gender showed no significant difference ($p > 0.05$), however, variations of miRNA-221 expression among patient and controls revealed significant differences ($p < 0.05$). There was a highly significant difference in the mean serum IL-10 levels among patients with CRC when compared with the control group ($p > 0.04$). While the highest disease risk was correlated to higher mi-RNA-221 with IL-10 levels related with poor prognosis.

Conclusion: The high expressions of mi-RNA-221 can act as prognostic markers in patients with colorectal cancer. Besides, the higher levels of miRNA-221 and IL-10 were correlated with worse prognosis.

Keywords: Colon cancer, Expression, IL-10, MiRNA-221, Prognosis.

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INTRODUCTION

The third most common disease diagnosed among men and women is colorectal cancer (CRC) with about 1.2 million investigation and more than 50% annual death worldwide.[1] MiRNA-221 has displayed higher expression in various tumors including colon cancer.[2] MiRNA-221 acts a special role related to that of oncogenes in tumor initiation and progression and is connected with various cancers including lung, breast, ovarian and papillary thyroid cancer.[3] It is hypothesized that the up-regulation of this miRNA-221 can afford to disease induction or progression.[4] The immune system performs an essential role in all neoplasms including colorectal cancer and chronic inflammation is a leading cause of CRC.[5] Chemokines and cytokines generated by cancer cells and cells supplied to tumor environment like mast cells and macrophages are the central cause of inflammation.[6] IL-10 is a cytokine whose status in CRC pathogenesis and treatment is of particular importance.[7] To fully understand the participation of miRNA-221 in colon cancer, this study aimed to analyze the plasma of colon cancer patients to show the role of IL-10 in CRC and to find the association of miR-221 and IL-10 levels with the grading of CRC.

MATERIALS AND METHODS

Sample Collection

During the period from March 2018 to February 2019, the present study was conducted at Shaheed Ghazi Al-Hariri hospital for surgical specialties in the Medical city, Baghdad. Data concerning age, gender and cancer's grade were taken from hospital registers. Histopathological grading depended upon the review of hematoxylin-eosin stained slides, and assigned as: (stage 1) for the well differentiated, (stage 2) for the moderately differentiated and (stage 3) for the poorly differentiated cancers. In our study, (105) subjects were enrolled, (55) of them (40 male and 15 female) whose ages ranged (30–80) years and histopathologically confirmed to have colorectal cancer, while (50) individuals (30 male and 20 female) whose ages ranged

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Table 1: Patient and control distribution in accordance with age

Age group no.	*Patient group*		*Control group*		*p-value*
	%	*No.*	*%*		
≤ 40 year	15	25	20	40	
> 40 year	40	75	30	60	
Total	55	100.0	50	100.0	
Mean ages (+SD)	(54.3 ± 14.8)		(45. 2 + 13.5)		0.680 NS
Age ranges	(30–80) yrs		(35–65) yrs		
Gender Males No. (%)	36 (65.5%)	30(60%)			p=0.758 NS
Gender Female No. (%)	19 (34.5%)	20(40%)			
Total No.	55	50			

NS: Non-significant

from (35–65) years and were not documented to have cancer and attended the same hospital as a control group. From CRC patients and healthy control individuals, 5 mL of venous blood specimens were withdrawn and placed in a sterile tube for serum isolation and kept at -70°C until use.

Molecular Detections of mi-RNA-221

In accordance with guidelines of (Taq-Man™ Micro-RNA assay, SM, Applied biosystems, USA), the mi-RNA-221 was detected.

Measurement of IL-10 by ELISA

The measurement of serum IL-10 levels of patients and healthy controls were managed according to cusabio Enzyme-linked immunosorbent assay (ELISA) kit.

Statistical Analysis

The statistical package (SPSS-25 program) was used for data analysis. Data were introduced as a (mean ± SD), percentage, median, and standard errors. Chi-square testing was used to assess the qualitative relation. The (≤0.05) *p*-value was regarded significant.

RESULTS

Demographic Characteristics of Study Groups

This study investigated (55) CRC patients for miRNA-221 and IL-10 levels and compared with (50) apparently healthy persons as a control group. The ages of colorectal cancer patients ranged from (30–80) years, with the mean age (54.3 ± 14.8) yrs. for patients, while mean ages for the healthy controls (45.2 + 13.5) years. No statistical significant variations ($p < 0.05$) among groups in regard to age. Males constituted 36 (65.5%)of the CRC patients, whereas the remaining 19 (34.5%) were females. Gender distribution in the control group showed that 30 (60%) were males and 20 (40%) were females. Results of gender distribution revealed non-significant differences ($p < 0.05$) between the patients and the controls.

Table 2: Serum Mi-RNA-221 levels among the study group

Mi-RNA-221	*Serum CRC*	*Controls*
Means	12.19	3.42
Standard Error of Mean	3.96	0.81
Median	7.24	1.32
CRC *vs* Control	0.001	

Mi-RNA-221 Level in the Study Group

The mean of log fold change value miR-221 gene expressions in the sera of CRC patient was higher when compared with the control group (12.19 *vs* 3.42, respectively) with a statistically significant variation; p = (0.001) as shown in the Table 2.

Levels of IL-10 in the Studied Groups

The close-fisted regard up of IL-10 in CRC patients reached (22.71 ± 4.34) pg/mL in comparison with (7.48 ± 2.76) pg/mL in healthy control group. A statistical significant variation in mean IL-10 concentration was found between CRC patients and the controls (P=0.001) as illustrated in Table 3.

Associations between CRC Grades and Studied Parameters

Results of the present study showed that the well-differentiated carcinomas had been observed in 16(32%) cases, moderately differentiated in 31(62%) cases and 3 (6%) poorly-differentiated tumors were noticed among only 3 CRC cases as seen in table 4. There was no statistically significant variation (p > 0.05) among CRC groups in accordance with cancer's grading. The highly-folded 221 concentrations reached (13.03 pg/mL) in poorly differentiated CRC patients compared with 9.23 and 12.19 in the well and moderately differentiated CRC patients,

Table 3: Serum levels of IL-10 in the studied groups

IL-10	*Serum CRC*	*Control*
Mean	22.71	7.48
Standard Error of Mean	4.34	2.76
Median	14.76	5.28
CRCs vs Controls	0.001	

Table 4: Associations between CRC gradings and the studied parameters

Marker		*Well differentiated 16(32%)*	*Moderately differentiated 31(62%)*	*Poorly differentiated 3(6)%*	*p-value*
Folding 221	Means folding (± SD)	9.23 ± 11.87	12.19 ± 16.83	13.03 ± 5.23	0.645
IL-10 (pg/mL)	M ± SD(pg/mL)	19.5 ± 20.3	21.2 ± 27.5	26.6 ± 38.7	0.619

respectively with (p=0.645). At the same time, IL-10 reached (19.5 ± 20.3) pg/mL in poorly-differentiated when compared with (21.2 ± 27.5) pg/mL and (26.6 ± 38.7) pg/mL among well and moderately differentiated cancer grading, respectively (p = 0.619).

DISCUSSION

Colorectal carcinoma (CRC) is one of the various public and threatening tumors in humans. The diagnosis of colorectal cancer rises reasonably after 40 years of age, with a progressive increase after age of 40 years, with an obvious growing after the age 50 years Ries *et al.,*[8] (2008). Over (90%) events of colorectal cancer develop in people with ages of 50 years or more (the National Institute of Health and National Cancer Institute, 1979).[9] The current results concerning age were in harmony with the Iraqi study conducted by Al-Hummadi[10] and Tahir[11] found that the mean age of CRC patients was (50) years, while Abdul Ghafoor[12] recently demonstrated that their mean age was (53) years. The mi-RNA-221 investigation involved in CRC pathogenicity as a diagnosis biomarker. The results in our study showed that log-folded miR-221 among the colon cancer patients was significantly higher in comparison with the healthy controls similarly to the results reported by.[13] In a study[14] that levels of expression of miRNA-221-3p, miRNA-342-3p and miRNA- 491-5p influenced the detection of early colon carcinoma patients. A further study reported that the deregulation of miRNA-221 indicated the growth prognosis and confirmed an oncogenic characteristic in CRC patients.[15] While another study found that high miRNA-221 expression enhanced CRC metastasis and cell invasion and through the targeting of RECK84-86 and CDKN1C. Besides, the miRNA-221-3p is up-regulated in colon carcinoma and is a pivotal prognostic biomarker for cancer detection.[16] A study by Yuan *et al*.[17] observed that the miRNA-221 passenger strand is low-expressed in CRC patients and promoted tumor suppressor-like characteristics. The Cai, *et al*[18] showed that the higher representation of miRNA-221 had low durability rates, therefore, miRNA-221 could assist as a molecular marker for the prognosis of colon cancer.

Interestingly, the analysis reported that highly-expressed miRNA-221 for poor prognosis of colon cancer patients may be considered as a risk factor for CRC and this finding coincides with a prior study that revealed a negative correlation with the overall durability time of patients as the lower the miRNA-221 expression level the greater the overall survival time, and vice versa.[19] Hence, in this study, it was observed that S. IL-10 among CRC patients showed significantly higher values compared to the control group and was higher in patients with poor prognoses. Several observations found that S. IL-10 levels are increased during the CRC progression and increased S. IL-10 levels were related to poorly CRC patient survival.[19,20] The Galizia *et al*.[21] study showed that a higher circulating (IL-10) level was related to worse results among CRC patients. Furthermore, Wang *et al*.[22] reviewed a prognosis usage of IL-10 serum levels among patients with CRC, enduring surgery and proved that circulating IL-10 levels were among the variables affecting disease-free survival rate. The interpretation by Zhao *et al* reported a tight correlation between high levels of S. IL-10 with poor prognosis among patients with carcinoma, which agrees with our findings.[23]

CONCLUSION

The high level of miRNA-221 and IL-10 can have major roles among patients with CRC. In contrast, those with high miRNA-22 expressions and IL-10 levels were associated with poor prognosis. Thus, they can serve as biomarkers for the prognosis of colon cancer.

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