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Research Article

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Dual Role of miR-150 in Colorectal Cancer Progression: A Quantitative Real-Time PCR Study

Maryam Jasim Hasan[1], Maryam Mohammed Bakheet[1], Hiba Mohssin Ali[1], Mohanad Kareem Aneed Al-Saedi[2], Maryam Qasim Mohammed*[1]
[1]*Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq;* [2]*Al-Nahrain University, Baghdad, Iraq*

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Abstract

Background: Colorectal cancer (CRC) is the second leading cause of cancer-related deaths and the third most common cancer globally. Non-coding RNAs, including miRNAs, regulate the tumor microenvironment of CRC and play key roles in its progression. Abnormal levels of miR-150 are associated with cancer cell migration, invasion, and angiogenesis. The expression of miR-150 in fundamental biological processes is influenced by cancer cell expression profiles. ***Objectives***: The present study aimed to estimate the level of *miR-150-3p* and *miR-150-5p* expression in CRC patients. ***Methods***: The study involved 50 CRC patients and 50 control participants. Liver enzyme levels and renal functions were evaluated. miRNA was extracted from blood samples, followed by complementary DNA synthesis. The gene expression levels of miR-150-3p and miR-150-5p were measured. ***Results***: The study found a significant increase in ALT and ALP levels in CRC patients, with highly significant differences of 0.01 and 0.001, respectively, while AST levels showed no significant difference between groups. Urea and creatinine levels also showed no significant differences. Gene expression analysis revealed that miR-150-3p levels were similar between patients and controls (non-significant fold change of 1.161), whereas miR-150-5p expression was reduced in CRC patients (fold change of 0.88). ***Conclusions***: miR-150-5p is downregulated in CRC patients, highlighting its potential as a diagnostic biomarker. However, no significant changes were observed in miR-150-3p levels. The results may be affected by factors such as treatment protocols and disease stages.

Keywords: Colorectal cancer, Gene expression, *miR-150-3p*, *miR-150-5p*, MicroRNA, Pearson correlation.

الدور المزدوج ل miR-150 في تطور سرطان القولون والمستقيم: دراسة كمية في الوقت الفعلي PCR

الخلاصة

الخلفية: سرطان القولون والمستقيم (CRC) هو السبب الرئيسي الثاني للوفيات المرتبطة بالسرطان وثالث أكثر أنواع السرطان شيوعا على مستوى العالم. تنظم الحمض النووي الريبي غير المشفرة، بما في ذلك miRNAs ، البيئة المكروية للورم في CRC وتلعب أدوارا رئيسية في تطوره. ترتبط المستويات غير الطبيعية من miR-150 بهجرة الخلايا السرطانية والغزو وتكوين الأوعية. يتأثر التعبير عن miR-150 في العمليات البيولوجية الأساسية بملف تعبير الخلايا السرطانية. **الأهداف**: هدفت الدراسة الحالية إلى تقدير مستوى تعبير *miR-150-3p* و *miR-150-5p* في مرضى سرطان القولون والمستقيم. **الطرائق**: شملت الدراسة 50 مريضا بسرطان الخلايا المزمنة و 50 مشاركا في التحكم. تم تقييم مستويات إنزيمات الكبد ووظائف الكلى. تم استخراج miRNA من عينات الدم، متبوعا بتخليق الحمض النووي التكميلي. تم قياس مستويات التعبير الجيني ل miR-150-3p و miR-150-5p. **النتائج**: وجدت الدراسة زيادة معنوية في مستويات ALT و ALP في مرضى CRC ، مع اختلافات كبيرة للغاية تبلغ 0.01 و 0.001 على التوالي، بينما لم تظهر مستويات AST فرقا كبيرا بين المجموعات. كما لم تظهر مستويات اليوريا والكرياتينين أي فروق ذات دلالة إحصائية. كشف تحليل التعبير الجيني أن مستويات miR-150-3p كانت متشابهة بين المرضى والضوابط (تغيير طية غير كبير قدره 1.161)، بينما انخفض تعبير miR-150-5p في مرضى CRC (تغيير الطية 0.88). **الاستنتاجات**: يتم تقليل تنظيم miR-150-5p في مرضى CRC، مما يسلط الضوء على إمكاناته كمؤشر حيوي تشخيصي. ومع ذلك، لم يلاحظ أي تغييرات كبيرة في مستويات miR-150-3p. قد تتأثر النتائج بعوامل مثل بروتوكولات العلاج ومراحل المرض.

* ***Corresponding author***: Maryam Q. Mohammed, Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq; Email: maryamqasim.ms.c.mic.2020@uomustansiriyah.edu.iq
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INTRODUCTION

The second most common cause of cancer-related deaths and the third most common cancer is colorectal cancer (CRC). The surrounding environment and colorectal tumors collaborate and exchange information, establishing the tumor microenvironment (TME) associated with CRC [1]. CRC continues to be the second leading cause of cancer-related mortality, accounting for around 10% of all newly diagnosed cancers worldwide [2]. The cellular environment whereby a particular microRNA is expressed determines whether it functions as an oncogene or a tumor suppressor. MicroRNA expression is frequently modulated in CRC, and the expression patterns of these microRNAs are linked to

the disease's diagnosis, prognosis, and treatment effectiveness [3,4]. The majority of investigations concern coding or non-coding genes, and CRC is thought to have a very complicated process at the molecular level; numerous miRNAs, including miR-150, have the potential to function as either tumor suppressors, thereby preventing tumor development and metastasis, or oncogenes, stimulating tumor development and invasion [5,6]. The miRNAs can function as a CRC promoter or suppressor and are crucial for some biological processes, depending on the cell milieu in which the information is expressed [7,8]. According to recent research, miRNAs perform a vital role in controlling both cell development and function and have distinctive expression profiles in both innate and adaptive immune system cells. Additionally, it was demonstrated that abnormal expression of miRNAs can contribute to immune system pathological disorders such as cancer and autoimmunity [9,10]. Likewise, miRNAs can be useful as prognostic and diagnostic indicators of disease in terms of severity [11]. The miR-150 was thoroughly investigated in both varieties of cancer and normal physiology. MiR-150 is one of the most down-regulated miRNAs in an assortment of cancers, including colorectal, pancreatic, liver, and neck squamous cell carcinoma, according to miRNA expression studies. It is hypothesized that in the previously mentioned malignancies, miR-150 functions as a tumor suppressor gene. In metastatic cells, aberrant expression of miR-150 was identified, which is closely associated with angiogenesis, invasion, and migration of cancer cells. It was also demonstrated that miR-150 tends to modulate the epithelial-mesenchymal transition (EMT) process, which is a crucial step in the migration and metastasis of tumor cells. Levels of miR-150 and putative target genes in the basic biological process can vary depending on the type of cancer cells and their gene expression profile [12]. Improving insight into the molecular mechanisms behind CRC metastasis is essential to enhancing therapeutic approaches for this illness and eventually enhancing patient survival rates. While the mechanisms mediated by miRNAs in colorectal carcinogenesis are well recognized, the mechanisms influencing metastasis remain to be explored [13]. The *miR-150* gene in humans is located on the 19q13.33 chromosome with one exon (Gene ID: 406942). MiR-150 targets cell receptors that participate in apoptosis and proliferation, thereby controlling the growth of cells. By directly and adversely affecting ZEB1, miR-150 may have tumor suppressor properties and influence the proliferation and invasion of epithelial ovarian cancer cells. A low expression of miR-150 was observed in colorectal cancer. One proposed indicator of the course of the HIV/AIDS epidemic and therapies is microRNA-150 [14,15]. Alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) have been demonstrated to represent the body's immunological and nutritional health along with liver metabolism, biosynthesis, and detoxifying capacity. Particularly absent is a thorough assessment of liver function markers associated with the risk of cancer. Previous studies that assessed a few chosen liver function markers concerning CRC risk provided only conflicting results [16]. It has been demonstrated that miR-150 can regulate the TME process, which is a crucial step in the migration and metastasis of tumor cells. Levels of miR-150 and presumed target genes in the basic biological process can vary concerning the type of cancer cells and their gene expression profile. The current study aimed to demonstrate the expression levels of *miR-150-3p* and *miR-150-5p* in individuals with colorectal cancer and assess certain liver and renal function tests.

METHODS

Study design and setting

In the current study, one hundred participants were enrolled. The samples were collected from Oncology Hospital, Medical City Department, Baghdad, Iraq, from 5 January 2023 to 20 October 2023. Fifty of them were patients with colorectal cancer (CRC) undergoing chemotherapy with ages ranging between 28 and 75 years (58% male and 42% female), while the remaining fifty individuals ranged in age from 25 to 66 years (54% male and 46% female), as apparently healthy controls. The mean and standard deviation for the CRC patients and the healthy controls were 57.51±9.5 and 55.32±8.6, respectively.

Inclusion criteria

Individuals diagnosed with colorectal cancer (CRC) at varying stages of disease progression, including those undergoing chemotherapy, will be included in the patient group, while the control group will consist of apparently healthy individuals with no prior diagnosis of any type of cancer.

Exclusion criteria

Individuals with a history of any other cancer type will be excluded from the patient group. Similarly, individuals in the control group will be excluded if they have a family history of cancer to ensure a comparison with those who are entirely free of oncological predispositions.

Biochemical investigation

A liver function test was performed to evaluate alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP), while a renal function test included urea and creatinine measured using a biochemical analyzer (Thermo Indiko Plus, Thermo Scientific, USA).

MicroRNA extraction

The miRNA was promptly extracted from whole blood samples preserved in Trizol using the Easy Pure® miRNA Kit (ER601-01, TransGen Biotech

Company, China). MiRNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) to ensure suitability for subsequent RT-qPCR analysis. The miRNA concentrations ranged from 48 to 67 ng/µl, with absorbance readings around 2.0, indicating high purity.

Complementary DNA synthesis

The methodology in EasyScript® One-Step gDNA Removal and cDNA Synthesis Super Mix was employed to perform the cDNA synthesis (TransGen Biotech, China) with Cat number (AE311-02). Reaction mix, random primer, anchored oligo (dT), genomic DNA remover, RNase-free water, reverse transcriptase, and the extra miRNA were the items needed to conduct cDNA synthesis. The thermal cycler's steps were illustrated by the following three stages: 25°C for 10 minutes for random primer binding, 42°C for 15 minutes for oligo dT binding and RT-enzyme activation, and 85°C for 5 seconds to inactivate the enzyme. The primer sequences (Table 1) that were used in this study were recommended by Alpha DNA Company, Canada, in a lyophilized form, where Nuclease Water (NFW) was used to dissolve them. Thus, a stock solution was obtained with a 100 picomole concentration, and then NFW was added to reach a concentration of 10 picomoles by preparing a solution termed as a work solution.

Table 1: Primer sequence used in the current investigation

Gene	Primer Sequence (5′→3′ direction)	Primer size (bp)	Tm (°C)	Ref.
miR-150-3P	CTGGTACAGGCCTGGGGGACA	21	70	[17]
miR-150-5P	TCTCCCAACCCTTGTACCAGTG	22	68	[18]
U6	AGAGAAGATTAGCATGGCCCCT	22	60	[19]
miR universal reverse primer	GCGAGCACAGAATTAATACGAC	22	58	[20]
Universal miR	CAGGTCCAGTTTTTTTTTTTTTTVN	26		[21]

Gene expression levels

The process was performed in a reaction volume of 20 µl following the manufacturer's instructions. 10 µl of master mix (TransStart® Top Green qPCR Super Mix, TransGen, biotech. AQ131-01), 3 µl of cDNA, 1.0 µl of forward primer, 1 µl of reverse primer, and 5 µl of nuclease-free water were identified to be the volume of components required to prepare the appropriate amount of reactions. The gene's expression stages and temperature were found to be 94°C for 1 minute, which acts as a hold temperature to activate the polymerase; subsequently, a 35–40 cycle consisted of 94°C for 10 seconds (denaturation), 58°C for 15 seconds (annealing), and 72°C for 20 seconds as (extension phase).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) was utilized to calculate the mean and standard error of biochemical parameters. Data are presented as mean ± standard error (SE). The ΔCt and ΔΔct calibrator methods were employed for estimating the fold of miR-150 and their internal control expression of genes [22–24]. Statistical significance was determined using *p*-values <0.05.

RESULTS

Table 2 provides a detailed overview of the clinical and demographic characteristics of fifty CRC patients, offering insights into their age distribution, BMI, disease stage, and chemotherapy regimens. The mean age of the cohort is 57.51±9.5 years, with an age range spanning 28 to 75 years, reflecting a diverse patient population.

Table 2: Clinical and demographic characteristics of CRC patients

Parameters	Type	Mean±SE	n(%)
Age	--	57.51±9.5	--
Age range	--	(28-75)	--
BMI (Kg/m^2)	--	24.69±3.53	--
Male	--	--	29(58)
Female	--	--	21(42)
Stage of Disease	Stage I	--	2(4)
	Stage II	--	8(16)
	Stage III	--	23(46)
	Stage IV	--	17(34)
Chemotherapy	Folflox	--	10(20)
	Xeloda	--	23(46)
	Oxaliplatin	--	4(8)
	Avastin	--	13(26)

Values are expressed as number and percentage or mean±SE.

The average body mass index (BMI) is 24.69±3.53 kg/m², suggesting a relatively normal BMI range within the group. In terms of gender distribution, 29 patients (58%) are male, while 21 patients (42%) are female. The distribution of disease stages among CRC patients highlights a variation in the severity of the condition at the time of diagnosis. A total of 2 patients (4%) were diagnosed at Stage I, indicating an early stage with localized disease. 8 patients (16%) were diagnosed with Stage II, where the disease has progressed but remains confined to the colon or rectum. The majority of patients, 23 individuals (46%), were diagnosed at Stage III, reflecting advanced disease with regional lymph node involvement. Furthermore, 17 patients (34%) were diagnosed with Stage IV, indicating metastatic disease where the cancer has spread to distant organs. Regarding the chemotherapy regimens administered,

the table reveals a diverse treatment approach. 10 patients (20%) were treated with Folflox, a combination therapy commonly used for advanced colorectal cancer. 23 patients (46%) received Xeloda®, an oral chemotherapy drug frequently prescribed for both early and advanced stages of the disease. Additionally, 4 patients (8%) were treated with oxaliplatin, a platinum-based chemotherapy agent often combined with other drugs. Finally, 13 patients (26%) were treated with Avastin, a targeted therapy designed to inhibit tumor angiogenesis and often used in combination with other chemotherapeutic agents. Liver enzyme levels, including alkaline phosphatase (ALP), alanine transaminase (ALT), and aspartate transaminase (AST), were assessed. The results revealed significantly elevated levels of ALT in patients (16.95±2.1) compared to controls (11.06±0.99), with a significant difference (p= 0.016). Similarly, ALP showed elevated levels in patients (80.1±2.1) compared to controls (66.6±3.2), with a highly significant difference (p= 0.001). In contrast, AST levels exhibited no significant difference (p= 0.9) between patients (22.85±2.6) and controls (23.19±1.6) (Figure 1).

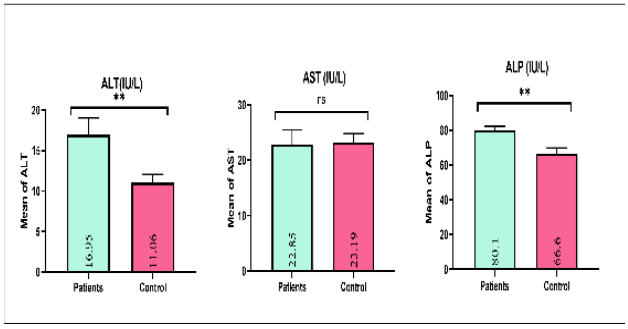

Figure 1: Comparison of liver function test parameters between CRC patients and controls.

The levels of urea and creatinine, key biomarkers for assessing kidney function, were measured and compared between the patient and control groups. The analysis indicated no statistically significant differences in either parameter. Specifically, urea levels were slightly lower in patients (25.49±1.5 mg/dL) compared to controls (28.93±1.3 mg/dL), though this difference was not significant (p= 0.1). Similarly, creatinine levels were comparable between patients (0.87±0.04 mg/dL) and controls (0.86±0.02 mg/dL), with no statistically significant difference (p= 0.8) (Figure 2).

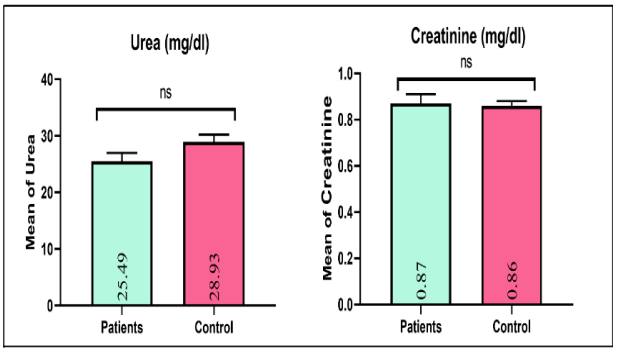

Figure 2: Comparison of renal function parameters between CRC patients and controls.

The gene expression levels of *miR-150-3p* and *miR-150-5p* genes were estimated using a real-time PCR device, and then the results were calculated using two equations: the $2^{-\Delta Ct}$ method and the ΔΔCt calibrator method. Gene expression analysis revealed that *miR-150-3p* expression levels were comparable between patients and healthy controls. The mean cycling threshold (Ct) for patients was 28.566, while the mean Ct for healthy people was 28.658, with a non-significant fold change of 1.161. Conversely, a decrease in *miR-150-5p* expression was observed in patients compared to controls, where the mean Ct was 25.844, while the mean Ct for the control was 25.543, with a fold change of 0.88 (Table 3).

Table 3: Gene expression levels of miR-150-3p and miR-150-5p in CRC patients and controls using the $2^{-\Delta Ct}$ Method

Groups	Means Ct of gene	Means Ct of GAPDH	ΔCt	$2^{-\Delta Ct}$	Exp group/Control group	Fold expression	*p*-value
miR-150-3P							
Patient	28.566	17.385	11.181	0.0004	0.0004/0.0003	1.161	0.4
Control	28.658	17.262	11.396	0.0003	0.0003/0.0003	1.00	
miR-150-5P							
Patient	25.844	17.385	8.459	0.0028	0.002/0.003	0.88	0.8
Control	25.543	17.262	8.281	0.0032	0.003/0.003	1.00	

Table 4 shows the fold expression of the miR-150-3p and miR-150-5p genes in both CRC patients and controls, calculated using the ΔΔCt calibrator method. The data is presented with details for each group and gene. The analysis of miR-150-3p expression in CRC patients and controls revealed no significant difference between the two groups.

Table 4: Fold expression of miR-150-3p and miR-150-5p genes in CRC patients and controls using the ΔΔCt calibrator method

Groups	Means Ct of gene	Means Ct of GAPDH	ΔCt	Δct calibrator	ΔΔct	2^-ΔΔct	Fold expression	*p*-value
miR-150-3P								
Patient	28.566	17.385	11.181	12.481	-1.3	2.462	1.161	0.4
Control	28.658	17.267	11.396	12.481	-1.085	2.121	1.00	
miR-150-5P								
Patient	25.844	17.385	8.459	9.198	-0.739	1.669	0.88	0.8
Control	25.543	17.262	8.281	9.198	-0.917	1.888	1.00	

In the patient group, the mean Ct value for miR-150-3p was 28.566, with a ΔCt of 11.181 and a fold change of 1.161. The *p*-value for this gene expression was 0.4, indicating no significant difference when compared to the control group. In the control group, the mean Ct for *miR-150-3p* was 28.658, with a ΔCt of 11.396 and a fold change of 1.00, serving as the baseline for comparison. Similarly, for *miR-150-5p*, the fold change in the patient group was 0.88, while in the control group, it was 1.00. The p-value for *miR-150-5p* expression in the patient group was 0.8, which also indicated no significant difference between patients and controls. In both cases, the ΔCt calibrator value remained consistent across both groups, confirming that there were no substantial differences in the expression levels of these miRNAs in CRC patients compared to controls. In conclusion, the data suggests that there is no significant variation in the expression of both *miR-150-3p* and *miR-150-5p* between the CRC patient and control groups based on the ΔΔCt method. Figure 3 illustrated the amplification plot for *miR-150-5p* was generated using qPCR samples from all investigation groups, with CT values ranging from 24.69 to 29.09.

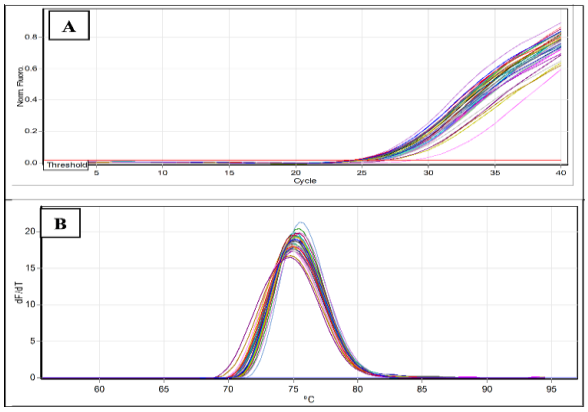

Figure 3: **A**) The amplification plot for the *miR-150-5p* gene was generated with qPCR samples. **B**) The gene dissociation curves that displayed melting temperatures confirmed the specificity of the PCR products.

These values reflect variations in gene expression, where lower CT values indicate higher expression levels and higher CT values suggest lower expression. This data provides insight into the relative expression of *miR-150-5p* across different groups. Additionally, gene dissociation curves for miR-150-5p showed melting temperatures (Tm) ranging from 73°C to 79°C, confirming the specificity of the PCR products. These results were obtained using the Qiagen Rotor-Gene Q qPCR device, ensuring reliable amplification and dissociation analysis. Figure 4 shows the amplification plot for *miR-150-3p* was generated using qPCR samples from all research groups, with CT values ranging from 27.6 to 29.65. These values indicate variations in gene expression, where lower CT values suggest higher gene expression and higher CT values indicate lower expression levels. Gene dissociation curves for miR-150-3p obtained through qPCR showed melting temperatures (Tm) ranging from 81°C to 86°C, confirming the specificity of the PCR products. These images were captured using the Qiagen Rotor-Gene Q qPCR device, ensuring accurate and reproducible amplification and dissociation analysis.

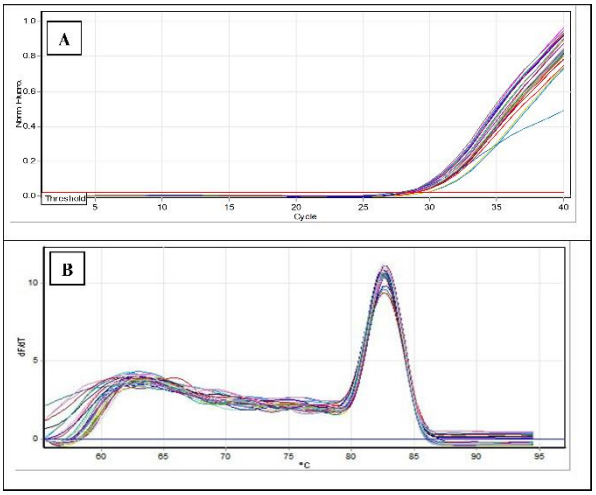

Figure 4: **A**) Amplification of the *miR-150-3p* gene was visualized using qPCR samples from different research groups. **B**) Gene dissociation curves for miR-150-3p were obtained using qPCR, with the melting temperature observed.

The Pearson correlation analysis (Figure 5) conducted in this study revealed a moderate positive correlation between the expression levels of *miR-150-5p* and *miR-150-3p*.

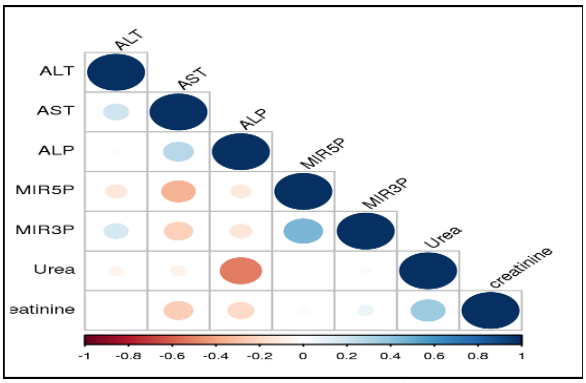

Figure 5: Pearson Correlation Analysis of Study Parameters Including ALP, ALT, AST, Urea, Creatinine, miR-150-3p, and miR-150-5p.

The correlation coefficient (r^2) for these two miRNAs was 0.454, which suggests a moderate degree of association between their expression patterns. The p-value of 0.003 indicates that this correlation is statistically significant. This suggests that the expression of *miR-150-5p* and *miR-150-3p* is linked, possibly due to their shared biological roles or regulatory pathways in the context of the studied conditions. On the other hand, the analysis showed no significant correlations between the expression levels of *miR-150-5p* or *miR-150-3p* and liver enzymes, including AST and ALT, or renal function indicators like urea and creatinine. These findings suggest that, within this study, the expression of these miRNAs does not appear to be directly related to liver or kidney function as measured by these specific biomarkers. Interestingly, a negative correlation was observed between urea levels and ALP levels, with an r^2 of -0.522 and a *p*-value of 0.001. This indicates a moderate inverse relationship

between these two factors, meaning as urea levels increase, ALP levels tend to decrease, or vice versa. A *p*-value of 0.001 indicates that this negative correlation is statistically significant, pointing to a potential relationship between renal and hepatic functions that warrants further exploration in future studies.

DISCUSSION

In patients with advanced colorectal cancer, when metastasis to other organs, particularly the liver and kidneys, is more prevalent, evaluation of liver and kidney function is crucial [25]. The kidney and liver play vital roles in detoxification and metabolism, and miR-150 is a key regulator of cellular processes such as proliferation, differentiation, and cell death in both organs. Research has established a significant relationship between miR-150 expression levels and the health of these organs. Alterations in miR-150 expression have been linked to the development of kidney fibrosis, liver diseases, and various inflammatory conditions. Furthermore, treatments for colorectal cancer, including chemotherapy and radiation, can have adverse effects on liver function as short-term side effects. In some cases, elevated liver enzymes and an increased risk of colon cancer may be attributed to an undiagnosed underlying disease that affects multiple organs. miR-150, particularly its 5p and 3p isoforms, serves as a crucial regulator of gene expression across different tissues, including the liver and kidneys. Dysregulation of miR-150 levels has been associated with various hepatic and renal disorders, including hepatitis, hepatocellular carcinoma, chronic kidney disease, and other related conditions. The precise biochemical mechanisms through which miR-150 contributes to these disorders are complex and involve intricate interactions with multiple genes and cellular processes. These interactions may influence critical pathways related to inflammation, fibrosis, cell apoptosis, and tissue regeneration, which collectively impact the progression and severity of these diseases [26–28]. The observation of elevated liver enzymes in the blood may be a sign of inflammation or injury to the liver. Liver enzymes are proteins that promote and accelerate particular chemical processes in the human body. Furthermore, several conditions affecting the colon, such as irritable bowel syndrome (IBS), have been shown to elevate liver enzymes. However, further research must be conducted to verify this association [29]. The elevated levels of ALT and ALP enzymes observed in patients suggest that liver function is adversely affected when a defect occurs in the colon, indicating a potential link between colorectal cancer (CRC) and liver dysfunction. However, no significant differences in renal function, as measured by urea and creatinine levels, were found between the two groups in this study. This suggests that CRC may not significantly disrupt renal function in the early stages. To further validate these findings, future studies should focus on exploring the correlation between liver enzymes and renal function, particularly by increasing the sample size and focusing on more advanced disease stages (Stage III and IV) [30,31]. According to the latest findings, miRNAs play essential functions in controlling both cell development and function while exhibiting distinct expression profiles in both innate and adaptive immune system cells. Likewise, it was demonstrated that abnormal expression of miRNAs can contribute to immune system pathological disorders such as cancer and autoimmunity. Additionally, miRNAs can be useful as prognostic and diagnostic indicators of disease in terms of severity [32,33]. Aberrant miR-150 expression levels are consistently detected in metastatic cancer cells, correlating with enhanced migratory, invasive, and angiogenic capacities. The miRNA's ability to regulate epithelial-mesenchymal transition (EMT), a pivotal process in tumor cell metastasis, has been extensively studied. Notably, miR-150 levels and the identity of its target genes in fundamental cellular processes can vary significantly based on the specific cancer type and its unique gene expression profile. Furthermore, the intricate interplay between miR-150 and other non-coding RNAs, such as long non-coding RNAs and circular RNAs, can profoundly influence the behavior of metastatic cells. Given its significant role in cancer metastasis, miR-150 emerges as a promising therapeutic target for the prevention and treatment of metastatic disease [12]. Depending on the cancer cell type and gene expression profile, levels of miR-150 and potential target genes in the fundamental cellular process can be different [34]. The current study revealed that the low level of gene expression *miR-150-5p* in CRC patients is a good indicator for diagnosing the disease, while the absence of significant differences between the two groups of the current study in gene expression *miR-150-3p* may deleteriously be afflicted by the low gene expression of the *miR-150-5p* and perhaps owing to the limitations of our investigation in terms of the number of samples and not following all the standards when collecting samples in terms of the stage of the disease and the type of chemotherapy, due to the difficulty of dealing with patients during the process of obtaining the sample as a consequence of the patient's health and psychological condition. Supportive to Min and their colleagues, miRNAs identified in exosomes, including *Let-7b-3p*, *miR-139-3p*, *miR-145-3p*, and *miR-150-3p*, have been validated in large cohorts comprising 134 participants and can be utilized as biomarkers for early CRC monitoring. A potential biomarker to identify CC early on has been proposed, based on the unique miRNA profile of circulating small extracellular vesicles (sEVs) enriched fractions in CC patients [35]. Regarding the growth and propagation of tumor cells, miR-150 serves as one of the miRNAs that have a vital role. Numerous pieces of evidence indicate that miR-150 has dual effects on cancerous cells, having the ability to prevent the development of tumors or promote malignancies. These contradictory effects on metastatic cells can be clarified by reviewing the function of miR-150 and its interactions with regulatory and signaling

networks. Metastatic cells that are closely associated with the migration, invasion, and angiogenesis of cancer cells exhibit aberrant levels of miR-150 [12]. The previous study investigated the role of miR-150 in CRC and demonstrated that miR-150 expression was decreased in CRC tissues and cells. Overexpression of miR-150 inhibited CRC cell growth and proliferation. Mechanistically, miR-150 targeted β-catenin, leading to decreased β-catenin levels and downstream targets. In vivo experiments confirmed the tumor-suppressive role of miR-150. These findings suggest that miR-150 could be a potential therapeutic target for CRC [5]. According to previous investigations, for patients with CRC, exosomal *miR-150-5p* functions as a prospective non-invasive diagnostic and risk assessment tool. In colorectal cancer, miR-150-5p was related to TP53 repression and has a favorable prognostic characteristic. This interaction tends to serve a role in the processes of invasion, migration, and apoptosis inhibition [5,36]. Correspondingly compatible with previous findings conducted by Koshizuka and colleagues, the analysis of miRNA expression patterns by RNA sequencing revealed that both pre-miR-150 strands (*miR-150-5p* and *miR-150-3p*) were significantly downregulated in head and neck squamous cell carcinoma (HNSCC) cells and that these miRNAs functioned as antitumor miRNAs in HNSCC cells that are consistent with current investigations in terms of down-regulation [37]. Li and their team accomplished a study utilizing data from the Cancer Genome Atlas Colon Adenocarcinoma (TCGA-COAD). The investigators employed a comprehensive strategy of data mining, computational biology, and real-time reverse transcription PCR (qRT-PCR) experiments to determine the relative levels of miR-150-3p in colon cancer (CC) tissues and cells. The results indicated that miR-150-3p is downregulated in CC tissues and cell lines [38]. Ultimately, the results of Sur and their team's systematic review and meta-analysis on the diagnostic and prognostic significance of miRNA-150 in CRC indicate that miRNA-150 is not a statistically relevant indicator for the prognosis of patients with CRC. It's conceivable that this is since there weren't sufficient studies included in the meta-analysis to determine the prognostic significance. As previously indicated, low expression of miR-150 in CRC patients was established in other articles as a potential diagnostic and predictive biomarker. Ultimately, they disclosed that miR-150 may be useful as a diagnostic biomarker for patients with CRC, but little evidence was discovered regarding miR-150 for prognosis [5]. The previous study, which aligned with the current result, showed a significant inverse correlation was observed between miR-150 expression levels and CRC progression. In all three tissue groups (normal, adenoma, and cancer), a consistent decrease in miR-150 expression was evident as the degree of malignancy increased. Both in situ hybridization (ISH) and quantitative real-time PCR (qRT-PCR) analyses revealed significantly reduced miRNA-150 expression in tumor tissues compared to paired non-cancerous tissues, further supporting the association between *miR-150* levels and CRC. Furthermore, patients with low miR-150 expression in their tumors exhibited poorer overall survival and a diminished response to adjuvant chemotherapy compared to those with high miR-150 expression [39]. In targeting certain gene issues, Feng's study reveals that miR-150 plays a critical role in suppressing colorectal cancer tumorigenesis and progression. We demonstrate that miR-150 directly targets c-Myb, a key oncogene implicated in colorectal cancer [40], while He and their colleagues found that miR-150 expression was significantly decreased in CRC tissues and cell lines compared to normal controls. A negative correlation was observed between miR-150 and β-catenin expression. Overexpression of miR-150 in CRC cells inhibited cell viability, proliferation, and colony formation. Mechanistically, miR-150 directly targeted β-catenin mRNA, leading to decreased β-catenin protein levels and subsequent downregulation of downstream targets such as c-Myc and Cyclin D1. In vivo experiments confirmed the tumor-suppressive role of miR-150 in CRC. These findings suggest that miR-150 could be a potential therapeutic target for CRC [41]. According to a previous investigation, Li and their colleagues found that miR-150 was reduced in CRC samples, while a protein called iASPP was increased. When we increased miR-150 levels in cancer cells, we observed decreased cell growth, cell death, and reduced ability to spread. Conversely, when we reduced iASPP levels, we saw similar effects. We confirmed that miR-150 directly targets iASPP, meaning that miR-150 can regulate iASPP expression. These findings suggest that miR-150 plays a crucial role in suppressing CRC and may be a valuable biomarker for predicting patient outcomes [42], while Mahmoudivar and their colleagues establish that certain miRNAs were altered in the blood of people with CRC. Specifically, miR-150 was decreased. These changes could help doctors distinguish between people with and without CRC. Interestingly, miR-150 levels changed after surgery, suggesting they might also be useful for monitoring the disease [43]. Finally, through targeting genes essential for carcinogenesis and encouraging apoptosis, *miR-150*, in particular its 5p isoform, has been suggested as a tumor suppressor in colon cancer. The 3p isoform, on the other hand, has a more context-dependent function and may function as an oncogene as well as a tumor suppressor. The present study has not consistently found substantial differences in miR-150 expression between normal and malignant tissues, despite its potential importance.

Study limitations

The present study has several limitations that may be attributed to several factors. Firstly, the complex regulatory networks involving miR-150 may ambiguate different expression patterns, complicating the identification of consistent differences between normal and malignant tissues. Secondly, inter-individual variability in miR-150

expression levels may contribute to observed inconsistencies, making it challenging to establish constant expression levels across samples. Thirdly, the limited sample size may have reduced the statistical power of the study, potentially hindering the detection of significant differences. Lastly, the functional role of miR-150 may undergo dynamic modifications at various stages of disease progression, further influencing its expression patterns. These limitations highlight the need for larger, well-stratified studies to elucidate the precise biological role of miR-150 in malignancy.

Conclusion

This is the first study that investigates *miR-150-5p* and *miR-150-3p* expression levels in CRC patients from Iraq due to their crucial role in gene regulation across various body tissues and their dual role in both promoting and suppressing tumor growth and metastasis. The downregulation of *miR-150-5p* in CRC patients suggests its potential role as a diagnostic biomarker. However, *miR-150-3p* levels showed no significant difference among the study groups. Variation in expression levels may be influenced by factors such as treatment protocols and disease stage heterogeneity. Further studies are needed to validate these findings and establish *miR-150-5p* as a reliable clinical biomarker.

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Conflict of interests

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Data sharing statement

Supplementary data can be shared with the corresponding author upon reasonable request.

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