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Four microRNA gene polymorphisms are associated with Iraqi patients with colorectal cancer

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Abstract

Background Colorectal cancer (CRC) is a major cause to global cancer-related mortality. The development of colorectal cancer is linked to hereditary variables that exhibit variability.

Objectives The objective of this investigation was to assess the potential correlation between microRNA gene polymorphisms and colorectal cancer (CRC) throughout the Iraqi population.

Methods DNA samples were obtained from a cohort of 100 individuals diagnosed with the (CRC) disease, as well as 100 samples as control group. Four primers were designed to amplify four specific high-frequency variants found within microRNA molecules. These variants include Mir146a G\C, Mir423 A\C, Mir196a2, and Mir370. The genotyping of the PCR fragments was performed using the single-strand conformation polymorphism (SSCP) method, followed by direct sequencing of each genotype.

Results Genotyping experiments confirmed the variability of four targeted variants, namely Mir146a G\C, Mir 423 A\C, Mir196a2, and Mir370 tend to exhibit a significant association with (CRC). Individuals with Mir146a: GC and Mir 423 A\C genotype showed a possible association with the increased risk of (CRC), respectively ($P=0.001$; OD 0.50; CI 95% 0.33–0.76; $P=0.002$; OD 0.53; CI 95% 0.36–0.80). Individuals with Mir196a2: TT and Mir370 GG genotype exhibited a potential association with (CRC) ($P=0.017$; OD 0.44; CI 95% 0.22–0.86; $P\leq 0.001$; OD 0.24; CI 95% 0.11–0.50).

Conclusions The study reveals that single nucleotide polymorphisms (SNPs) in microRNA have a notable and distinct correlation with the heightened susceptibility to colorectal cancer (CRC).

Keywords MicroRNA, Variations, CRC, Mir196a2, Mir370

Background

Colorectal cancer (CRC) ranks as the third most prevalent form of cancer globally, with a reported incidence of over 1.84 million new cases in 2018. This places CRC behind only lung and breast cancers in terms of frequency [1, 2]. Colorectal cancer (CRC) has a higher prevalence in males compared to women, with an approximate difference of 30%. The incidence of CRC is generally low in adults below the age of 50, regardless of gender, but significantly rises with advancing age [3, 4]. Approximately 70% of people develop sporadic colorectal cancer (CRC) in the normal epithelial lining of the colon and rectum, without any hereditary predisposition [5]. Less than 10% of individuals diagnosed with colorectal cancer (CRC) exhibit a hereditary susceptibility, including conditions such as Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC) [6, 7].

The completion of the human genomic project has recently drawn the attention of biomedical researchers

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to single nucleotide polymorphisms (SNPs) as a potential factor in the development of colorectal cancer (CRC) [8, 9]. Single nucleotide polymorphisms (SNPs) are inherent variations in DNA sequences that distinguish them from gene mutations. These variations are present in the general healthy population at a minimum frequency of 1% [6]. Single nucleotide polymorphisms (SNPs) are present in nearly all genes at varying rates and have the potential to impact the protein products of genes, potentially leading to an increased vulnerability to cancer. Among the genes that are currently recognized to possess single nucleotide polymorphisms (SNPs), a subset of particular interest is constituted by those that encode for microRNAs (miRNAs). MicroRNAs (miRNAs) are a group of small, single-stranded noncoding RNAs that have significant involvement in the control of gene expression by translational repression or degradation of target mRNA. These miRNAs have a profound impact on essential activities in a wide range of physiological processes [10–12]. Dysregulation of microRNAs (miRNAs) has been implicated in a diverse array of human disorders and can exert influence on the initiation and advancement of tumors through modulation of proto-oncogenes and tumor suppressor genes [13, 14]. Current research findings indicate that the manifestation of microRNAs (miRNAs) is correlated with the progression or prognostication of colorectal cancer (CRC) [15, 16]. Hence, it is probable that microRNAs (miRNAs) possess significant use as biomarkers in colorectal cancer (CRC). The occurrence of single nucleotide polymorphisms (SNPs) has been seen in genes associated with the miRNA biogenesis pathway, including those involved in primary miRNA, pre-miRNA, and mature miRNA sequences [17].

One of the most commonly investigated single nucleotide polymorphisms (SNPs) connected with microRNA (miRNA) in cancer research is rs11614913, which is located in the pre-miRNA region of miR-196-a2. The association between a single nucleotide polymorphism (SNP) of Mir196a2 (rs11614913) and extended survival in Chinese persons diagnosed with lung cancer has been shown. This specific SNP influences the efficacy of Mir196a2 in binding to its target messenger RNA (mRNA) [18]. The study revealed a substantial association between the overexpression of Mir196 and the malignant advancement of gliomas, as well as unfavorable survival outcomes [19]. In recent times, two studies with conflicting findings were published, examining the association of rs11614913 with the susceptibility to colorectal cancer in the Chinese population. The first study reported a significant association between the T allele and an increased risk of colorectal cancer (odds ratio [OR] 1.320; confidence interval [CI] 1.056–1.649, P value = 0.014), while the second study found no significant association between the C allele and the risk of colorectal cancer (OR 1.065; CI 0.803–1.414, P value = 0.665) [20].

The Mir146a gene is situated on the 5q34 (14) region of the chromosome. According to a recent study, there is evidence suggesting that Mir146a functions as a regulator of both the innate and adaptive immune responses [21]. The presence of mir-146a variant alleles has been associated with an earlier onset of breast and ovarian cancer in patients, as these alleles are projected to target genes such as BRCA1 and BRCA2 [22]. The chromosomal location of Mir423 is on chromosome 17. It was observed in untreated HL-60 leukemia cells during tests that induced differentiation using 2-O-tetra-decanoylphorbol-13-acetate. This suggests that the mature miRNA plays a role in the processes of cellular differentiation [23]. The study conducted by [24] demonstrates that Mir423 plays a crucial role in stimulating cellular proliferation and controlling the G1/S transition in hepatocellular carcinoma (HCC) through its interaction with p21Cip1/Waf1.

The precise biological function of Mir370 in colon cancer and the molecular mechanism involved have not yet been clearly established. However, a recent study has provided evidence suggesting that Mir370 acts as a tumor suppressor in laryngeal squamous cell carcinoma (LSCC) by downregulating FoxM1 [25]. The down regulation of Mir 370 in ovarian cancer is attributed to hypermethylation, while the overexpression of Mir 370 has been found to limit ovarian cancer cell viability, decrease colony formation, and increase the susceptibility of ovarian cancer cells to cisplatin (cDDP). This effect is achieved through the direct targeting of Endoglin (ENG) by Mir 370, as indicated by previous research [26]. The findings of Wu et al. indicate that miR 370 has a significant impact on the proliferation of human prostate cancer cells. This effect is achieved through the direct suppression of the tumor suppressor FOXO1, which is known to be involved in crucial cellular processes such as cell growth, differentiation, apoptosis, and angiogenesis [27, 28].

Several studies have presented contradictory findings on the correlation between the aforementioned miRNAs' different genotypes and the risk of colorectal cancer (CRC). These discrepancies may be indicative of geographical and demographic factors influencing the outcomes. Regrettably, there has been a lack of research conducted in Iraq thus far about the incidence of these single nucleotide polymorphisms (SNPs) within our community, as well as their potential correlation with the risk of colorectal cancer (CRC). Enhanced comprehension of single nucleotide polymorphisms (SNPs) within microRNAs (miRNAs) may consequently enhance comprehension of disease causes and facilitate the development of robust risk-assessment models [29, 30].

Nevertheless, the identification of the role played by several microRNA single nucleotide polymorphisms (SNPs) in the development of colorectal cancer (CRC) remains unexplored within the Iraqi population. Given the aforementioned data, the objective of this study was to examine the potential correlation between four single nucleotide polymorphisms (SNPs) in microRNAs (miRNAs), specifically Mir146a, Mir423, Mir370, and Mir196a2, and the heightened susceptibility to colorectal cancer (CRC) within our population. Additionally, we sought to determine whether any association exists between these genetic variations and the clinicopathological characteristics of CRC subjects.

Material and methods

Study subjects

This study employed a case–control design and involved a sample of 100 patients who were diagnosed with colorectal cancer (CRC) and were admitted to the Middle-Euphrates Cancer Center in Najaf governorate and the Merjan Cancer Center in Babil governorate. The diagnosis of CRC was confirmed through histopathological examination. From April 2021 until March 2023, the individuals exhibited varying types of colorectal cancer (CRC). The individuals that were included in the study had a wide age range, spanning from 25 to 75 years old. The histological types, stages, and grades of the patients were observed at different stages of their treatment and clinical progression. The study included a total of 100 individuals, consisting of 65 males and 35 females. The age of the participants ranged from 32 to 72 years, with a mean age of 53 years. One hundred control subjects, who were healthy and matched in terms of age and sex, were enrolled from individuals visiting the hospital outpatient clinics and who did not have cancer. The clinicopathological information of both the patient and control participants were obtained from their respective hospital records.

A survey instrument was developed that encompassed demographic data such as patients' gender, age, tumor type, location, stage, and grade at the time of diagnosis. Additionally, supplementary information was collected.

The experiments undertaken in this study adhered to the ethical guidelines outlined in the Helsinki Declaration for research involving human beings. The biochemical research involving human participants received approval from the Institutional Review Board (IRB) at the University of Babylon, with the reference numbers IECIH/UOK 088/2020 and CAAE 08802212.

Genomic DNA extraction

The genomic DNA samples were acquired from the peripheral blood samples using a Blood/Cell DNA Mini Kit (Cat. No. GB100, Geneaid Co., Taipei, Taiwan). The confirmation of the purity of the isolated genomic DNA was conducted using a Nanodrop spectrophotometric technology, which was provided by Biodrop Co., UK. The assessment of the isolated genomic DNA's integrity was conducted by the utilization of agarose gel electrophoresis, using established standard techniques [31].

Single nucleotide polymorphism (SNP) genotyping

Four PCR-specific primers were generated utilizing the NCBI primer BLAST program for the purpose of amplifying four unique PCR fragments inside the microRNA [32]. In the process of PCR design, the focus was on four specific high-frequency single nucleotide polymorphisms (SNPs), namely Mir146a, Mir423, Mir370, and Mir196a2. These SNPs were targeted inside DNA fragments of varying lengths, specifically 257 bp, 215 bp, 306 bp, and 319 bp, respectively. The oligonucleotide sequences for PCR are presented in Table 1. The PCR experiments were carried out using a lyophilized PCR AccuPower PreMix (Promega Co., USA), with a final volume of 20 mL for each amplified fragment. Following the execution of polymerase chain reaction (PCR) tests, it was verified that the resulting PCR products exhibited sizes consistent with the anticipated values using electrophoresis conducted on agarose gels.

SSCP technique

The genotyping studies were performed with the PCR-single-strand conformation polymorphism (SSCP) technique. Subsequently, the PCR products were put into polyacrylamide gels with an 8% concentration, and the samples were allowed to migrate until they reached the bottom of the gels. The gels were subjected to staining with Ethidium bromide in accordance with the prescribed methodology [33]. The SSCP banding patterns were confirmed using Sanger dideoxy-sequencing, following the recommended protocols provided by Macrogen Inc., a company based in South Korea. The DNA chromatogram of each genotype was viewed utilizing Snap Gene Viewer version 4.0.4, a software produced by Insightful Science in Canada. The alignment of the reported variation with the matching DNA sequences was conducted using Bio Edit software, specifically version 7.1.

Statistical and functional analysis

The chi-square test was employed to assess the disparities in genotype distribution between the subjects and the control group. The odds ratio (OR) and 95% confidence interval (95% CI) were computed to assess the relative risk of colorectal cancer (CRC) and to examine the associations between the single nucleotide polymorphism (SNP) and clinicopathological features of individuals

Table 1 The specific PCR primers designed for the amplification of the microRNA SNP

Mir146A G\C	SNP locus in the amplicon	Gene Bank Acce.no	5–3	PCR product	Annealing TM
Forward	140	KR606822.1	5-ACCAGGCTTTTCACTCTTGT-3	257	58
Reverse			5-CTGTCTCCAGTCTTCCAAGC-3		
Mir 423 A\C					
Forward	57	NG_087026.1	5-TCGTCAAGGGTAGAGACGGG-3	215	61
Reverse			5-AAACTCAAGCGCGGGTTAGG-3		
Mir196a2					
Forward	112	AF490843.1	5-CCCTTCCCTTCTCCTCCAGA-3	306	60
Reverse			5-AGAGGACGGCATAAAGCAGG-3		
Mir370 G\A					
Forward	166	NM_001375797.2	5-CAAACATTGGGCCCATGCAG-3	319	60
Reverse			5-GCAGAGCAACATGGAGTCCT-3		

CR primers were designed based on the Gene Bank accession numbers

diagnosed with colorectal cancer. The predetermined level of significance was established at a value less than 0.05. The statistical analyses were conducted using SPSS version 28.

Results

Demographic characteristics of study subjects

The research encompassed a comprehensive sample size of 100 cases of colorectal cancer (CRC) and 100 control subjects. The clinical and pathological attributes of these individuals are detailed in Table 2. Significant statistical differences were seen between the patients and controls in relation to smoking status, gender, age, and alcohol intake. There were no statistically significant differences found between the two groups in terms of inclination and education level. The postoperative pathological stages were categorized as follows: stage I, with a total of 50 cases accounting for 50% of the sample; stage II, with 25 cases representing 25% of the sample; stage III, with 20 cases accounting for 20% of the sample; and stage IV, with 5 cases representing 5% of the sample.

Genotyping analysis

Four single nucleotide polymorphisms (SNPs) with the highest frequency were individually chosen. This study selected four single nucleotide polymorphisms (SNPs) as microRNAs (miRNAs): Mir146a, Mir423, Mir370, and Mir196a2. The analysis revealed four distinct banding patterns corresponding to the selected SNPs, indicating that the Mir146a, Mir423, Mir370, and Mir196a2 SNPs exhibit three genotypes each. The sequencing studies that were done successfully confirmed the presence of the three anticipated genotypes for all high-frequency single nucleotide polymorphisms (SNPs) that were examined. The electropherograms of the four examined single

Table 2 Clinicopathological characteristics of CRC cases and controls

Variable	Subjects n100 (100%)	Control n100 (100%)	*P* value
Gender			
Male	65 (56%)	50 (50%)	0.032*
Female	35 (35%)	50 (50%)	
Living in			
Urban	40 (40%)	45 (45%)	0.47
Countryside	60 (60%)	55 (55%)	
Education level			
Up to high school	70 (70%)	60 (60%)	0.13
Beyond to high school	30 (30%)	40 (40%)	
Age			
< 50 years	75 (75%)	45 (45%)	0.003*
≥ 50 years	25 (25%)	55 (55%)	
Smoking			
Smoker	80 (80%)	53 (53%)	< 0.001*
Non-smoker	20 (20%)	47 (47%)	
Location of tumor			
Colon	85 (85%)		
Rectum	15 (15%)		
Alcohol consumption			
Yes	78 (78%)	49 (49%)	< 0.001*
No	22 (22%)	51 (51%)	
Family history			
Yes	57 (57%)		
No	43 (34%)		
TNM stage			
I	50 (50%)		
II	25 (25%)		
III	20 (20%)		
IV	5 (5%)		

n number of subjects

*$P < 0.05$

nucleotide polymorphisms (SNPs) exhibited the following genotypes: Mir146a (GG: GC: CC), Mir 423 (AA: AC: CC), Mir370 (GG: GA: AA), and Mir196a2 (CC: CT: TT) (Fig. 1).

In order to assess the Hardy–Weinberg equilibrium (HWE) of the research population, an analysis of the genetic diversity of the discovered polymorphic single nucleotide polymorphisms (SNPs) was conducted. Based on the chi-square values, the compatibility of the polymorphisms of all four discovered polymorphic single nucleotide polymorphisms (SNPs) with the Hardy–Weinberg equilibrium (HWE) was confirmed in both the control and colorectal cancer (CRC) groups at a significance level of 0.05 (see Table 3).

The distribution of Mir146a genotype in patients with colorectal cancer (CRC) was seen as follows: 45 individuals (45%) exhibited the homozygous GG genotype, 40 individuals (40%) exhibited the homozygous CC genotype, and 15 individuals (15%) exhibited the heterozygous GC genotype. The distribution of the Mir146a polymorphism genotypes in the control group was as follows: 65 participants (65%) had the homozygous GG

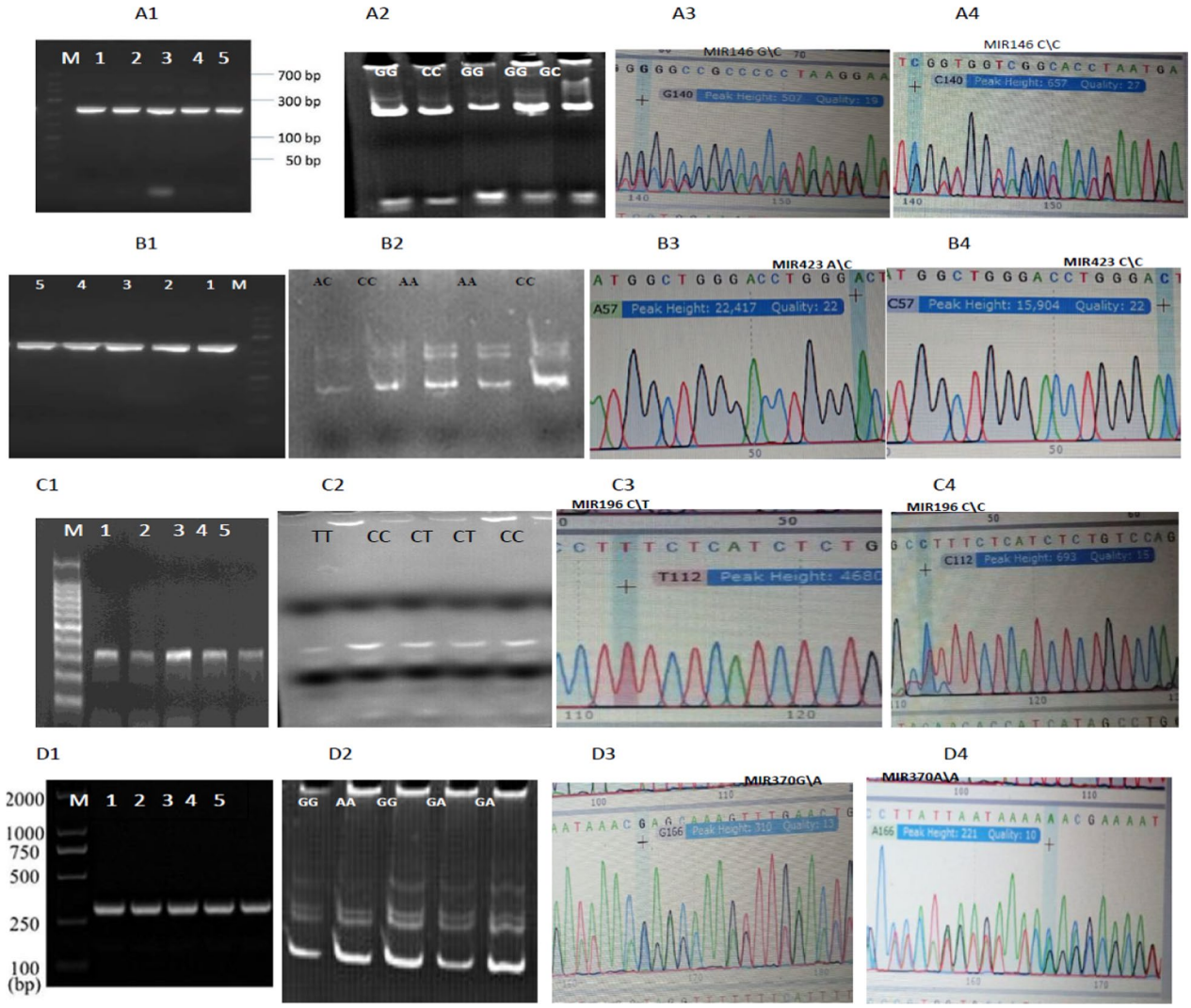

Fig. 1 The present work presents a schematic diagram illustrating the process of microRNA genotyping through the utilization of the PCR-SSCP-sequencing approach. In this study, we design four unique primers, denoted as **A1**, **B1**, **C1**, and **D1**. These primers were designed to amplify DNA fragments of certain lengths, 257 bp, 215 bp, 306 bp, and 319 bp. The purpose of these amplifications was to flank the regions associated with Mir146a, Mir423, Mir196a2, and Mir370, respectively. The genotyping technique employed in this study was PCR-SSCP as shown in figures **A2**, **B2**, **C2**, **D2**, specifically targeting SNPs. It was observed that all the targeted SNPs exhibited three distinct patterns of nucleic acid changes. The sequencing reactions of the targeted loci, as positioned in the amplified PCR fragments, are denoted as **A3**-**A4**, **B3**-**B4**, **C3**-**C4**, and **D3**-**D4**

Table 3 Hardy–Weinberg equilibrium (HWE) for Mir146a, Mir 423 A\C, Mir196a2 C\T, and Mir370 A\G SNPs in patients and control groups

SNP	Patients		Controls	
	Observed	Expected	Observed	Expected
Mir146a G\C				
GG	45	27.56	65	46.92
GC	15	49.87	7	43.15
CC	40	22.56	28	9.92
Chi-square value	27.7709		38.9128	
P value	**< 0.0001**		**< 0.0001**	
Mir 423 A\C				
AA	40	25	60	42.25
AC	20	50	10	45.5
CC	40	25	30	12.25
Chi-square value	19.7802		33.1593	
P value	**0.0001**		**< 0.0001**	
Mir196a2 C\T				
CC	50	34.81	70	56.25
CT	18	48.38	10	37.5
TT	32	16.81	20	6.25
Chi-square value	21.9994		24.6003	
P value	**< 0.0001**		**< 0.0001**	
Mir370 A\G				
AA	40	27.56	70	60.06
AG	25	49.87	15	34.87
GG	35	22.56	15	5.06
Chi-square value	5.0275		13.1319	
P value	0.0810		**0.0014**	

The *P* value with statistical significance is in bold

CL confidence interval

genotype, 28 subjects (28%) had the homozygous CC genotype, and 7 subjects (7%) had the heterozygous GC genotype. The frequencies of G and C alleles in the subjected group were 52% and 57.5%, respectively, whereas in the control group, they were 68.5% and 31.5%, respectively. The distribution of genotypes for the miR146a gene in both patient and control groups is presented in Table 3.

In the patient group with colorectal cancer (CRC), the frequencies of the homozygous alleles AA and CC for the Mir423 polymorphism were observed to be 40% each. Additionally, the frequency of the heterozygous allele AC for this polymorphism was found to be 20% in the same patient group. In contrast, the healthy control group exhibited frequencies of 60%, 10%, and 30% for the polymorphic alleles AA, CC, and AC, respectively. The frequencies of the A and C alleles in the patient group were 50% and 50%, respectively, whereas in the control group, the rates were 65% and 35%, respectively.

In the study, the frequency of Mir370 polymorphism genotypes (AA, AG, and GG) was detected in both the patient and control groups. In the patient group, the observed frequencies were 40% for AA, 25% for AG, and 35% for GG genotypes. Conversely, in the control group, the observed frequencies were 70% for AA, 15% for AG, and 15% for GG genotypes. The frequencies of the A and G alleles in the sick group were 52.5% and 47.5%, respectively. In contrast, the control group had frequencies of 77.5% and 22.5% for the A and G alleles, respectively.

The frequencies of the Mir196a2 polymorphism alleles CC and TT in the patient group with colorectal cancer (CRC) were 50% and 32%, respectively, while the frequency of the heterozygous allele (CT) was 18%. In contrast, the healthy control group had frequencies of 70%, 15%, and 15% for the polymorphic alleles CC, TT, and CT, respectively, as indicated in Table 3 (Tables 4, 5).

Discussion

The high death rates observed in diverse populations for colorectal cancer (CRC) can be attributed to the significant number of cases being diagnosed at later stages. It is worth noting that CRC is the fourth most prevalent kind of cancer globally [34]. The issue at hand is a significant public health concern across several populations [35]. According to the World Health Organization (WHO), it has been projected that the incidence of new cases of colorectal cancer (CRC) and the associated mortality rates will see a significant rise of 77% and 80%, respectively, by the year 2030 [36, 37]. MicroRNA (miRNA) has been extensively studied and shown to have a strong correlation with the initiation and advancement of many cancer types. A number of microRNA (miRNA) biomarkers have been discovered as possible risk factors for colorectal cancer (CRC) [38, 39]. The increasing prevalence of colorectal cancer (CRC) among various populations prompts inquiries into the potential risk linked to certain alleles of MicroRNA (miRNA) in affected individuals. The objective of this study was to investigate the correlation between microRNA (miRNA) polymorphism and colorectal cancer (CRC) in the Iraqi population by employing genotyping techniques to analyze four frequently seen single nucleotide polymorphisms (SNPs) in microRNA (miRNA) sequences.

MicroRNAs (miRNAs) have a crucial role in regulating a wide range of biological and physiological processes. Consequently, their dysregulation, which can be caused by mutations, transcriptional changes, and epigenetic modifications, appears to be associated with this phenomena [40, 41]. Among the various genetic alterations, single nucleotide polymorphism (SNP) stands out as the prevailing form, exhibiting associations with illness risk, population diversity, and individual responses to

Table 4 Genotype frequencies of Mir146a, Mir423, Mir196a2, and Mir370 in both the control (*n* = 100) and patient subjects (*n* = 100) and their association with CRC risk

SNP	Patients (n = 100)	Controls (n = 100)	Chi-square	*P* value	Odd (95% CI)
Mir146a G\C					
GG	45 (45%)	65 (65%)	References		
GC	15 (15%)	7 (7%)	5.50	0.019*	0.32 (0.12–0.85)
CC	40 (40%)	28 (28%)	5.40	0.02*	0.48 (0.26– 0.89
Allele					
G	105 (52.5%)	137 (68.5%)	10.71	0.001*	0.50 (0.33–0.76)
C	95 (57.5%)	63 (31.5%)			
Mir 423 A\C					
AA	40 (40%)	60 (60%)	Reference		
AC	20 (20%)	10 (10%)	6.60	0.01*	0.33 (0.14–0.78)
CC	40 (40%)	30 (30%)	4.85	0.028*	0.50 (0.26–0.92)
Allele					
A	100 (50%)	130 (65%)	9.20	0.002*	0.53 (0.36–0.80)
C	100 (50%)	70 (35%)			
Mir196a2 C\T					
CC	50 (50%)	70 (70%)	Reference		
CT	18 (18%)	10 (10%)	4.67	0.031*	0.39 (0.16–0.93)
TT	32 (32%)	20 (20%)	5.74	0.017*	0.44 (0.22–0.86)
Allele					
C	118 (59%)	150 (75%)	11.57	< 0.001*	0.48 (0.31–0.78)
T	82 (41%)	50 (25%)			
Mir370A\G					
AA	40 (40%)	70 (70%)	Reference		
AG	25 (25%)	15 (15%)	8.16	0.004*	0.34 (0.16–0.72)
GG	35 (35%)	15 (15%)	15.61	< 0.001*	0.24 (0.11–0.50)
Allele					
A	105 (52.5%)	155 (77.5%)	27.47	< 0.001*	0.32 (0.20–0.94)
G	95 (47.5%)	45 (22.5%)			

* The *P* values are statistical significance. *CL* confidence interval

medication [42, 43]. In recent years, several researches have been conducted to examine the impact of miRNA single nucleotide polymorphisms (SNPs) on colorectal cancer (CRC) susceptibility. However, the findings of these studies have not yet provided conclusive evidence and exhibit variability across different populations. Given the lack of previous investigation into the prevalence of single nucleotide polymorphisms (SNPs) in the frequently disrupted Mir146a, Mir196a2, Mir370, and Mir423 in the Iraqi population, as well as their potential association with colorectal cancer (CRC), our research aims to address this gap. Specifically, our study focuses on determining the frequency of these aforementioned microRNAs and their potential correlation with CRC risk and various pathological characteristics of cancer.

The present study aimed to elucidate the association between genetic variations in microRNAs (MiRNAs) and the occurrence of colorectal cancer (CRC). The correlation identified between the investigated fragments of MiRNAs was determined by genotyping four high-frequency single nucleotide polymorphisms (SNPs) using polymerase chain reaction-single-strand conformation polymorphism (PCR-SSCP) [44]. The selection of the PCR-SSCP method in the present genotyping strategy was based on its cost-effectiveness and high sensitivity in identifying genetic difference within the 200–350 bp range [45].

This study revealed a significant association between Mir146a and colorectal cancer (CRC) within the sample group. The potential correlation between these single nucleotide polymorphisms (SNPs) and the initiation and progression of colorectal cancer (CRC) can be clarified by further examination. The current investigation revealed that individuals with the GG genotype of the Mir146a gene exhibited an increased vulnerability to colorectal cancer (CRC). The observed result is not unexpected, as

Table 5 Genotype frequencies of mir-146a, mir-423 and mir-196a2, mir370 in patient subjects ($n=100$) and their association with tumors stage

Gene\ genotype	Stage		OR (95% CI)	*P* value
	I & II n (%)	III & IV n (%)		
Mir146a GG	40 (53.3%)	15 (60%)	Reference	
Mir146a GC	25 (33.3%)	2 (8%)	0.21 (0.04–1.01)	0.037*
Mir146a CC	10 (13.3%)	8 (32%)	2.13 (0.70–6.42)	0.17
Mir 423 AA	35 (46.6%)	10 (40%)	Reference	
Mir 423 AC	10 (13.3%)	10 (40%)	3.50 (1.13–10.76)	0.025*
Mir 423 CC	30 (40%)	5 (20%)	0.58 (0.17–1.89)	0.36
Mir 196 CC	30 (40%)	8 (32%)	Reference	
Mir 196 CA	20 (26.6%)	7 (28%)	1.31 (0.41–4.19)	0.64
Mir 196 AA	25 (33.3%)	10 (40%)	1.50 (0.51–4.37)	0.45
Mir370 AA	40 (53.3%)	12 (48%)	Reference	
Mir370 AG	20 (26.6%)	10 (40%)	1.66 (0.61–4.51)	0.31
Mir370 GG	15 (20%)	3 (12%)	0.66 (0.16–2.69)	0.56

* The *P* values are statistical significance

The number and the percentage are within the same genotype

previous studies have demonstrated that the activity of Mir146a, Additionally, it has been found that individuals with the GG genotype have higher expression levels of Mir146a, leading to an increase in its activity [46, 47]. Several investigations have been undertaken on colon, lung, breast, and hepatic cancer, which support our discovery of an elevated cancer risk associated with the Mir146a CG genotype [48–50]. The study on colon cancer did not acknowledge this particular correlation [51]. A subsequent investigation was conducted on a community in Italy, wherein it is widely acknowledged that cancer arises as a result of the interplay between genetic factors and environmental influences. On the contrary, a meta-analysis conducted on single nucleotide polymorphism (SNP) studies of colorectal cancer (CRC) microRNAs (miRNAs) revealed that individuals of Asian descent exhibit a tendency toward a correlation between the mir-146a SNP and an elevated risk of CRC [52].

Several studies have demonstrated that Mir146a, along with its prevalent polymorphism, rs2910164, which involves a G to C substitution, is found inside the sequence of the Mir146a precursor. In the present work, we want to investigate this miRNA hotspot in colorectal cancer (CRC) for the first time. The single nucleotide polymorphism (SNP) in question converts a G:U base pair to a C:U mismatch. As a consequence, there is a decrease in the levels of both pre- and mature Mir146a [53, 54]. In this case–control study, we examined the association between the Mir146a polymorphism, rs2910164, and the risk of colorectal cancer (CRC). Our study represents the first investigation into the function of this specific polymorphism in CRC risk, and we observed a statistically significant association. While our findings do not demonstrate a significant association between the aforementioned miRNA-associated SNPs and the risk of colorectal cancer (CRC), we posit that a more thorough and comprehensive examination of miRNA SNPs will enhance our comprehension of the specific miRNAs implicated in the initiation and advancement of CRC. This understanding is crucial for the advancement of innovative diagnostic and therapeutic methodologies aimed at combating this life-threatening ailment.

The subsequent focus of this study involved Mir423, wherein it was observed that the AA and CC genotypes constituted the predominant genotypic variations, consistent with the genetic makeup of the Chinese population. Similarly, another investigation conducted on a distinct cohort of African-American individuals also identified CC and AA genotypes as the prevailing genetic variations. According to previous investigations, it has been observed that Mir423 has the ability to enhance the transition from the G1 phase to the S phase of the cell cycle, hence promoting cell proliferation [55, 56]. The majority of research conducted on Mir423 has been focused on investigating its expression patterns rather than its genotypic characteristics. In two separate analyses, it was shown that there was no observed elevation in the likelihood of developing hepatocellular and bladder cancer associated with any of the genotypes of the miRNA single nucleotide polymorphisms (SNPs) [57, 58]. Another study asserts that the Mir423 single nucleotide polymorphism (SNP) plays a protective effect in relation to the risk of breast cancer [22]. Our study conducted an observation of an elevated susceptibility to colorectal cancer (CRC) in individuals with the Mir423 single nucleotide polymorphism (SNP) CC genotype. A study conducted by [58] demonstrated an elevated susceptibility to colorectal cancer (CRC) when Mir 423 is accompanied by other mutations. These findings demonstrate a multifaceted interplay between single nucleotide polymorphisms (SNPs), other genetic variations, and the combined effects of environmental and ethnic factors.

The current study aimed to examine the genetic polymorphism of Mir 370 in colorectal cancer (CRC). Specifically, we explored the mir-370 single nucleotide polymorphism (SNP) rs2279398G > A in case–control studies to assess its association with CRC risk. Our findings revealed that this association exhibited diverse directions [59, 60]. Numerous studies have demonstrated that the rs2279398 polymorphism, which is situated in the 3'-UTR region of the DOK3 gene, has the ability to impact the efficacy of miRNA binding. Consequently, this polymorphism may have a role in the modulation of

tumor suppression, while previous studies have suggested that the findings presented here could offer valuable SNP information for elucidating the specific biological implications of cancer progression and treatment resistance in individuals with colorectal cancer (CRC). In a similar vein, Zhang and colleagues have documented that the presence of the KRAS 3'-UTR polymorphism has the potential to serve as a predictive factor for the response of patients with wild-type KRAS colorectal cancer who are undergoing cetuximab monotherapy [61, 62]. However, despite the growing body of evidence indicating the significance of DOK3 in several facets of carcinogenesis, there remains a lack of clarity on the potential impact of the DOK3 (rs2279398) polymorphism on protein expression.

While rs11614913 SNP may not be as widely recognized as rs2279398 SNP, it has also been associated with the progression of colorectal cancer. Additionally, it has been found to be associated with elevated susceptibility to various other types of cancer [63]. Based on this discovery, rs11614913 has been extensively investigated as one of the most commonly researched single nucleotide polymorphisms (SNPs) linked to microRNAs (miRNAs) in case–control studies involving several types of solid tumors [61, 62, 64, 65]. In more recent times, two studies conducted in China have examined the correlation between this single nucleotide polymorphism (SNP) and its impact on the susceptibility and advancement of colorectal cancer (CRC) [19, 63].

In a scholarly publication authored by Liu et al. [66], a report was presented. The study demonstrated a significant association between the CT and TT genotypes, as well as the T allele, with an elevated risk of colorectal cancer (CRC) when compared to the CC genotype and C allele. Specifically, the CT genotype exhibited an odds ratio (OR) of 7.34 (95% confidence interval [CI] 3.76–14.34, $P<0.001$) compared to the CC genotype, while the TT genotype had an OR of 1.99 (95% CI 1.63–2.42, $P<0.001$) compared to the CC genotype. Contrary findings were seen in the study conducted by Zhan et al., whereby they discovered a substantial association between the CC genotype and C allele and an elevated risk of colorectal cancer (CRC) in comparison with the TT genotype and T allele. Jiang et al. [67] and Zhu et al. [68] conducted studies on the Chinese population, in which they identified the CC genotype as a risk genotype for colorectal cancer (CRC). It is noteworthy that a separate study conducted on the Chinese population did not identify any association between Mir196a-2 single nucleotide polymorphism (SNP) and risks of colorectal cancer (CRC) [69]. According to a recent meta-analysis comprising seven research, it was shown that the genetic variant rs11614913 may potentially play a role in decreasing the susceptibility to colorectal cancer [70].

The objective of this study was to evaluate the frequencies of alleles and genotypes of the rs11614913 polymorphism in the Mir196a2 gene among Iraqi patients diagnosed with colorectal cancer (CRC). The aim was to investigate any potential correlation between the rs11614913 polymorphism, considered as a genetic component, and the occurrence of CRC in this population. The findings of our study indicate that individuals with the CC genotype had a greater susceptibility to colorectal cancer (CRC) compared to those with CT or TT genotypes. Furthermore, the incidence of colorectal cancer (CRC) was found to be higher in those carrying the C allele compared to those carrying the T allele. No substantial connection between the miR-196a2 polymorphism and the risk of colorectal cancer (CRC) was identified in previous studies. Hezova et al. [21, 71] conducted a study in Europe, examining a cohort of 197 patients diagnosed with non-hereditary colorectal cancer (CRC) and 212 control subjects. No significant association was observed between the rs11614913 polymorphism of the miR196a2 gene and the susceptibility to colorectal cancer (CRC). The results of their study were in agreement with the data collected by Chen et al. [69]. A previous study conducted in Iran shown a noteworthy correlation, albeit without the inclusion of equilibrium calculation [44, 72].

The main limitation of this study is the small sample size, which may affect the reported results by not fully reflecting the actual effects on the development and advancement of colorectal cancer (CRC). The utilization of a small sample size in this study may potentially affect the statistical significance of the findings. Therefore, it is advisable to reproduce the aforementioned technique using a larger sample size in order to acquire more reliable conclusions generated from a thorough investigation.

In summary, the genotyping investigations conducted on four variants within microRNAs demonstrated a significant association between the polymorphisms of Mir146a, Mir196a2, Mir370, and Mir423 single nucleotide polymorphisms (SNPs) and colorectal cancer (CRC) in the Iraqi people. Individuals harboring the Mir423: AC and Mir370: AG genotypes exhibited a heightened vulnerability to colorectal cancer (CRC). Both single nucleotide polymorphisms (SNPs) possess the potential to serve as biomarkers for the evaluation of colorectal cancer (CRC) in the Iraqi population being studied. However, it is imperative to do additional research in order to substantiate the molecular diagnostic capabilities that have been alluded to on a more extensive level.

Conclusions

The study of our aggregated data reveals that single nucleotide polymorphisms (SNPs) in microRNA have a notable and distinct correlation with the heightened susceptibility to colorectal cancer (CRC). Nevertheless, this study proposes the utilization of a more comprehensive range of examined samples in order to obtain additional insights into the association between both single nucleotide polymorphisms (SNPs) and the susceptibility to colorectal cancer (CRC).

Abbreviations

SNPs	Single nucleotide polymorphisms
CRC	Colorectal cancer
mir	MicroRNA
3′-UTR	3 Untranslated region
SSCP	Single-strand conformation polymorphisms
PCR	Polymerase chain reaction

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Author contributions

The process of collecting samples and conducting genotyping was supervised by zahraa Isam Jameel.

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Availability of data and materials

The data presented in this study are classified as confidential, in accordance with the regulations set forward by the Institutional Review Board of the University of Babylon. The analysis of the medical record was conducted with the authorization of the medical board at Merjan Cancer Center. The genotyping data can be obtained upon request from the Laboratory of Biotechnology, under the supervision of Dr. Zahraa Isam., zahraa.isam@science.uoqasim.edu.iq).

Declarations

Ethical approval and consent to participate

Institutional Ethics Committee Involving Humans of the University of Babylon (Babel, Iraq) ratified this study (IECIH/UOK 088/2020; CAAE 08802212). All subjects signed a written informed consent form before participation, and the study was conducted in accordance with Helsinki's Declaration.

Competing interests

No conflict of interest is reported by the author.

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