# MicroRNA Sequence Polymorphism and the Risk of Colorectal Cancer

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## Abstract:

***Introduction:*** Colorectal cancer (CRC) is the 3rd common malignant disease. Prediction of its risk depending on the individuals' genetic makeup is an essential step for screening and personalized medicine. Different studies implicated MicroRNAs (miRNAs) as potential predictors for CRC risk and prognosis with different findings in various demographic settings. To the present knowledge, no similar work was conducted, so far, to study these relations in our community.
***Aim:*** To determine the prevalence of single nucleotide polymorphisms (SNPs) in genes coding for the miRNAs: mir-146a, mir-423 and mir-196a2 in patients with sporadic CRC. The association of these SNPs with increased CRC risk or advanced tumor stage or grade at diagnosis was also explored.
***Materials and Methods:*** In this case-control study, we enrolled 150 patient subjects with sporadic CRC and 140 sex and age matched cancer free control subjects. Genomic DNA was extracted from venous blood samples and screened for the presence of SNPs in mir-146, mir-423 and mir-1962a genes using High Resolution Melting Analysis (HRMA) method, and then results were confirmed by direct sequencing. The SPSS statistical software (version 17) was used to test for the association between the presence of the SNP and the risk of CRC, its stage and grade.
***Results:*** The frequency of the CG genotype of mir-1946a was significantly higher ($P < 0.01$) in the patient subjects (44%) compared to the control ones (30.7%). This genotype was associated with increased risk of CRC (OR= 1.741, 95 % CI= 1.050 -2.886, P=0.031), late stage cancer (III and V of TNM system, OR=3.562, 95%CI= 1.578-8.039, P=0.002) and poor tumour differentiation (OR=5.267, 95%CI= 1.105- 25.109, P=0.037). Individuals carrying CC genotype showed increased CRC risk compared to the common GG genotype (control: 10%, patients: 15.3%, OR= 2.235, 95 % CI= 1.064- 4.694, P=0.004). Additionally it was associated with late stage tumor (OR= 8.522, 95 % CI= 2.899- 25.049, P= 0.0001) but not tumor grade (OR= 2.950, 95 % CI= 0.389-22.339, P= 0.295). None of the genotypes of mir-423 and mir-196a2 showed any association with neither increased risk of CRC nor late stage or poor differentiation.
***Conclusion:*** Various SNPs of miRNAs showed different correlations with CRC and its pathological characteristics which reflect a complex interaction between the genes and the cellular milieu- that is influenced by the demographic qualities of the studied population. Mir-146 can represent a potential target for screening of CRC and a prognostic marker. Further evaluation in a large scale studies will give a better insight on the effects of SNPs on CRC risk and prognosis.
***Key words:*** MicroRNA, Colorectal Cancer, Polymorphisms, Risk.

## Introduction:

Colorectal Cancer (CRC) is the 3rd most frequent type of malignancy and the 4th commonest cause of cancer morbidity worldwide [1]. Most patients (~70%) develop sporadic CRC in normal epithelial lining of the colon and rectum without any genetic predisposition [2]. Fewer than (10%) of patients with CRC have a genetic predisposition such as Familial Adenomatous Polyposis (FAP) and Hereditary Non-Polyposis Colorectal Cancer (HNPCC) [2]. The rest of the cases occur, for yet unknown reason, in families at higher rate than normal population but not in a define cancer genetic syndrome [2, 3]. If CRC is detected at early stage, excellent outcomes can be obtained by surgery alone. In contrast, in advanced disease, the prognosis is poor, with little chance of cure [4].

The recent completion of the human genomic project brought Single Nucleotide Polymorphisms (SNPs) to the attention of biomedical researchers as a potential contributor to the pathogenesis of CRC [5-7].

SNPs are naturally occurring DNA sequence variations, which differ from gene mutations, and occur in the 'normal' healthy population at a frequency of at least (1%) [3]. SNPs occur in almost all genes at variable frequencies and influence the protein product of genes in a way that might increase susceptibility to cancer.

Of the genes that are known to have SNP, are those coding for MicroRNAs (miRNAs). miRNAs are abundant, endogenous non coding, 20-25 nucleotides RNA that modulate the expression of their target genes post-transcriptionally [8, 9]. They function through post-transcriptional regulation of gene expression via base-pairing with complementary sequences in target mRNAs, resulting in translational suppression of imperfectly matched mRNAs or degradation of perfectly matched mRNAs [8, 10]. They influence almost all the activities of the cells such as; differentiation and cell cycle and tumor initiation and progression [11, 12].

miRNAs related SNPs can deregulate biological pathways in a variety of ways such as disruption of microRNA; binding sites, expression and processing which may alter an individual's susceptibility to CRC [3]. Mir-146a, mir-423 and mir-192a2 were thought to participate in cancer pathogenesis, however the complete understanding of their role is still absent [12, 13].

mir-146a gene is located on chromosome 5q34 [14]. A recent study provided evidence that mir-146a acts as a modulator of the innate immune response and of the adaptive immune response as well [14]. Due to the fact that the predicted mir-146a target genes include BRCA1 and BRCA2, it was found that breast and ovarian cancer patients who had at least one mir-146a variant allele were diagnosed at an earlier age [15].

mir-423 is situated on chromosome 17 and was detected in untreated HL-60 leukaemia cells during 2-O-tetra-decanoylphorbol-13-acetate induced differentiation experiments; indicating that the mature miRNA is involved in the differentiation processes [16]. mir-423 promotes cell growth and regulates G1/S transition by targeting p21Cip1/Waf1 in hepatocellular carcinoma (HCC) [17].

Recently, many studies have demonstrated that mir-196 plays a critical roles in normal development and

in the pathogenesis of human diseases such as cancer (18). One SNP of mir-196a2 (rs11614913) has been associated with prolonged survival in Chinese individuals with lung cancer, this particular SNP affects the binding efficiency of mir-196a2 to its target mRNA [18]. The upregulation of mir-196 was found to be significantly correlated with the malignant progression of gliomas and poor survival rates [19].

Different studies showed conflicting results with regard to the association between the above miRNAs various genotyping and CRC risk which might shows geographical and demographic influences. Unfortunately, no study was done so far in Iraq neither to determine the prevalence of these SNP in our population nor to determine their correlation with the risk of CRC.

Better understanding of SNP in miRNAs, in turn, will improve both understanding of the mechanisms of disease and the design of effective risk-assessment models [20].

The aim of this study is to examine whether the presence of SNPs in miRNAs; 146a, 432 and 196a2 is associated with increased risk of CRC in our population and identify the presence of any association between the polymorphisms and the clinicopathological characteristics of our CRC patients.

## Material and Methods:

### Study subjects:

This case–control study included 150 patients with histopathologically confirmed CRC who attended the Oncology Department at Azadi Teaching Hospital, Kirkuk for treatment or follow up during the period from April -2014 to March -2015. The patients had CRC of different histological types, stages and grades and were at various points in their treatment and clinical course. The patient participants were 87 males and 63 females with age range of 52±20 years (mean age 53 years). Patients with family history of colon cancer or polyposis or having inflammatory bowel disease were excluded from the study, those with history of recent blood transfusion (less than one month) were also disqualified.

One hundred forty healthy age and sex match were enrolled as control subjects from cancer- free visitor to the hospital outpatient clinics. Clinicopatholigcial details for the patient and control subjects were taken from their hospital records.

A questionnaire was formulated; which included information about patients' sex, age, marital status, type and location of tumor with its stage and grade at presentation, other information were also obtained.

The study was performed after obtaining ethical approval from Kirkuk Health Authority and the ethics committee at Kirkuk Medical College. Written consent was obtained from each participant after full explanation of the study aim, procedure and risks.

### SNP Genotyping:

We collected five millilitres of venous blood in Ethinyl Diamine Tetraacetic Acid (EDTA) containing tubes from the patient and the control subjects for genotyping. DNA samples extracted from the blood of the patients and the control subjects were screened for SNPs using Polymerase Chain Reaction (PCR) followed by High Resolution Melting Analysis (HRMA). Ten samples were sent for direct sequencing for confirmation of HRMA results and

obtaining positive control for each SNP genotype for following experiments. An example for a positive control for GC genotype of mir-146a is shown in Figure 1.

## DNA Extraction, HRMA and Direct Sequencing:

Genomic DNA was extracted from the blood samples using the QIAamp® DNA Blood Mini Kit (Cat. No. 51104, Qiagen, GmbH) following the manufacturer's protocol. The concentration and the purity of the DNA were assessed by NanoVue™ Plus Spectrophotometer (GE Health Care, USA). Previously designed and tested primers were used [16, 21, 22] to perform the PCR for HRMA and direct sequencing, they were synthesiszed by the Genetic and Molecular Research Unit of Koya University, Iraq. The primers were selected to ensure that they are short to improve the results of HRMA (as melting curve of small amplicons gives better picture for different sequences) yet long for subsequent direct sequencing to be feasible [23]. The tested miRNAs genes and the sequences of the used primers, the amplicon size and the annealing temperatures of the PCR are shown in Table 1.

The PCR reaction was composed of 12.5 Micro liter (µl) 2X Type-it® HRM PCR Kit (Qiagen Medical Ltd, GmBH), each primer at final concentration of 0.7µM and 20 Nanogram (ng) template DNA, all made up to a final volume of 25 µl with PCR grade water. Non template control (NTC) PCR was included to detect DNA carry over and PCR contamination.

The thermal cycling of the PCR was done at private laboratory in Kirkuk by Rotor-Gene Q machine (Qiagen Medical Ltd, GmBH). This machine has HRM capabilities beside thermal cycling performance. The cycling programme consisted of one holding of 95°C for 5 minutes (min), followed by 45 cycles of [(95°C for 10 second (s)/ (55 °C for 30s)]/ (72 °C for 30s). A final melting curve stage of one cycle; rapid ramping from 65°C to 95°C, at ramping up rate of 0.1°C /s with a hold of 2s at each step. The data were analyzed using the machine custom resolution melting software. For sequencing purposes, the PCR products were purified using AccuPrep®PCR purification Kit (BIONEER Corp, Korea) according to the supplier guidelines. Appropriate concentration of purified PCR products and the corresponding primers were sent to Macrogen Company, Korea, and sequenced using Applied Biosystems BigDye® Terminator v3.1 Cycle Sequencing Kit and 3130xl Genetic Analyzer. The chromatograms were viewed by using the Chromas Lite software version 2.01.

## Statistical analysis:

Fisher's exact test was used to test for the differences in the genotype distribution between the patient subjects and the control ones. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated for the estimation of the relative risk of CRC and estimate the relations between the SNP and clinicopathological characteristics of colorectal cancer patients. The level of significance was set at $<0.05$. All statistical analyses were performed with the SPSS version 17.

## Results:

### Study subjects' clinicopathological characteristics:

Of the 150 subjects with colorectal cancer, the majority 87(58%) were male and 63 (42%) were females. The control and patient subjects were not significantly different with regard to age, gender or educational status ($P$>0.05). Most of the tumors ($n$=106, 70.6%), were located in the colon and the rest ($n$=44, 29.4%) were positioned in the rectum. Stages I and II (TNM system) represented the majority ($n$=95, 63.3%) of the cases at the time of diagnoses. The vast majority of patients had tumors of the adenocarcinoma type that were moderately differentiated ($n$=123, 82%). The detailed clinicopathological characteristics of patient and the control subjects are shown in Table 2 .

**Table (1):** Genes and their SNP ID and the sequences of the primers. The amplicon size and the PCR annealing temperature for each primer pair are shown. Additionally, the melting temperature of each product is displayed.

| Gene | SNP ID | Primer Sequences | Product Size | Annealing Temp (°C) | HRM Temp Range |
|---|---|---|---|---|---|
| **mir-146a** | rs2910164 | **F:** GGAGGGGTCTTTGCACCATC **R**:TGCCTTCTGTCTCCAGTCTTCC | 210bp | 57ºC | 77ºC-87ºC |
| **mir-423** | rs6505162 | **F:**GGGCAGAGAGCGAGACTTT **R:** GGAAGCCAGGAACTGTCTCT | 137bp | 60ºC | 79˚C-89ºC |
| **mir-196a2** | rs11614913 | **F:** AACCCCCTTCCCTTCTCCTC **R:** GCTTGTCCTCCTTGGTCTGC | 257bp | 59ºC | 65ºC-88ºC |

ID: identity, SNP: Single nucleotide polymorphism, Temp: Temperature, bp: base pair

**Table (2):** The clinicopathological characteristics of the study subjects, CRC patients ($n$=150) and control subjects ($n$=140).

| Characteristic | CRC Patients | Controls | *P* value |
|---|---|---|---|
| **Total no.** | 150 | 140 | |
| **Mean age ± SD, years** | 52±20 | 53±18 | >0.05 |
| **Sex** | | | |
| Male | 87(58%) | 80(57.1%) | >0.05 |
| Female | 63(42%) | 60(42.9%) | >0.05 |
| **Education level** | | | |
| Up to high school | 100(66.6%) | 83(59.3%) | >0.05 |
| Beyond high school | 50(33.3%) | 57(40.7%) | >0.05 |
| **Location of tumor** | | | |
| Colon | 106(70.6%) | | |
| Rectum | 44(29.4%) | | |
| **TNM stage** | | | |
| I | 60(40%) | | |
| II | 35(23.3%) | | |
| III | 30(20%) | | |
| IV | 25(16.7%) | | |
| **Differentiation** | | | |
| Well-differentiated | 12(8%) | | |
| Moderately differentiated | 123(82%) | | |
| Poorly differentiated | 15(10%) | | |

**Table (3):** Genotype frequencies of mir-146a, mir-423 and mir-196a2 in both the control (*n*=140) and patient subjects (*n* =150) and their association with CRC risk.

| miRNA name | Genotype | Control *n*, % | Patients *n*, % | OR (95%CI) | *P* Value |
|---|---|---|---|---|---|
| **mir-146a** | GG | 83(59.3%) | 61(40.7%) | 1 (Reference) | |
| | CG | 43(30.7%) | 66(44%) | 2.088 (1.258- 3.466) | 0.031 |
| | CC | 14(10%) | 23(15.3%) | 2.235(1.064- 4.694) | 0.004 |
| **mir-423** | AC | 74(52.8%) | 82(54.6%) | 1(Reference) | |
| | AA | 38(27.2%) | 42 (28%) | 0.997 (0.581 -1.711) | 0.992 |
| | CC | 28 (20%) | 26 (17.4%) | 0.838 (0.451 -1.556) | 0.576 |
| **mir-196A2** | CC | 67(47.8%) | 80(53.3%) | 1 (Reference) | |
| | CT | 65(46.5%) | 59(39.4%) | 0.760 (0.470- 1.227) | 0.262 |
| | TT | 8(5.7%) | 11(7.3%) | 0.660 (0.248- 1.752) | 0.404 |

n: number of subjects, OR: Odd ratio, CI: confidence interval.

**Table (4):** Genotype frequencies of mir-146a, mir-423 and mir-196a2 in patient subjects (*n* =150) and their association with tumours stage. The number and the percentage are within the same genotype.

| Gene/Genotype | Stages | | OR (95%CI) | *P* value |
|---|---|---|---|---|
| | *I& II n (%)* | *III& IV n(%)* | | |
| mir-146a GG | 50(74.6%) | 11(25.4% ) | 1(Reference) | |
| mir-146a CG | 37(56% ) | 29(44%) | 3.562 (1.578-8.039) | 0.002 |
| mir-146a CC | 8(47.1% ) | 15(52.9%) | 8.522 (2.899- 25.049) | 0.0001 |
| mir-423 AC | 54(65.8%) | 28(34.2%) | 1(Reference) | |
| mir-423 AA | 28(66.7%) | 14(33.3%) | 0.964(0.438- 2.119) | 0.927 |
| mir-423 CC | 13(50%) | 13(50%) | 1.928 (0.788- 4.715) | 0.149 |
| mir-196a2 GC | 56(70%) | 24(30%) | 1(Reference) | |
| mir-196a2 CT | 34(57.7%) | 25(42.3%) | 1.715 (0.848-3.468) | 0.132 |
| mir-196a 2 TT | 5(45.5) | 6(54.5) | 2.800 (0.778-10.066) | 0.114 |

**Table (5):** Genotype frequencies of mir-146a, mir-423 and mir-196a2 in patient subjects (*n* =150) and their association with tumor grade. The number and the percentage are within the same genotype.

| Gene/Genotype | Grade/ differentiation | | OR (95%CI) | *P* value |
|---|---|---|---|---|
| | *Well& moderately n (%)* | *Poor n(%)* | | |
| mir-146a GG | 59(96.7%) | 2(3.3% ) | 1(Reference) | |
| mir-146a CG | 56(90.6% ) | 10(9.4%) | 5.267 (1.105-25.109) | 0.037 |
| mir-146a CC | 20(69.6% ) | 3(30.4%) | 2.950(0.389 -22.339) | 0.295 |
| mir-423 AC | 76(92.6%) | 6(7.4%) | 1(Reference) | |
| mir-423 AA | 37(88%) | 5(12%) | 1.711(0.490-5.975) | 0.399 |
| mir-423 CC | 22(84.6%) | 4(15.4%) | 2.3030 (0.596- 8.894) | 0.226 |
| mir-196a2 GC | 71(87.5%) | 9(12.5%) | 1(Reference) | |
| mir-196a2 CT | 56(95%) | 3(5%) | 0.422(0.109-1.634) | 0.212 |
| mir-196a2 TT | 8(72.7%) | 3(27.3%) | 2.958(0.661-13.221) | 0.155 |

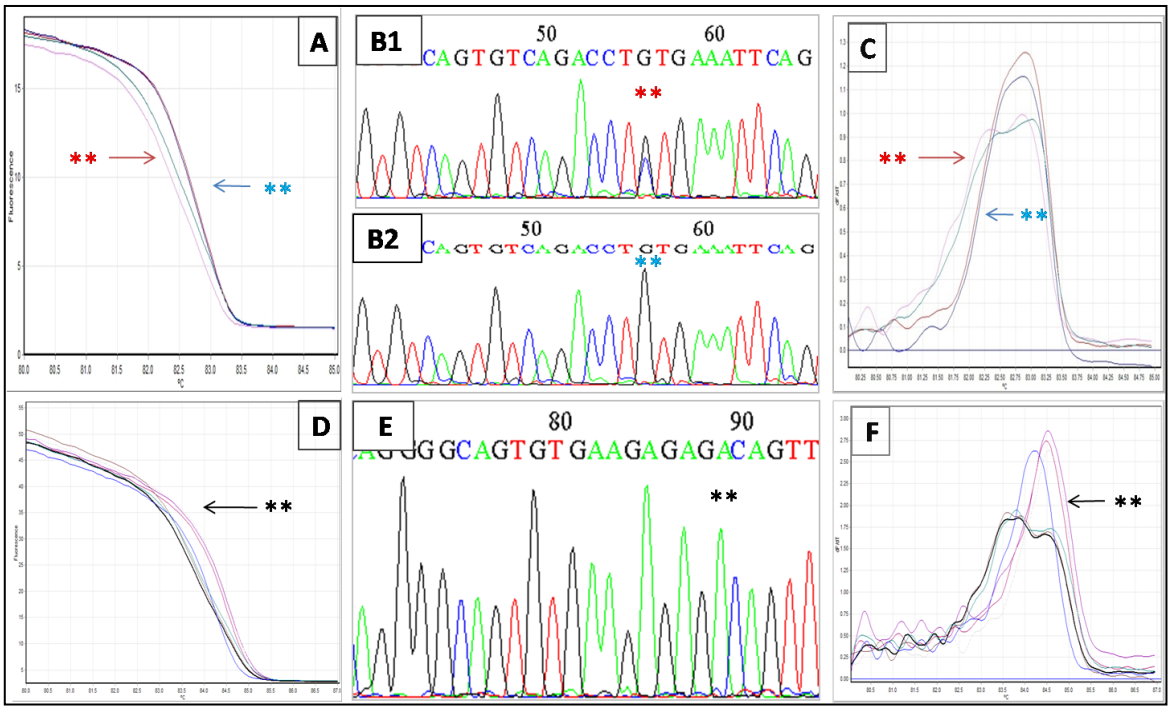


**Figure (1):** Screening for SNP in mir-146a and mir-423 by HRMA using the Rotor Gene Q system.
**(A)** Melting curve of the samples for the mir-146a, different genotypes can be determined by variation in the shape of the melting curve, those samples clustering with known common genotype were called as wild type(Blue asterisk ) whilst the remainder were called as SNP (red asterisk). In **(B1)** a display of direct DNA sequencing of mir-146a showing CG heterozygous genotype (red asterisks). Figure **(B2)** shows GG genotype sequencing (Blue asterisks). In figure **(C)** the results are shown as derivative plot with arrows directed toward the two mutant samples (red asterisk). The melting peak represents the state at which DNA melting is maximum. Normalisation is not essential requirement prior to this analysis. Figures **D, E** and **F** show the melting curve, DNA direct sequencing and derivative plot of mir-423 gene, respectively. The derivative plot represents the negative derivative of the fluorescence on the Y-axis and temperature on the X-axis

## SNP identification and confirmation:

By applying the genotyping strategy, HRMA was able to correctly call all the various SNPs, the heterozygous SNPs were easily identified. For all the other samples, we did 1:1 mix of, by direct sequencing, confirmed major genotype to any sample which showed a melt curve pattern of the major genotype. This was done since HRMA has limited ability to identify homozygous SNPs. An example of the SNPs that were identified by HRMA and confirmed by direct sequencing is shown in Figure 1.

## Association between the SNP and the cancer pathological characteristics:

Genotyping of mir-146a gene for polymorphisms (rs2910164) indicated a significant shift (P=0.035) in the genotypic frequencies in CRC, reflected by increase in CG genotype to (44%) and a decrease in the GG genotype down to (30.7%) , which is the major genotype in the control subjects. The same genotype (CG) was associated with increased CRC risk (OR= 1.741, 95 % CI= 1.050-2.886, P=0.031). CG genotype was associated with late stages (stages III and V) of CRC (OR=3.562, 95%CI= 1.578-8.039,

P=0.002) and poor tumor differentiation (OR=5.267, 95%CI= 1.105- 25.109, P=0.037). More details can be found in Tables 4&5. The CC genotype is the least common form of mir-146a (10% and 15.3% of the control and patient subjects sequentially, P<0.05). And it was associated with increased CRC risk (OR= 2.235, 95 % CI= 1.064- 4.694, P= 0.004) and late stage cancer (OR= 8.522, 95% CI= 2.899-25.049, P= 0.0001) but not with poor differentiation (OR= 2.950, 95% CI= 0.389 -22.339, P= 0.295) (Tables 4&5).

Genotyping of mir-423 for rs6505162 polymorphism revealed that the AC genotype is the commonest genotype in both the control and patient subjects (52.8% and 54.6% respectively). The AA genotype came next (27.2% &28% of the control and patient subjects correspondingly) and had no significant association with higher risk of developing CRC (OR=0.982, 95% CI=0.581-1.658, P=0.946). Furthermore, no association was found between the AA genotype and the examined pathological parameters (differentiation and TNM' stage) of colorectal cancer, statistical details can be seen in (Tables 4&5). The minor genotype (CC) of mir-423 (20% and 17.4% of the control and patient subjects respectively, P>0.05) did not confer higher risk of CRC (OR=0.838, 95% CI=451 -1.556, P=0.576). Additionally it was not associated with higher stage of the tumor or lower degree of differentiation (Tables 4&5).

For the genotype polymorphisms of mir-196a2 (rs11614913), the CRC risk of individuals with CT genotype was similar to that of individuals with the major genotype CC (OR= 0.760, 95%CI=0.470- 1.227, P=0.262). And that of individuals with TT genotype showed no increase in CRC chance compared to CC sequence (OR 0.660, 95% CI=0.248-1.752, P=0.404). The above genotypes (CT and TT) were not associated with higher stage of the disease or the level of differentiation. Tables 4 and 5 give more numerical details.

## Discussion and Conclusion:

Despite the progress that has been made so far to crack the code of CRC pathogenesis, there is still much to be known with regard to the factors that make some people more susceptible to this malignancy. MicroRNA (miRNA) deregulation due to genetic and epigenetic mechanisms seems to have an influence on susceptibility to cancers [24].

Of these genetic changes, Single Nucleotide Polymorphism (SNP) is the most common type, that is associated with disease susceptibility, population diversity, and individual response to medicine [25]. Recently, a number of studies have investigated the role of miRNA SNPs in CRC susceptibility, but the results remain inconclusive and vary from population to another. Since no attempt was done so far to explore the prevalence of SNP in the commonly de-arranged mir-146a, mir-196a2 and mir-423 in Iraqi population or their association with CRC, we got motivated to conduct this work which focuses on frequency of the above MicrRNA and their association with CRC risk and some cancer pathological characteristic.

In the present study it was found that the CG genotype of the mir-146a conferred a higher susceptibility to CRC and leads to advanced stage and poor differentiation of the tumors at the time of diagnosis. This finding is not odd;

since mir-146a activity increases cell proliferation and survival by affecting Toll like and cytokine receptors, and the CG genotype is associated with increased expression of mir-146a and consequently its activity [22, 26]. Different studies conducted on colon, lung, breast and hepatic cancer strengthen our finding of increased risk of cancer in mir-146a CG genotype [25, 27-30]. A new paper on colon cancer failed to recognize such association [31]. The later study was done on Italian population, and cancer is well recognized to be the outcome of genetic-environmental interaction. On the other side, Meta-analysis of CRC miRNAs SNP studies found that the Asian have a trend toward an association between mir-146a SNP and increased CRC [32].

The next target of this work was mir-423, and it was found that the CA genotype was the major genotype similar to the Indian population whereas CC and AA genotype were the major genotypes in China and Africa based studies respectively [33]. This similarity to the Indian genotype might reflect a genetic closeness to them. mir-423 has been found so far to promote cell proliferation by enhancing G1/S cell cycle transition [34] Most of the studies that were carried out on mir-423 focused on its expression rather than its genotyping. The research on its genotyping displayed conflicting results. In two investigations, no increased risk of hepatcellular and bladder cancer was found with any of the genotypes of the miRNA SNPs [17, 35]. Another work claims protective role of mir-432 SNP on breast cancer risk [16]. Our research observed no increased risk of CRC cancer in the rs6505162 CC genotype. The results further exacerbate the confusion with regard to the impact of SNP on cancer risk. One study showed increased risk of CRC when BRCA mutation occur in conjugation with mir-423SNP [36]. These reflect a complex interaction of SNP and other genetic variation with influence of environmental and ethnic backgrounds.

mir-196a2 SNP (rs11614913) was investigated in several case-control studies with respect to CRC risk and the association had different directions. A report by Lv et al., 2012. Showed that CT, TT genotypes and T allele were associated with an increased risk of CRC compared with the CC genotype and C allele (CT vs. CC: OR = 7.34, 95% CI 3.76–14.34, P < 0.001; TT vs. CC: OR = 1.99, 95% CI 1.63–2.42, P < 0.001, respectively). Opposite results were shown by Zhan et al.,. who found that CC genotype and C allele were associated with a significantly increased risk of CRC compared with the TT genotype and T allele. Similarly, the CC genotype was considered as a risk genotype for CRC by Min et a l., .[37] and Zhu Let al., [38] these investigations were carried out on Chinese population. Interestingly, another study enrolling Chinese population did not observe any link between miR-196a-2 SNP and CRC risks in [39]. A recent meta-analysis of seven studies suggested that rs11614913 might contribute to reduced risk of CRC [40]. Our results showed no correlation between mir-196a2 polymorphism and CRC risk. These conflicting results stem from the complexity of the cancer pathogenesis per se, in addition to the technical factors; such as sample selection and size and variation in the genotyping methods.

In conclusion, the progressive advance and affordability of genotyping; makes mir-146a a good target for cancer

screening at population level and might serve as a prognostic marker in our population. Other miRNAs showed no association with CRC or its pathological characteristics. We acknowledge that our sample size is modest and larger study might reveal different outcome. Since SNPs characteristic are demographically dependent, it might be justifiable to investigate the predictive and prognostic role of the above SNP in our people.

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