

## RESEARCH ARTICLE

Editorial Process: Submission:01/02/2022 Acceptance:04/23/2022

# Association between Genetic Polymorphisms in MicroRNAs 196a2 (rs11614913) and 34 b/c (rs4938723) and Risk of Hepatocellular Carcinoma in Egyptian Patients

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## Abstract

**Background:** Hepatocellular carcinoma (HCC) is a common cancer with substantial cancer-related deaths worldwide. Deregulation of some genetic polymorphisms has been identified in HCC. **Objective:** We aimed to demonstrate the frequency of miRNA 196a2 rs11614913 and miRNA 34 b/c rs4938723 gene polymorphisms in HCC patients and their correlation with the clinical features and laboratory findings at diagnosis. **Subjects and methods:** The study was performed on 40 patients with newly diagnosed HCC and 40 patients with liver cirrhosis in addition to 40 age and sex-matched healthy controls. Detection of miRNA 196a2 rs11614913 and miRNA 34 b/c rs4938723 gene polymorphisms was determined by PCR-RFLP. **Results:** HCC patients had significantly higher frequency of miR-196-2a rs11614913 CC genotype when compared with cirrhotic patients (60.0 % versus 30.0 %,  $p=0.013$ ). In spite of the fact that HCC patients also had higher frequency of miR-196-2a rs11614913 CC genotype in comparison to controls, the difference fell short of statistical significance (60.0 % versus 42.5 %,  $p=0.18$ ). No significant differences were found between the studied groups regarding the frequency of miR-196-2a alleles. miR34 b/c rs4938723 CC genotype was the only identified genotype in all participants in the three studied groups. No significant associations were found between the different clinical and laboratory variables and genotypic variations in HCC patients. **Conclusions:** This study identified miR-196a2 rs11614913 CC genotype as a risk factor for HCC development while we failed to document similar relation for miR-34b/c rs4938723 polymorphism.

**Keywords:** Hepatocellular carcinoma- MicroRNA- miR-196a2- miR-34b/c

*Asian Pac J Cancer Prev*, **23** (4), 1373-1377

## Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the second most frequent cause of cancer-related death worldwide (2018). HCC has a strong male predominance, with a male to female ratio estimated to be 2–2.5:1.8 (Akinyemiju et al., 2017). Unfortunately, most cases of HCC are detected at an advanced stage with overall survival not exceeding one year in many situations (El Mahdy et al., 2019).

In Egypt, liver cancer represents 23.8% of the total malignancies and HCC constitutes 70.48% of liver tumors among. The incidence of HCC is highest due to high prevalence of hepatitis C and hepatitis B (El Mahdy et al., 2019).

MicroRNAs (miRNAs) are small non-coding RNAs (17–23 nucleotides) that control mRNA post-transcriptionally. Several miRNAs have been reported to be potential prognostic biomarkers in many

cancers (Karakatsanis et al., 2013).

The deregulation of specific miRNAs has been identified in HCC, including miR-148a, miR-203, miR-138, miR-122 and miR-124. Therefore, these miRNAs may become potential therapeutic targets or candidates for HCC treatment (Yin et al., 2015).

MiRNA-196A is transcribed in two genomic locations, the HOXC (Chr12 in humans, gene miR196A2) and HOXB (Chr17 in humans, gene miR196A1) loci, downstream of HOXC10 and upstream of HOXB9 respectively (Simonson and Das, 2015).

The miR-34 family consists of three miRNAs (a,b,c) which contain identical seed regions and show variable tissue expression. In somatic cells, miR-34 is an integral component of the p53 network, impeding cell cycle progression and proliferation by silencing oncogenic targets (Soni et al., 2013).

In our study, we demonstrated the frequency of miRNA 196a2 rs11614913 and miRNA 34 b/c rs4938723 gene

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polymorphism in HCC patients and correlated to the clinical features and laboratory findings at diagnosis.

## Materials and Methods

### Subjects and Methods

This study was conducted on at Beni-Suef University Hospital, Beni-Suef, Egypt. in the period between April 2017 and April 2019. The study protocol was approved by the Ethical Committee of Beni-Suef Faculty of Medicine and all subjects provided informed consent to participate in the study.

The study was performed on 40 patients with newly diagnosed HCC and 40 patients with liver cirrhosis in addition to 40 age and sex-matched healthy controls. HCC was diagnosed on the basis of at least two imaging methods (ultrasound, CT) and biochemistry (alpha fetoprotein). Patients were excluded if they had criteria were associated or previous cancer, any metastasized cancer or submission to radiotherapy or chemotherapy.

All the patients were subjected to full history taking, clinical examination and laboratory investigations including complete blood count, prothrombin time and concentration, serum albumin, total bilirubin, alanine transaminase (ALT), aspartate transaminase (AST) and alpha fetoprotein (AFP). Abdominal ultrasound and abdominal CT were performed to assess tumor size, number of lesions and portal vein status.

### Detection of miRNA 196a2 rs11614913 and MiRNA 34 b/c rs4938723 gene polymorphism

Five ml of venous blood were collected from participant in sterile EDTA vials. Genomic DNA was isolated from peripheral blood samples using QIAamp DNA Mini Kit (Cat. no. 51114, QIAGEN) according to the manufacturer's protocol. Amplification of MiRNA 34 b/c and miRNA-196a2 single nucleotide polymorphisms was achieved polymerase chain reaction (PCR). Genotyping of both polymorphisms was determined by a restriction fragment length polymorphism (RFLP) assay using specific primer sequences and restriction enzymes. The following pairs of primers were used for amplification of miRNA 196a2: forward 5'-CCC CTT CCC TTC TCC TCC AGA TA-3' and reverse 5'-CGA AAA CCG ACT GAT GTA ACT CCG-3'. In case of MiRNA 34 b/c, forward and reverse primers were 5'- CTCACCTCCTCTGGGAACCTT-3' and 5'-AAGGCCATACCATCAAGACAGTAT-3', respectively. The PCR reaction was performed in 25µl of reaction mixture containing 5.5 µl nuclease-free water, 1 µl of both primers, 12.5 µl Dream Taq green PCR master mix, and approximately 5 µl DNA. The PCR cycling was: denaturation at 95°C for 5 min; 35 cycles of denaturation for 30 s at 95°C, then annealing at 60°C for 30 s, extension at 72°C for 2 min; completed by a final extension at 72°C for 10 min. After confirmation of successful PCR amplification by 1.5% agarose gel electrophoresis, each PCR product was digested overnight with 5U of MspI and TASI (New England Biolabs, Ipswich, MA, USA) for miR196a2 and MiRNA 34 b/c respectively at 37°C overnight.

For the miRNA-196a2 (rs11614913) polymorphism,

PCR product of 149bp was digested into fragments of 125bp and 24bp which was indicative of wild type genotype (CC), whereas the presence of three bands of 149bp, 125bp and 24bp represented the heterozygous (CT) genotype and an undigested 149-bp band represented the mutant genotype (TT).

In case of MiRNA 34 b/c, the C allele produced a 26- and 186-bp pattern, while the T allele was undigested and produced a 212-bp fragment. To ensure quality control, genotyping was performed without knowledge of the subjects' case/control status and a 15% random sample of cases and controls was genotyped twice by different persons; reproducibility was 100%.

### Statistical Analysis

Data obtained from the present study are presented as number and percent, mean and standard deviation (SD) or median and interquartile range (IQR). Categorical variables were compared using chi-square test while numerical variables were compared using one-way ANOVA or Kruskal-Wallis test as appropriate. All statistical tests were computed using SPSS 25 (IBM, USA) with p value less than 0.05 considered statistically significant.

## Results

The present included 40 HCC patients, 40 liver cirrhosis patients and 40 age and sex-matched healthy

Table 1. Clinical Findings in the Studied HCC Patients (n=40)

Variable	
Age (years) mean ± SD	58.5 ± 3.9
Male/female n	28/12
Smoking n (%)	12 (30.0)
Child Pugh classification n (%)	
Child A	20 (50.0)
Child B	13 (32.5)
Child C	7 (17.5)
Tumor size n (%)	
<2cm	16 (40.0)
>2cm	24 (60.0)
Solitary lesions n (%)	21 (52.5)
Portal vein thrombosis n (%)	14 (35.0)
Ascites n (%)	13 (32.5)
Laboratory findings mean ± SD/ median (IQR)	
Hb (gm/dl)	11.5 ± 1.7
Platelets (×10 <sup>3</sup> /ml)	167.8 ± 75.9
ALT (IU/L)	55.5 ± 47.5
AST (IU/L)	94.5 ± 102.7
Bilirubin (mg/dl)	2.01 ± 1.9
Albumin (gm/dl)	3.1 ± 0.66
AFP (ng/ml)	99.5 (7.1- 403.5)
Creatinine (mg/dl)	0.98 ± 0.23

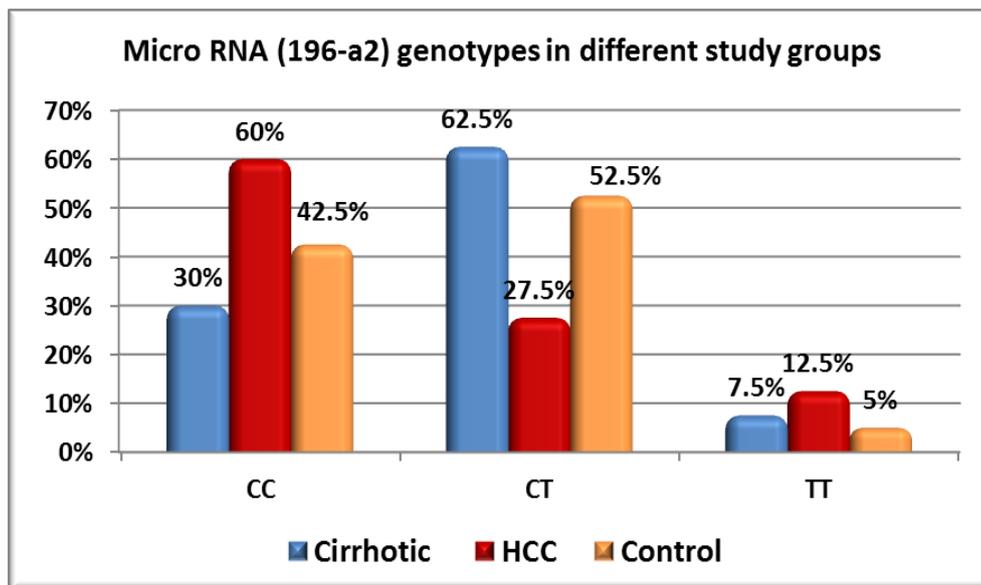


Figure 1. Micro RNA 196-a2 Genotypes in Different Study Groups

Table 2. Genotypic and Allelic Frequencies of miR-196-2a rs11614913 and miR-34 b/c rs4938723 in the Studied Groups

	HCC N=40	Cirrhosis N=40	Controls N=40	p value
miR-196-2a rs11614913 genotypes n (%)				
CC	24 (60.0)	12 (30.0)	17 (42.5)	0.027
CT	11 (27.5)	25 (62.5)	21 (52.5)	
TT	5 (12.5)	3 (7.5)	2 (5.0)	
miR-196-2a rs11614913 alleles n (%)				
C allele	59 (73.8)	49 (61.3)	55 (68.8)	0.23
T allele	21 (26.2)	31 (38.7)	25 (31.2)	
miR34 b/c rs4938723 genotypes				
CC	40 (100.0)	40 (100.0)	40 (100.0)	NA

controls. Clinical and laboratory data of HCC patients are shown in Table 1. Patients comprised 28 males and 12 females. There were 20 patients (50.0 %) classified as Child-Pugh A class while 13 patients (32.5 %) were classified as Child-Pugh B class and 7 patients (17.5 %) were classified as C class. In addition, there were 21 patients (52.5 %) with solitary lesions and 14 patients (35.0 %) had portal vein thrombosis and 13 patients (32.5 %) had ascites.

The identified genotypic and allelic of miR-196-2a and miR-34 b/c polymorphisms are shown in Table 2 and Figure 1. HCC patients had significantly higher frequency of miR-196-2a rs11614913 CC genotype when compared with cirrhotic patients (60.0 % versus 30.0 %,  $p=0.013$ ). In spite of the fact that HCC patients also had higher frequency of miR-196-2a CC genotype in comparison to controls, the difference fell short of statistical significance

Table 3. Relation between Genotypic Variations and Clinical and Laboratory Data

	CC N=24	CT N=11	TT N=5	p value
Age (years)	62.7 ± 5.6	60.5 ± 8.1	62.2 ± 7.4	0.66
Male/female n	14/10	1-Oct	1-Apr	0.13
Child-Pugh classification n (%)				
A	13 (54.2)	5 (45.5)	2 (40.0)	0.85
B	8 (33.3)	3 (27.3)	2 (40.0)	
C	3 (12.5)	3 (27.3)	1 (20.0)	
Laboratory findings mean ± SD/ median (IQR)				
Hb (gm/dl)	11.4 ± 1.9	11.8 ± 1.07	11.2 ± 1.5	0.7
Platelets ( $\times 10^3$ /ml)	173.4 ± 76	151.6 ± 53.2	176.2 ± 121.7	0.7
ALT (IU/L)	60.3 ± 49.1	53.7 ± 52.4	36.4 ± 25.3	0.6
AST (IU/L)	115.1 ± 122.8	71.6 ± 59.7	46.2 ± 17.3	0.3
Bilirubin (mg/dl)	1.8 ± 1.6	2.5 ± 2.7	2.02 ± 1.8	0.7
Albumin (gm/dl)	3.1 ± 0.66	3.04 ± 0.69	3.38 ± 0.71	0.5
AFP (ng/ml)	56.0 (5.7-306)	68.0 (9.2-517.5)	261.5 (88.0-426.5)	0.65
Creatinine (mg/dl)	0.97 ± 0.24	0.99 ± 0.022	1.08 ± 0.22	0.5

(60.0 % versus 42.5 %,  $p=0.18$ ). miR34 b/c rs4938723 CC genotype was the only identified genotype in all participants in the three studied groups. No significant associations were found between the different clinical and laboratory variables and genotypic variations in HCC patients (Table 3).

## Discussion

Emerging evidence indicates that polymorphisms in miRNA genes may alter the expression, regulation and function of miRNAs (Esquela-Kerscher and Slack, 2006). The present study assessed the prevalence of miRNA-196a2 SNPs (rs11614913) polymorphisms in Egyptian HCC patients.

HCC patients had significantly higher frequency of miR-196-2a CC genotype when compared with cirrhotic patients. In spite of the fact that HCC patients also had higher frequency of miR-196-2a CC genotype in comparison to controls, the difference fell short of statistical significance.

Our findings are in accordance with other studies conducted on different populations. In one Chinese study, miR-196a2C>T polymorphisms were found to be associated with development of HCC in male patients with chronic hepatitis B virus infection (Qi et al., 2010). In another study from the same country, it was found that TC and CC genotypes of miR-196a2 T>C were associated with an elevated risk of HCC compared to the TT genotype. Moreover, the TC+CC genotype of miR-196a2 T>C was correlated with an increased risk of HCC compared to the wide-type genotype (Yan et al., 2015). In another Chinese work, (Xu et al., 2016) evaluated the relationship between the miR-196a2 SNP and the risk of HCC recurrence after liver transplantation. They revealed that inheritance of the homozygous CC genotype of miR-196a2 was associated with higher miR-196a2 expression than inheritance of the TT genotype. Similar conclusions were reported by the studies of (Farokhizadeh et al., 2019) from Iran and (Akkız et al., 2011) from Turkey.

In a recent meta-analysis, (Zhang et al., 2020), the authors identified a total of 20 studies providing 5,337 HCC cases and 6,585 controls. Pairwise analysis found that miR-196a2 rs11614913 was significantly associated with the susceptibility of HCC with individuals with TC/CC were more susceptible.

In our study on Egyptian HCC patients, the identified miR34 a/b genotypes were restricted to the CC genotype. In contrast to these findings, the Chinese studies of (Chen et al., 2016) and (Liu et al., 2017) found an association between pri-miR-34b/c rs4938723 polymorphism and higher risk of HCC.

In conclusion, this study identified miR-196a2 rs11614913 as a risk factor for HCC development in Egyptian patients while we failed to document similar relation for miR-34b/c rs4938723 polymorphisms. These findings highlight the clinical significance of genetic risk factors in the development of HCC. Treatment plans should be tailored based on comprehensive identification

of susceptibility genes in affected individuals.

## Author Contribution Statement

All authors equally shared in formulating the idea, conception, and data collection statistics, writing and drafting the manuscript.

## Acknowledgments

### Ethical approval

This study was conducted on at Beni-Suef University Hospital, Beni-Suef, Egypt. In the period between April 2017 and April 2019. The study protocol was approved by the Ethical Committee of Beni-Suef Faculty of Medicine and all subjects provided informed consent to participate in the study.

### Data Availability Statement

Data of this research will be available upon reasonable request.

### Conflict of Interests

None.

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