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# Association between miR-196a2 polymorphism and the development of hepatocellular carcinoma in the Egyptian population

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## Abstract

**Background:** Hepatocellular carcinoma (HCC) is one of the most prevalent cancers worldwide. Circulating microRNAs (miRNAs) are endogenous, small (17–25 nucleotides) non-coding RNAs that are overexpressed in many human cancers including HCC. Single-nucleotide polymorphisms (SNPs) of miRNAs play an important role in the pathogenesis of HCC. In our study, we aimed to evaluate the role of miR-196a2 rs11614913 polymorphism in the development of HCC. A total of 200 subjects, including 80 HCC patients, 60 patients with liver cirrhosis, and 60 healthy controls were selected. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was taken to determine miR-196a2 rs11614913 polymorphism.

**Results:** The genotype distribution of the TC and CC, TC + CC genotypes, and the C allele were significantly higher in HCC patients than control and cirrhotic groups ( $P = 0.02$ ,  $P = 0.005$ , and  $P = 0.003$ , respectively). Compared with the wild-type TT genotype, both the variant TC, CC, TC + CC genotypes were associated with an elevated risk of HCC (OR = 2.77, 95% CI = 1.27–6.04), (OR = 4.94, 95% CI = 1.74–14.07), (OR = 3.24, 95% CI = 1.55–6.78) respectively. Moreover, the C allele was correlated with an increased risk of HCC (OR = 2.30, 95% CI = 1.40–3.76) compared to the wide-type T allele. Also, there is no significant correlation between the different miR-196a2 genotypes and either the clinico-pathologic features of HCC or its aggressiveness.

**Conclusion:** Our results suggest that the miR-196a2 rs11614913 polymorphism is associated with an increased risk of HCC in the Egyptian population.

**Keywords:** Hepatocellular carcinoma, MiR-196a2, Polymorphism

## Background

HCC represents a global health problem. It is one of the most common malignant tumors and the third cause of cancer-related mortality per year with high incidence worldwide [1].

The etiology of HCC is complex; there are many risk factors such as infection with hepatitis B or C virus (HBV, HCV), alcohol abuse, nonalcoholic steatohepatitis (NASH) and aflatoxin exposure. HCC usually develops in patients with liver cirrhosis due to chronic inflammation and advanced fibrosis [2].

MicroRNAs (miRNAs) are a class of small non-coding RNAs, approximately ~ 22 nucleotides long, that perform important roles in the regulation of mammalian gene expression via post-transcriptional repression by directly binding to the 3' untranslated region (UTR) of messenger RNAs (mRNAs), resulting in downregulation of their expression [3].

MiRNAs have been showed to play important roles in regulating different biological processes, including cell differentiation, proliferation, and apoptosis. SNPs of miRNAs may influence their functions through altering miRNA expression, maturation, and/or efficiency of targeting and, thereby, contribute to the risk of cancer [4].

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The miR-196a-2 gene is located in a region between homeobox (HOX) clusters HOXC10 and HOXC9 on chromosome 12. The miR-196a-2 is thought to be over-expressed in HCC tissues and plays important roles in the pathogenesis and development of HCC [5].

Several studies demonstrated the association of miR-196a-2 gene with many cancers including colon, prostate, pancreatic, lung, breast, urinary bladder, and kidney cancer [6].

The present study was designed to evaluate the role of miR-196a2 rs11614913 polymorphism in the development of HCC in the Egyptian population.

## Methods

The present study was conducted at the clinical pathology department, National Liver Institute, Menoufia University, in the duration between September 2017 and December 2018. A total of 200 subjects were enrolled in this case-control study, including 80 patients with HCC, 60 patients with cirrhosis with no radiological evidence of HCC and 60 apparently healthy individuals matched in age and sex as a control group, with no previous history of liver or malignant diseases and negative for hepatitis viral markers. Patients with HCC (diagnosed according to definitive criteria in triphasic computed tomography (CT) with contrast showing arterial enhancement and delayed venous washout) were excluded if they have inflammatory diseases, hematological malignancy, and cancer of any organ other than the liver. The study protocol was approved by the local ethics committee of the National Liver Institute, Menoufia University. Informed consent was taken from both the patients and control group subjects after explaining the aim and concerns of the study.

For all subjects, the followings were done: collection of relevant clinical data, basic laboratory tests including liver function tests (Cobas-6000 auto analyzer, Roche Diagnostics, Germany), Alpha-fetoprotein (AFP) (Cobas e411 immunoassay analyzer, Roche Diagnostics, Germany), prothrombin time (Coagulometer CA - 1500, Siemens, Germany). Hepatitis serology (HBsAg and HCV Ab) (Cobas e411 immunoassay analyzer, Roche Diagnostics, Germany). Molecular testing for miR-196a2 rs11614913 polymorphism was done by PCR-RFLP assay.

## DNA extraction and genotyping

Total DNA was extracted from EDTA treated blood samples using Zymo Quick-gDNA™ MiniPrep DNA Purification Kit (Zymo Research, CA, USA).

After ethanol precipitation, the DNA was purified and dissolved in double distilled water and frozen at  $-20^{\circ}\text{C}$  until use. The miR-196a2 genotype was determined by polymerase chain reaction-restriction fragment length polymorphism assay (PCR-RFLP). The PCR primers

(Thermo scientific) were as follows: forward 5'-CCCCTTCCCTTCTCCTCCAGATA-3' and reverse 5'-CGAAAACCGACTGATGTAACCTCCG-3'. PCR cycling conditions were 5 min at  $94^{\circ}\text{C}$ , followed by 30 cycles of 30 s at  $94^{\circ}\text{C}$ , 30 s at  $63^{\circ}\text{C}$ , and 60 s at  $72^{\circ}\text{C}$ , with a final elongation step at  $72^{\circ}\text{C}$  for 10 min. For restriction fragment length polymorphism, the PCR products were digested with 5 units MsPI enzyme (New England Biolabs, USA) at  $37^{\circ}\text{C}$  and visualized by electrophoresis on 2% agarose under ultraviolet (UV) illumination. The allele types were determined as follows: a single 149 bp fragment for the TT genotype, 2 fragments of 24 and 125 bp for the CC genotype, and 3 fragments of 24, 125, and 149 bp for the TC genotype.

## Statistical analysis

Statistical analysis of the present study was conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Data was expressed into two phases: descriptive and analytical study chi-square test, one-way ANOVA test, Kruskal-Wallis test, Fisher's exact test, odds ratio (OR), and confidence interval (CI) test were used.  $P$  value  $> 0.05$  was considered statistically non-significant.  $P$  value  $< 0.05$  was considered statistically significant.  $P$  value 0.000 ( $< 0.001$ ) was considered statistically highly significant.

## Results

### Baseline characteristics of the study subjects

There is no significant difference between the three statistically studied groups as regard age ( $P = 0.06$ ) and gender ( $P = 0.88$ ). However, there is a statistically highly significant difference between the three groups regarding smoking ( $P = < 0.001$ ) and family history of HCC ( $P = < 0.001$ ) (Table 1). Also, there was a statistically significant difference between studied groups as regarding alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, serum albumin, and international normalized ratio (INR) (Table 2).

### MiR-196a2 genotypes and alleles distribution among study subjects

The frequency distributions of the different genotypes for miR-196a2 polymorphism are shown in (Fig. 1 and Table 3). There was a statistically significant difference between HCC patients and each of the other groups. HCC patients had a higher incidence of CC and TC genotypes when compared to cirrhotic patients and healthy controls;  $P = 0.02$ ,  $0.005$ , respectively. Allele frequencies showed a statistically higher incidence of C allele in HCC patients compared to cirrhotic patients and healthy controls;  $P = 0.003$ .

**Table 1** Socio-demographic data of the studied groups

	Control N = 60	Cirrhosis N = 60	HCC N = 80	$\chi^2$	P value			
Age (years)								
X ± SD	52.61 ± 7.5	56.92 ± 6.45	54.04 ± 8.34					
Min-max	24–60	42–70	43–65	*F = 2.89	0.06			
	<b>No.</b>	<b>%</b>	<b>No.</b>	<b>%</b>	<b>No.</b>	<b>%</b>		
Gender								
Male	30	50.0	29	48.3	42	52.5	0.25	0.88
Female	30	50.0	31	51.7	38	47.5		
Smoking								
Yes	0	0.0	17	28.3	38	47.5		
No	60	100.0	41	68.3	42	52.5	43.75	< 0.001
Ex	0	0.0	2	3.3	0	0.0		
Family history								
Yes	0	0.0	9	15.0	22	27.5	19.81	< 0.001
No	60	100.0	51	85.0	58	72.5		

X = mean, SD = standard deviation,  $\chi^2$  = chi-square, P probability of error. No = number, % = percentage \*F = one-way ANOVA test

#### MiR-196a2 gene polymorphism and the risk for HCC

To evaluate the risk of HCC according to the miR-196a2 genotype using the TT genotype as the reference genotype. Table 4 showed that the TC genotype was associated with a 2.77-fold increased risk of HCC when compared with the TT genotype (OR = 2.77, 95% CI = 1.27–6.04). Moreover, the CC genotype was also associated with a 4.94-fold increased risk of HCC compared with the TT genotype (OR = 4.94, 95% CI = 1.74–14.07). Furthermore, the TC + CC genotypes were associated with a 3.24-fold increased risk of HCC when compared with the TT genotype (OR = 3.24 and 95% CI = 1.55–6.78). Comparing C allele versus T allele distributions in the studied groups, C allele was significantly associated with an increased risk of HCC with 2.30-fold (OR = 2.30, 95% CI = 1.40–3.76) (Table 4).

Additionally, Table 5 showed that there was no statistically significant difference between HCC and cirrhotic groups as regarding genotypes and alleles  $P = 0.13$ ,  $0.09$  respectively. The TC + CC genotypes were significantly associated with 2.15-fold increased risk of HCC when compared with the TT genotype (OR = 2.15, 95% CI = 1.01–4.54).

#### Relation between the different genotypes and clinico-pathological data among HCC patients

There was no statistically significant difference between the different genotypes as regards clinical findings (spleen, liver, ascites, child classification, and encephalopathy) (Table 6). Also, there was no statistically significant difference between the different genotypes as

regard laboratory investigation (ALT, AST, albumin, total and direct bilirubin, PT Conc%, and INR and AFP) (Table 7).

#### Relation between the different genotypes and the aggressiveness of HCC (size and number of focal lesions and AFP level) among HCC patients

There was no statistically significant difference between the different genotypes regarding either the level of AFP ( $P$  value, 0.76) or the size and number of the focal lesions ( $P$  value, 0.99) (Table 8). However, 66.7% of patients with the CC genotype had a high level of AFP. Moreover, 61.9% of patients with the CC genotype had multiple focal lesions.

#### Discussion

HCC is the sixth most common cancer worldwide; also it is the third most common cause of mortality and poor-survival due to the recurrence of HCC and metastasis [7].

The incidence of liver cancer is one of the highest cancers in Egypt, also it is the fifth most common cancer in both genders and the prognosis for patients with HCC is generally poor [8]. So, early diagnosis of HCC is mandatory for the development of specific curative therapies.

MiRNAs are small, non-coding RNAs which involved in many biological functions, they are critical post-transcriptional regulators in gene expression, modulating cell metabolism, and cell survival [9]. MiRNAs variation may affect its structure and expression. Also, deregulated miRNA and its associated post-transcriptional gene silencing or gene expression suggested being an important part of the pathogenesis of HCC [10].

MiR196 family consists of miR-196-a1, miR196-a2, and miR-196b. Two mature miRNAs, miR-196a-5p, and miR-196a-3p are generated from hsa-mir-196a-2 with the studied polymorphism, rs11614913, residing in the 3' arm. Thus, the potential targets of miR-196a could be influenced by its altered expression patterns [11].

The role of miR-196 in different cancer types is mostly unknown. Although many studies not only suggest the oncogenic function of miR-196, but also it is suggested that miR-196 may play a tumor-suppressive action. If the miR-196 has a dominant action on the inhibition of oncogenic molecules, it will play a tumor suppressor function. However, if the miR-196 mainly targets tumor suppressors, it will play an oncogenic effect [12].

Many studies suggest that miR-196a could play an important role in pathogenesis and malignant behavior of HCC by targeting many genes, such as HOX gene, HMGA2, and annexin A1 [13]. HOX proteins disorders were suggested to play an important role in malignant transformation and metastasis of HCC. ANXA1 involved in many biological processes by acting as a mediator of

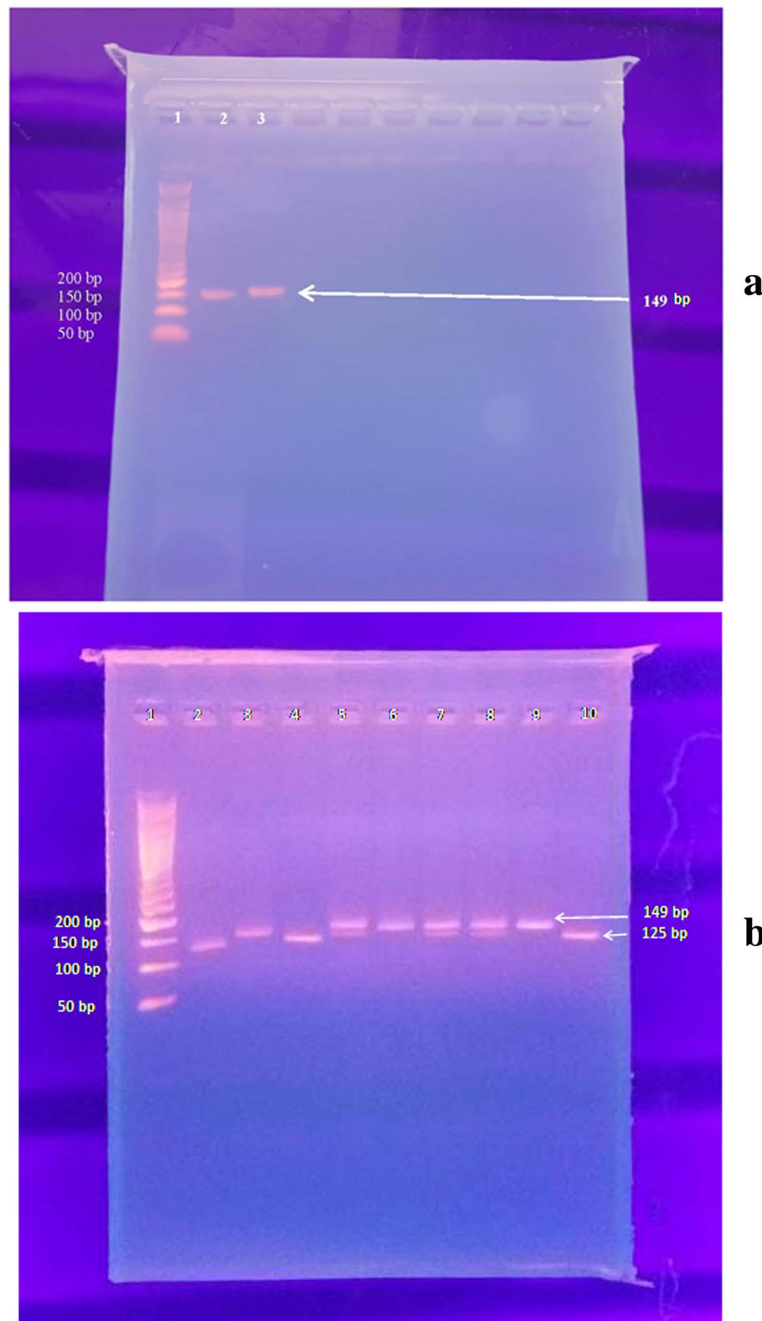
**Table 2** Lab investigations of the studied groups

	Control N = 60		Cirrhosis N = 60		HCC N = 80		Kruskal–Wallis	P value	Post hoc
AST(U/L)									
Min-max	10.0–29.00		12.0–226.00		21.0–540.0				P1 = < 0.001
X ± SD	17.30 ± 4.50		60.56 ± 43.95		124.26 ± 204.04		85.26	< 0.001	P2 = 0.02
Median	17.00		45.50		68.50				P3 = < 0.001
ALT(U/L)									
Min-max	10.00–27.00		11.0–121.00		11.0–379.0				P1 = < 0.001
X ± SD	18.01 ± 4.81		75.30 ± 18.73		79.32 ± 71.51		86.56	< 0.001	P2 = < 0.63
Median	17.00		54.00		55.00				P3 = < 0.001
Albumin (g/dL)									
Min-max	3.70–5.0		1.0–4.00		2.0–4.0				P1 = < 0.001
X ± SD	4.42 ± 0.05		1.74 ± 0.09		2.62 ± 0.66		114.31	< 0.001	P2 = 0.32
Median	4.40		3.00		3.00				P3 = < 0.001
Total bilirubin (mg/dl)									
Min-max	.20–.80		1. 2–15.0		1.0–20.00				P1 = < 0.001
X ± SD	0.267 ± 0.021		3.20 ± 0.413		3.45 ± 0.38		121.93	< 0.001	P2 = 0.31
Median	.54		1.20		3.0				P3 = < 0.001
Direct bilirubin (mg/dl)									
Min-max	.06–.20		1.00–11.00		1.00–16.00				P1 = < 0.001
X ± SD	0.038 ± 0.004		3.22 ± 0.90		3.33 ± 0.37		51.30	< 0.001	P2 = 0.30
Median	.105		1.00		1.50				P3 = < 0.001
Alpha fetoprotein (ng/mL)									
Min-max	-----		1–100		6–20,949				
X ± SD			13.85 ± 1.78		3770.10 ± 421.51		9.78*	< 0.001	-----
Median			4.00		526.00				
Prothrombin conc.									
Min-max	83–100		40–93		19–102				P1 = < 0.001
X ± SD	93.67 ± 4.534		56.63 ± 13.467		58.37 ± 16.95		112.96	< 0.001	P2 = 0.87
Median	92.50		50.60		57.40				P3 = < 0.001
PT.INR									
Min-max	1.00–1.11		1.02–1.83		.95–3.59				P1 = < 0.001
X ± SD	1.03 ± 0.028		1.42 ± 0.18		1.48 ± 0.434		113.35	< 0.001	P2 = 0.65
Median	1.0200		1.43		1.38				P3 = < 0.001
	No	%	No	%	No	%			
HCV Ab									
Negative	60	100.0	1	1.7	7	8.8			
Positive	0	0.0	59	98.3	73	91.2	167.15	< 0.001	
HBV sAg									
Negative	60	100.0	60	100.0	78	97.5			
Positive	0	0.0	0	0.0	2	2.5	1.96**	0.33	

\*Mann–Whitney test

\*\*Fisher's exact test

- P1 = comparison between cirrhosis and control
- P2 = comparison between cirrhosis and HCC
- P3 = comparison between control and HCC



**Fig. 1 a** Agarose gel electrophoresis for miR-196a2 gene amplification bands correspond to ladder band size (149 bp). **b** Agarose gel electrophoresis showing PCR-RFLP analysis of miR-196a2 gene after addition restriction enzyme (MspI). Lanes (ladder): lanes 3, 6, and 9 (TT) band (149); lanes 5, 7, and 8 (TC) band (149,125, and 24 bp); and lanes 2, 4, and 10 (CC) band (125 and 24 bp)

apoptosis and inhibitor of cell differentiation. So, ANXA1 may participate in the pathogenesis of HCC [14].

SNPs in the miR-196a2 (rs11614913) affect the development of cancer susceptibility due to their targeting on several vital genes [15]. Therefore, the present study was designed to evaluate the role of miR-196a2 rs11614913 polymorphism in the development of HCC in the Egyptian population.

The present study shows that the HCV-positive patients accounted for 73 (91.2%) and HBs Ag about 2 (2.5%) of HCC patient group. Dessouky et al. reported that more than 75% were positive for HCV-antibody among Egyptian patients with HCC [16].

Considering miR-196a2 rs11614913 polymorphism, the results of the present study show that, genotype distribution among the studied groups showed a

**Table 3** Distribution of the genotypes and allele frequencies in three studied groups

	Control N = 60		Cirrhosis N = 60		HCC N = 80		$\chi^2$	P value
	No.	%	No.	%	No.	%		
Polymorphism								
TT	28	46.7	22	36.7	17	21.3		
TC	25	41.6	25	41.6	42	52.4	11.7	0.02
CC	7	11.7	13	21.7	21	26.3		
Polymorphism								
TT	28	46.7	22	36.7	17	21.3	10.33	0.005
(TC + CC)	32	53.3	38	63.3	63	78.7		
Allele								
T	81	67.5	69	57.5	76	47.5	11.23	0.003
C	39	32.5	51	42.5	84	52.5		

statistically significant difference between HCC patients and each of the other groups. HCC patients had a higher incidence of CC and TC genotypes when compared to cirrhotic patients and healthy controls ( $P = 0.02, 0.005$ ), respectively. Allele frequencies showed a statistically higher incidence of C allele in HCC patients compared to cirrhotic patients and healthy controls ( $P = 0.003$ ).

These results are similar to the results obtained by Li et al. and Yan et al. who found that there was a significant difference in the distribution of miR-196a2 genotypes and alleles between HCC cases and the two other groups [17, 18].

In contrast with the present study, Chu et al. stated that the miR-196a2 genotype distribution among HCC patients was not significantly different from that among the two other groups [19].

**Table 4** Comparison of polymorphism between Control and HCC groups

	Control N = 60		HCC N = 80		$\chi^2$	P value	OR(95%CI)
	No.	%	No.	%			
Polymorphism							
TT	28	46.7	17	21.3			TT is a reference
TC	25	41.6	42	52.4	11.38	0.003	CC = 4.94 [1.74–14.07]
CC	7	11.7	21	26.3			TC = 2.77 [1.27–6.04]
Polymorphism							
TT	28	46.7	17	21.3	10.15	0.001	3.24 [1.55–6.78]
(TC + CC)	32	53.3	63	78.7			
Allele							
T	81	67.5	76	47.5	11.14	0.0008	2.30 [1.40–3.76]
C	39	32.5	84	52.5			

**Table 5** Comparison of polymorphism between Cirrhosis and HCC groups

	Control N = 60		HCC N = 80		$\chi^2$	P value	OR(95%CI)
	No.	%	No.	%			
Polymorphism							
TT	22	36.7	17	21.3			TT is a reference
TC	25	41.6	42	52.4	4.06	0.13	CC = 2.09 [0.82–5.34]
CC	13	21.7	21	26.3			TC = 2.17 [0.97–4.86]
Polymorphism							
TT	22	36.7	17	21.3	4.05	0.04	2.15 [1.01–4.54]
(TC + CC)	38	63.3	63	78.7			
Allele:							
T	69	57.5	76	47.5	2.75	0.09	1.50 [0.93–2.41]
C	51	42.5	84	52.5			

Also in a meta-analysis done by Liu et al. [20], it has been found that the miR-196a2 genotypes were associated with a decreased susceptibility of HCC frequency.

On the other hand, Tian and his colleagues reported that the distribution of the miR-196a2 (rs11614913) polymorphism did not affect HCC susceptibility [5].

In the present study, the comparison of the polymorphism between the control and the HCC groups confirmed that both TC genotype and CC genotype were associated with a significantly increased risk of HCC when compared with the TT genotype.

Moreover, when comparing (C) allele versus (T) allele distributions in the studied groups, C allele was found to be associated with a significantly increased risk of HCC with 2.30-fold (OR = 2.30, 95% CI = 1.40–3.76).

Our results are comparable to the results obtained by Zhao et al. [21], who found that both CC genotype and C allele is at increased risk for HCC “CC vs TT (OR = 1.302, 95% CI = 1.019–1.663) and C vs. T (OR = 1.130, 95% CI = 1.004–1.272)”.

Also, Yan et al. [18] reported that individuals carrying the TC and CC genotypes of miR-196a2 were found to be associated with an elevated risk of HCC compared to the TT genotype, with an adjusted odds ratio of 1.50 (1.03–2.17) and 2.86(1.60–5.16), respectively.

In contrast to the present study, Chu et al. [19] suggested that the interaction between studied gene polymorphisms and cancer risk factors was not statistically significant.

The present study shows that the TC + CC genotypes were significantly associated with 3.24-fold increased risk of HCC when compared with the TT genotype (OR = 3.24, 95% CI = 1.55–6.78).

Li et al. documented that the TC + CC genotypes of rs11614913 polymorphism were significantly associated with an increased risk of HCC (TT vs. TC + CC: OR = 2.52, 95% CI = 1.18–4.19;  $P < 0.05$ ) [22].

**Table 6** Relation between genotypes and socio-demographic data among HCC patients

Studied variable	Genotype						Chi-square test	P value
	CC N = 21		TC N = 42		TT N = 17			
Age (years) X ± SD	52.50 ± 4.628		54.48 ± 4.77		56.06 ± 6.329		2.96*	0.06
	<b>No.</b>	<b>(%)</b>	<b>No.</b>	<b>(%)</b>	<b>No.</b>	<b>(%)</b>		
Gender								
Male	10	(47.6)	20	(47.6)	12	(70.6)	0.49	0.78
Female	11	(52.4)	22	(52.4)	5	(29.4)		
Smoking								
Yes	12	(57.1)	16	(38.1)	10	(58.8)	3.15	0.20
No	9	(42.9)	26	(61.9)	7	(41.2)		
Family history								
Yes	5	(23.8)	12	(28.6)	5	(29.4)	0.20	0.90
No	16	(76.2)	30	(71.4)	12	(70.6)		
Spleen								
Average	5	(23.8)	5	(11.9)	1	(5.9)	2.50**	0.25
Splenomegaly	16	(76.2)	37	(88.1)	16	(94.1)		
Liver								
Hepatomegaly	6	(28.6)	7	(16.7)	5	(29.4)	3.01	0.55
Cirrhotic	14	(66.7)	34	(81.0)	12	(70.6)		
Shrunken	1	(4.8)	1	(2.4)	0	(0.0)		
Ascites								
Present	16	(76.2)	38	(90.5)	13	(76.5)	3.20	0.20
No	5	(23.8)	4	(9.5)	4	(23.5)		
Encephalopathy								
Present	12	(57.1)	28	(66.7)	10	(58.8)	0.66	0.71
Absent	9	(42.9)	14	(33.3)	7	(41.2%)		
Child score								
A	7	(33.3)	13	(31.0)	4	(23.5)	1.61**	0.80
B	6	(28.6)	17	(40.5)	6	(35.3)		
C	8	(38.1)	12	(28.6)	7	(41.2)		

\*ANOVA test

\*\*Fisher's exact test

Also, Chen et al. [12] stated that the rs11614913 polymorphism of miR-196a-2 carry a significant increased risk for HCC development (C vs T: OR = 1.14, 95% CI = 1.06–1.23,  $P = 0.001$ ; CC vs TT: OR = 1.31, 95% CI = 1.12–1.53,  $P = 0.001$ ; TC + CC vs TT: OR = 1.16, 95% CI = 1.03–1.31,  $P = 0.018$ ; CC vs TT: OR = 1.14, 95% CI = 1.00–1.30,  $P = 0.043$ ).

In the contrary, a meta-analysis done by Peng et al. [23] stated that there was no evidence of significant association between miR-196a2 rs11614913 polymorphism and HCC risk when all eligible studies were pooled into the meta-analysis (CC vs TT: OR = 1.287, 95% CI = 0.931–1.607,  $P = 0.226$ ; TC vs TT: OR = 1.055, 95% CI = 0.958–1.161,  $P = 0.278$ ; TC + CC vs TT: OR = 1.134, 95% CI = 0.974–1.320,  $P = 0.105$ ).

Additionally, the results obtained by Kim and his colleagues reported that the miR-196a-2 rs12304647 CC

genotype had a protective effect against the development of HCC in patients with chronic hepatitis B infection and cirrhosis [24].

In the present study, there is no statistically significant difference between HCC and cirrhotic groups as regarding genotypes and alleles  $P = 0.13$ ,  $0.09$ , respectively.

Study of the correlation between miR-196a2 genotypes and personal history and clinical data, including spleen, liver, ascites, child classification, encephalopathy, and tumor size, shows that there was no statistically significant difference data among HCC patients as regards personal history and clinical data.

Chu et al. [19] suggested that a significant association between miRNA499 SNPs and HCC is present. However, gene-environmental interactions of miRNA499 polymorphisms, smoking, and alcohol consumption might alter the HCC susceptibility.

**Table 7** Relation between genotypes and clinicopathological data among HCC patients

Studied variable	Genotype			Kruskal–Wallis test	P value
	CC N = 21	TC N = 42	TT N = 17		
AST (U/L)					
Min-max	12.0–302.0	21.0–540.0	14.00–258.00		
X ± SD	83.96 ± 70.88	158.04 ± 269.99	90.56 ± 78.90	0.38	0.82
Median	67.50	93.00	66.00		
ALT (U/L)					
Min-max	11.0–292.00	12.0–379.0	11.0–205.0		
X ± SD	80.14 ± 61.68	83.57 ± 83.45	67.82 ± 49.62	1.10	0.57
Median	64.000000	59.00	53.0		
ALB (g/dL)					
Min-max	2.0–4.0	2.0–4.0	2.0–4.0		
X ± SD	2.73 ± .70	2.54 ± .58	2.61 ± .67	1.25	0.53
Median	3.0	3.00	2.0		
BT (mg/dL)					
Min-max	1.0–16.0	1.0–20.0	1.0–9.0		
X ± SD	4.03 ± 3.96	3.66 ± 3.54	3.36 ± 2.43	0.05	0.97
Median	2.0	3.00	3.00		
BD (mg/dL)					
Min-max	1.0–16.0	1.0–15.00	1.0–8.0		
X ± SD	3.58 ± 4.46	2.55 ± 2.92	3.03 ± 2.56	0.94	0.62
Median	1.00	1.0	2.0		
AFP (ng/mL)					
Min-max	12–5200	11–20,949	6–19,007		
X ± SD	1586.48 ± 1513.68	1801.07 ± 4254.21	2052.71 ± 4561.92	3.35	0.18
Median	615.00	420.00	680.00		
Focal lesion					
Min-max	1.50–9.00	1.50–8.00	2.00–11.00		
X ± SD	4.81 ± 2.10	3.99 ± 1.72	4.75 ± 2.07	1.5	0.22
Median	5.00	4.0	4.50		
PT. conc					
Min-max	19–96	20–102	29–89		
X ± SD	56.26 ± 18.66	59.40 ± 16.54	58.43 ± 16.53	0.73	0.69
Median	55.00	63.00	57.00		
PT.INR					
Min-max	1.03–2.71	.95–3.59	1.08–2.40		
X ± SD	1.54 ± .45	1.45 ± .45	1.47 ± .37	0.61	0.73
Median	1.4150	1.3300	1.45		

Studying the correlation between miR-196a2 rs11614913 genotypes and laboratory characteristics, there was no statistically significant difference between the different genotypes among the HCC cases regarding lab investigations (ALT, AST, albumin, total and direct bilirubin, INR, and AFP).

In our analysis of the association between this polymorphism and the HCC aggressiveness as regards the AFP level and size and number of the focal lesions, we noticed that there was no statistically significant

difference between the different genotypes regarding either the level of AFP (*P* value, 0.76) or the size and number of the focal lesions (*P* value, 0.99). However, 66.7% of patients with the CC genotype had a high level of AFP. Moreover, 61.9% of patients with the CC genotype had multiple focal lesions.

Li and his co-workers reported that, in a subsequent analysis of the association between microRNA-196a2 and clinic-pathological characteristics, there was an association between rs11614913 genotype and



**Table 8** Relation between the different genotypes and the aggressiveness of HCC (size and number of focal lesions and AFP level) among HCC patients

Studied variable	Genotype						Chi-square test	P value
	CC		TC		TT			
	No.	(%)	No.	(%)	No.	(%)		
AFP								
<= 400	7	33.3	18	42.9	7	41.2	0.54	0.76
> 400	14	66.7	24	57.1	0	58.8		
Focal lesion size								
<= 3	9	42.9	18	42.9	7	41.2	0.01	0.99
> 3	12	57.1	24	57.1	10	58.8		
No. of focal lesion:								
Multiple	10	47.6	26	61.9	10	58.8	1.18	0.55
Single	11	52.4	16	38.1	7	41.2		

tumor size ( $P = 0.046$ ), but not with tumor number [17].

## Conclusion

The findings of this study suggested that miR-196a2 polymorphism is associated with HCC risk in the Egyptian population. More well-designed studies based on larger sample sizes and more ethnic groups are still needed in the future.

## Abbreviations

AFP: Alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; bp: Base pair; CT: Computed tomography; DNA: Deoxyribonucleic acid; EDTA: Ethylenediaminetetraacetic acid; HBV: Hepatitis B virus; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HOX: Homeobox; INR: International normalized ratio; miR: MicroRNA; NASH: Non-alcoholic steatohepatitis; PCR-RFLP: Polymerase chain reaction-restriction fragment length polymorphism; SNP: Single-nucleotide polymorphism

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## Authors' contributions

All authors read and approved the final manuscript. EG had selected the idea and design of the work and had made the final revision of data. GR did the data gathering, analysis, and manuscript preparation. EO and AS did the data gathering and manuscript preparation. EA did the design, data gathering, analysis, and manuscript preparation.

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## Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available due to confidential and institutional ethical issues but, are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

The study was conducted in accordance with the ethical principles of the 1975 Declaration of Helsinki, and it was reviewed and approved by the Research Ethics Committee of National Liver Institute, Menoufia University.

Written informed consent was obtained from all the participants after explaining the aim and concerns of the study (ethics committee reference number: not available).

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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