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Association of MicroRNA Polymorphisms With Hepatocellular Carcinoma in an Iranian Population

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Background: Single nucleotide polymorphisms (SNPs) can modulate various biological processes by influencing microRNA (miRNA) biogenesis and altering target selection. Common SNPs may alter the processing of miRNA and may be associated with hepatocellular carcinoma (HCC). We investigated the relationship between *miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C and HCC susceptibility, examining the interaction of the miRNAs with hepatitis B virus (HBV).

Methods: We evaluated the associations of *miR-499*A>G (rs3746444), *miR-149*C>T (rs2292832), *miR-196a2*T>C (rs11614913), and *miR-146a*G>C (rs2910164) with HCC susceptibility in 100 HCC patients (70 males and 30 females) and 120 healthy controls (70 males and 50 females), using the PCR-restriction fragment length polymorphism method.

Results: For *miR-499*A>G, the frequencies of the AG genotype and G allele were higher in female HCC patients than in female controls (P=0.02 and 0.045, respectively). The frequency of the A allele was higher in HBV-positive HCC patients than in controls (P=0.019). For *miR-149*C>T, the frequency of the CC genotype was higher in female HCC patients than in female controls (P=0.009). For *miR-196a2*T>C, the frequencies of the CT and CC genotypes and the C allele were higher in HBV-positive HCC patients than in controls (P<0.001, P=0.009, and P<0.001, respectively). The frequencies of *miR-146a*G>C polymorphisms did not differ between HCC patients and controls.

Conclusions: *miR-499*A>G, *miR-149*C>T, and *miR-196a2*T>C were associated with the development of HCC in women and/or that of HBV-related HCC. They can be considered genetic risk factors for the development of HCC among Iranians.

Key Words: Single nucleotide polymorphism, MicroRNA, Hepatocellular carcinoma, Hepatitis B virus

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the cause of more than half a million deaths each year, making it the third most common cause of cancer-related death worldwide [1]. This disease has several risk factors, including chronic hepatitis B virus (HBV) and hepatitis C virus infections, alcohol abuse, liver cirrhosis, and exposure to aflatoxin B1. However, most individuals with these risk factors never develop HCC, and many HCC cases develop in individuals without the above risk factors; thus, other factors, such as genetics, may play an important role in the development of HCC [2].

Recent studies have demonstrated that a new class of small regulatory RNA molecules, termed microRNAs (miRNAs), are

involved in HCC progression [3, 4]. miRNAs are a group of small noncoding RNA molecules that have been identified in many organisms. They can regulate the expression of genes in a variety of eukaryotic systems. miRNAs are transcribed from endogenous DNA and processed from primary transcripts (pri-miRNAs) to hairpin precursors (pre-miRNA) comprising two strands: the leading strand, which is used for production of mature miRNA, and the passenger strand, which is believed to be degraded. Mature miRNAs are involved in post-transcriptional gene expression by base pairing with target mRNAs of protein-coding genes [5]. miRNAs play essential roles in many physiological processes, such as development [6], cellular variation [7], proliferation, cell death, and metabolism [8-10]. Many studies have confirmed that alterations in miRNA expression are involved in cancer development by regulating the expression of proto-oncogenes or tumor-suppressor genes [5, 11, 12]. miRNA polymorphisms may be involved in hepatocarcinogenesis and can determine the susceptibility of an individual to the development of HCC.

Single nucleotide polymorphisms (SNPs) are common genetic variations in the human genome. SNPs in protein-coding genes can affect the functions of proteins and in turn influence susceptibility to a specific cancer [13, 14]. Recently, four well-known miRNA polymorphisms (*miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C) have been shown to contribute to different types of cancers, such as breast cancer, gastric cancer, cervical squamous cell cancer, and colorectal cancer [15-19].

We investigated the potential associations of *miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C with susceptibility to HCC and the interactions of these miRNAs with HBV in order to confirm whether these SNPs can serve as biomarkers for susceptibility to HCC in Iranian patients. We studied the interaction of the miRNAs with only HBV since HBV infection is a widespread risk factor for HCC in the Iranian population.

METHODS

Patients and controls

In this retrospective study, 100 patients diagnosed as having HCC (based on liver biopsy) between October 2008 and December 2013 were recruited. During the same period, 120 controls without clinical liver disease, recruited among random donors from a blood transfusion organization, were matched to the patients by age and sex. Of the HCC patients, 70 were male, and the median age was 36 (range: 10–60) years. Of the controls, 70 were male, and the median age was 38 (range: 25–60) years. All patients and controls were of Iranian descent and admitted to Namazi Hospital, Shiraz, Iran, during the study period. All completed a questionnaire on demographic characteristics, history of cancer, alcohol use, and tobacco use. Those with a history of cancer, alcohol use, or tobacco use were excluded. Patients' clinical characteristics, including tumor differentiation, tumor size, metastasis, cirrhosis, Child-Pugh class and chemotherapy, were collected from medical records. Their characteristics are shown in Table 1. Both patients and controls were at Hardy–Weinberg equilibrium for all polymorphisms ($P>0.05$).

The Ethics Committee of Shiraz University of Medical Sciences approved the study protocol, which conformed to the 1975 Helsinki Declaration. Written informed consent was obtained from all patients and controls.

DNA extraction and genotyping

Genomic DNA from patients was extracted from HCC biopsy tissue samples using a DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany), and genomic DNA from the control group was extracted from whole blood and purified using the phenol-chloroform extraction method. Different isolation methods were used for DNA from patients and controls because the samples were from different tissue sources, and we could not obtain biopsy tissues from the control group but rather had to use blood. The purity of the extracted DNA samples was confirmed by check-

Table 1. Characteristics of HCC patients (N=100) and controls (N=120)

Characteristics	N
HBV positive	35
HCV positive	5
Non-viral	60
Cirrhosis	58
α-fetoprotein (μg/L)	
<100	53
100–400	21
>400	26
TNM stage III–IV	87
Female controls	50
Male controls	70
Female HCC patients	30
Male HCC patients	70

Abbreviations: HBV, Hepatitis B virus; HCV, Hepatitis C virus; HCC, hepatocellular carcinoma.

ing the 260/280 ratio of each sample.

Polymorphisms of *miR-499*A>G (rs3746444), *miR-149*C>T (rs2292832), *miR-196a2*T>C (rs11614913), and *miR-146a*G>C (rs2910164) were analyzed using PCR-restriction fragment length

Table 2. Primer sequences used for miRNA amplification, restriction enzymes, and product sizes

Locus (rs)	Primers	Restriction enzymes	Product sizes
miR-499 A>G	Forward: 5′-CAAAGTCTTCACTTCCCTGCCA-3′	BclI	GG: 146 bp
(rs3746444)	Reverse: 5′-GATGTTTAACTCCTCTCCACGTGAT*C-3′		AG: 146+121+25 bp AA: 121+25 bp
miR-149 C>T	Forward: 5′-CGAGGCTCCCAGGCCTTC-3′	AluI	TT: 224+66 bp
(rs2292832)	Reverse: 5′-CTGCAGGTTCTGAGGGGCT-3′		CT: 224+153+71+66 bp CC: 153+71+66 bp
miR-196a2 T>C	Forward: 5′-CCCCTTCCCTTCTCCTCCAGATA-3′	MspI	TT: 149 bp
(rs11614913)	Reverse: 5′-CGAAAACCGACTGATGTAACTCCG-3′		CT: 149+125+24 bp CC: 125+24 bp
miR-146a G>C	Forward: 5′-CATGGGTTGTGTCAGTGTCAGAGCT-3′	SacI	GG: 147 bp
(rs2910164)	Reverse: 5′-TGCCTTCTGTCTCCAGTCTTCCAA-3′		AG: 147+122+25 bp AA: 122+25 bp

*The highlighted nucleotide was included to produce a restriction site to detect and digest by the corresponding restriction enzyme.
Abbreviation: miRNA, microRNA.

Table 3. Genotype and allele frequencies of the *miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C polymorphisms in HCC patients and controls

SNP (rs)	Genotype	HCC patients	Controls	*P**	OR (95% CI)
		N (%)	N (%)		
miR-499	GG	39 (39)	51 (42.5)	0.59	1.16 (0.65–2.06)
(rs3746444)	AG	40 (40)	32 (26.7)	0.52	0.55 (0.30–1)
	AA	21 (21)	37 (30.8)	0.09	1.68 (0.87–3.26)
	G allele	118 (59)	134 (55.8)	0.50	0.88 (0.59–1.31)
	A allele	82 (41)	106 (44.2)	0.50	1.14 (0.76–1.69)
miR-149	TT	42 (35)	35 (35)	1	1 (0.55–1.81)
(rs2292832)	TC	58 (48.3)	41 (48)	0.27	0.74 (0.42–1.31)
	CC	20 (16.7)	24 (24)	0.17	1.58 (0.77–3.23)
	T allele	142 (59.1)	111 (55.5)	0.43	0.86 (0.58–1.28
	C allele	98 (40.9)	89 (44.5)	0.43	1.16 (0.78–1.73)
miR-196a2	TT	17 (17)	20 (16.7)	0.94	0.98 (0.45–2.10)
(rs11614913)	TC	57 (57)	59 (49.2)	0.24	0.73 (0.41–1.29)
	CC	26 (26)	41 (34.2)	0.18	1.48 (0.79–2.77)
	T allele	91 (45.5)	101 (42)	0.47	0.87 (0.59–1.29)
	C allele	109 (54.5)	139 (58)	0.47	1.15 (0.77–1.71)
miR-146a	GG	64 (64)	74 (61.7)	0.72	0.90 (0.50–1.63)
(rs2910164)	GC	22 (22)	33 (27.5)	0.34	1.34 (0.69–2.62)
	CC	14 (14)	13 (10.8)	0.47	0.75 (0.31–1.79)
	G allele	150 (75)	181 (75.4)	0.91	1.02 (0.65–1.62)
	C allele	50 (25)	59 (24.6)	0.91	0.98 (0.62–1.55)

**P* values were obtained using chi-square tests and Fisher's exact tests by comparing corresponding rows with the sums of the other rows.
Abbreviations: OR, odds ratio; HCC, hepatocellular carcinoma; CI, confidence interval; SNP, single nucleotide polymorphism.

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polymorphism (RFLP). The four polymorphic regions were amplified using previously used primers [20] with slight modifications (Table 2) and the following PCR conditions: one cycle at 95°C for 5 minutes; 40 cycles at 95°C for 1 minute, 59°C for 1 minute, and 72°C for 1 minute; and one final cycle of extension at 72°C for 10 minutes. The *miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C polymorphisms were detected by digesting the PCR products with restriction endonucleases BclI, AluI, MspI, and SacI, respectively (Thermo Fisher Scientific, Waltham, MA, USA). The reaction products (5 μL) were run on 3.0% agarose gels stained with ethidium bromide and directly visualized under ultraviolet illumination. For each of the miRNA polymorphisms, 12 samples were randomly chosen for a second PCR assay followed by DNA sequencing to validate the RFLP findings.

Statistical analysis

Allele and genotype frequencies were calculated in the patients and controls by direct gene counting. Frequencies were compared between patients and controls using chi-square tests and Fisher's exact tests. All reported *P* values were two-tailed. $P<0.05$ was considered statistically significant. Bonferroni correction was performed for each test. Statistical analyses were performed using SPSS version 16 (IBM Corp., Armonk, NY, USA). Hardy–Weinberg equilibrium was measured using Arlequin V311 (http://cmpg.unibe.ch/software/arlequin3). The study power of each test was calculated using power SSC ver. 1 (Tarbiat Modares University, Tehran, Iran).

RESULTS

As shown in Table 3, the genotypic and allelic frequencies of *miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C polymorphisms did not differ between HCC patients and controls. After classification of patients and controls according to sex (Tables 4 and 5), the frequencies of the AG genotype and

Table 4. Genotype and allele frequencies of the *miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C polymorphisms in male HCC patients and controls

SNP (rs)	Genotype	HCC patients	Controls	*P**	OR (95% CI)
		N (%)	N (%)		
miR-499	GG	29 (41.5)	36 (51.4)	0.23	1.5 (0.73–3.09)
(rs3746444)	AG	22 (31.4)	15 (21.5)	0.18	0.6 (0.26–1.36)
	AA	19 (27.1)	19 (27.1)	1	1 (0.44–2.25)
	G allele	80 (57.1)	87 (62.1)	0.39	1.23 (0.74–2.04)
	A allele	60 (42.9)	53 (37.9)	0.39	0.81 (0.49–1.35)
miR-149	TT	26 (37.1)	33 (47.1)	0.23	1.51 (0.73–3.14)
(rs2292832)	TC	25 (35.7)	22 (31.4)	0.59	0.82 (0.38–1.77)
	CC	19 (27.2)	15 (21.5)	0.43	0.73 (0.31–1.70)
	T allele	77 (55)	88 (62.8)	0.18	1.38 (0.83–2.30)
	C allele	63 (45)	52 (37.2)	0.18	0.72 (0.44–1.20)
miR-196a2	TT	7 (10)	11 (15.7)	0.31	1.68 (0.55–5.19)
(rs11614913)	TC	42 (60)	33 (47.1)	0.12	0.59 (0.29–1.23)
	CC	21 (30)	26 (37.1)	0.37	1.38 (0.64–2.96)
	T allele	56 (66.6)	55 (39.2)	0.90	0.97 (0.58–1.61)
	C allele	84 (33.4)	85 (60.1)	0.90	1.03 (0.62–1.71)
miR-146a	GG	47 (61.7)	44 (62.9)	0.59	0.83 (0.39–1.76)
(rs2910164)	GC	13 (18.6)	17 (24.3)	0.41	1.41 (0.58–3.43)
	CC	10 (14.3)	9 (12.9)	0.80	0.89 (0.30–2.57)
	G allele	107 (76.4)	105 (75)	0.78	0.93 (0.52–1.66)
	C allele	33 (23.6)	35 (25)	0.78	1.08 (0.60–1.94)

**P* values were obtained using chi-square tests and Fisher's exact tests comparing the corresponding rows with the sum of the other rows.
Abbreviations: OR, odds ratio; HCC, hepatocellular carcinoma; CI, confidence interval; SNP, single nucleotide polymorphism.

Table 5. Genotype and allele frequenciwes of the *miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C polymorphisms in female HCC patients and controls

SNP (rs)	Genotype	HCC patients	Controls	*P**	OR (95% CI)
		N (%)	N (%)		
miR-499	GG	10 (33.3)	15 (30)	0.75	0.86 (0.29–2.53)
(rs3746444)	AG	18 (60)	17 (34)	0.02†	0.34 (0.12–0.97)
	AA	2 (6.7)	18 (36)	0.006‡	7.88 (1.54–53.98)
	G allele	38 (63.3)	47 (47)	0.045†	0.51 (0.25–1.04)
	A allele	22 (36.4)	53 (53)	0.045†	1.95 (0.96–3.96)
miR-149	TT	7 (23.3)	9 (18)	0.56	0.72 (0.21–2.51)
(rs2292832)	TC	13 (43.4)	36 (72)	0.010‡	3.36 (1.18–9.77)
	CC	10 (33.3)	5 (10)	0.009‡	0.22 (0.06–0.83)
	T allele	27 (45)	54 (54)	0.27	1.43 (0.27–2.87)
	C allele	33 (55)	46 (46)	0.27	0.70 (0.35–1.39)
miR-196a2	TT	10 (33.3)	9 (18)	0.11	0.44 (0.14–1.41)
(rs11614913)	TC	15 (50)	26 (52)	0.86	1.08 (0.40–2.96)
	CC	5 (16.7)	15 (30)	0.18	2.14 (0.62–7.82)
	T allele	35 (58.3)	44 (44)	0.07	0.56 (0.28–1.13)
	C allele	25 (41.6)	56 (56)	0.07	1.78 (0.89–3.59)
miR-146a	GG	17 (56.7)	30 (60)	0.76	1.15 (0.41–3.17)
(rs2910164)	GC	9 (30)	16 (32)	0.85	1.10 (0.37–3.29)
	CC	4 (13.3)	4 (8)	0.44	0.57 (0.11–3)
	G allele	43 (71.6)	76 (76)	0.54	1.25 (0.57–2.75)
	C allele	17 (28.3)	24 (24)	0.54	0.80 (0.36–1.76)

*For genotypes, *P* values were obtained using chi-square tests and Fisher's exact tests by comparing the corresponding rows with the sum of the other rows; †For alleles, $P<0.05$ was considered statistically significant; ‡For genotypes, $P<0.017$ was considered statistically significant (Bonferroni correction).
Abbreviations: OR, odds ratio; HCC, hepatocellular carcinoma; CI, confidence interval; SNP, single nucleotide polymorphism.

the G allele in the *miR-499*A>G polymorphism were higher in female patients than in female controls (OR=0.34, 95% confidence interval [CI]=0.12–0.970, $P=0.02$, study power=67%; OR=0.51, 95% CI=0.25–1.04, $P=0.045$, study power=72%, respectively). In contrast, the frequencies of the AA genotype and the A allele in *miR-499*A>G polymorphism were lower in female patients than in female controls (OR=7.03, 95% CI=1.36–48.47, $P=0.006$, study power=98%; OR=1.95, 95% CI=0.96–3.96, $P=0.045$, study power=62%, respectively).

In addition, for the *miR-149*C>T polymorphism, the frequency of the CC genotype was higher in female patients than in female controls (OR=0.22, 95% CI=0.06–0.83, $P=0.009$, study power=88%). In contrast, the frequency of the TC genotype was lower in female patients than in female controls (OR=3.36, 95% CI=1.18–9.77, $P=0.010$, study power=79%).

Further analysis was performed to determine the relationship between the four miRNA polymorphisms and HBV-related HCC (Table 6). For the *miR-499*A>G polymorphism, the frequencies of the G allele and the GG genotype were lower in HBV-positive HCC patients than in controls (OR=1.9, 95% CI=1.07–3.38, $P=0.019$, study power=65%; OR=2.49, 95% CI=0.98–6.54, $P=0.035$, study power=69%, respectively). In contrast, the frequency of the A allele was higher in HBV-positive HCC patients than in controls (OR=0.53, 95% CI=0.3–0.94, $P=0.019$, study power=68%).

In addition, for the *miR-196a2*T>C polymorphism, the frequencies of the CT and CC genotypes and the C allele were higher in HBV-positive HCC patients than in controls (OR=0.28, 95% CI=0.12–0.66, $P=0.001$, study power=89%; OR=0.3, 95% CI=0.11–0.85, $P=0.009$, study power=97%; OR=0.24, 95% CI=0.13–0.44, $P<0.001$, study power=99%). In contrast, the frequencies of the TT genotype and the T allele were lower in HBV-positive HCC patients than in controls (OR=9.65, 95% CI=3.29–30.68, $P<0.001$, study power=96%; OR=4.09,

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Table 6. Genotype and allele frequencies of the *miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C polymorphisms in HBV-positive HCC patients and controls

SNP (rs)	Genotype	HBV-positive HCC patients	Controls	*P**	OR (95% CI)
		N (%)	N (%)		
miR-499	GG	8 (22.8)	51 (42.5)	0.035†	2.49 (0.98–6.54)
(rs3746444)	AG	12 (34.3)	32 (26.7)	0.38	0.70 (0.29–1.69)
	AA	15 (42.9)	37 (30.8)	0.18	0.59 (0.26–1.38)
	G allele	28 (40)	134 (55.8)	0.019†	1.90 (1.07–3.38)
	A allele	42 (60)	106 (44.2)	0.019†	0.53 (0.30–0.94)
miR-149	TT	7 (20)	35 (35)	0.098	2.15 (0.79–6.05)
(rs2292832)	CT	16 (45.7)	41 (48)	0.63	0.83 (0.35–1.92)
	CC	12 (34.3)	24 (24)	0.23	0.61 (0.24–1.52)
	T allele	30 (42.8)	111 (55.5)	0.068	1.66 (0.93–2.99)
	C allele	40 (57.2)	89 (44.5)	0.068	0.60 (0.33–1.08)
miR-196a	TT	5 (14.3)	74 (61.7)	<0.001‡	9.65 (3.26–30.68)
(rs11614913)	CT	20 (57.1)	33 (27.5)	0.001‡	0.28 (0.12–0.66)
	CC	10 (28.6)	13 (10.8)	0.009‡	0.30 (0.11–0.85)
	T allele	30 (42.8)	181 (75.4)	<0.001‡	4.09 (2.26–7.42)
	C allele	40 (52.7)	59 (24.6)	<0.001‡	0.24 (0.13–0.44)
miR-146a	GG	17 (48.6)	74 (61.7)	0.16	1.70 (0.75–3.89)
(rs2910164)	GC	11 (31.4)	33 (27.5)	0.65	0.83 (0.34–2.03)
	CC	7 (20)	13 (10.8)	0.23	0.49 (0.16–1.50)
	G allele	45 (64.3)	181 (75.4)	0.06	1.70 (0.93–3.13)

*For genotypes, *P* values were obtained using chi-square tests and Fisher's exact tests by comparing the corresponding rows with the sum of the other rows; †For alleles, $P<0.05$ was considered statistically significant; ‡For genotypes, $P<0.017$ was considered statistically significant (Bonferroni correction).
Abbreviations: OR, odds ratio; HCC, hepatocellular carcinoma; CI, confidence interval; SNP, single nucleotide polymorphism; HBV, hepatitis B virus.

95% CI=2.26–7.42, $P<0.001$, study power=94%, respectively).

DISCUSSION

Identifying the genetic biomarkers of HCC susceptibility may contribute to lowering HCC-related mortality through early diagnosis, patient care, and personalized therapy [21]. We investigated the potential associations of *miR-499*A>G, *miR-149*C>T, *miR-196a2*T>C, and *miR-146a*G>C with HCC susceptibility and the interactions of these miRNAs with HBV infection in Iranian patients. We found that the genotypic and allelic frequencies of *miR-146a* and *miR-196a2* polymorphisms did not significantly differ between HCC patients and controls. However, the genotypic and allelic frequencies of *miR-499* and *miR-149* polymorphisms significantly differed between female patients and female controls. Further analysis showed that *miR-499* and *miR-196a2* polymorphisms were associated with the risk of HBV-related HCC.

He *et al*.'s [22] meta-analysis examined the potential associations between the four common SNPs (*miR-499*A>G, rs3746444; *miR-149*C>T, rs2292832; *miR-196a2*T>C, rs11614913; and *miR-146a*G>C, rs2910164) and cancer risk. They demonstrated that the rs11614913TT and the rs2910164C genotypes were significantly associated with decreased cancer risk, particularly colorectal and lung, esophageal, cervical, prostate cancers, and HCC; however, the rs3746444G allele was a risk factor for cancers in the Asian population. A previous study suggested that *miR-146a* rs2910164 and *miR-499* rs3746444 polymorphisms may not be associated with the risk of HCC, particularly in the Asian population [23]. Another study showed that *miR-196* family members inhibit cell migration, invasion, and metastasis, which are functionally linked to expression of homeobox protein HoxC8 (HOXC8) through a mechanism involving translational repression [24].

Importantly, our results showed that the frequencies of the C allele, the homozygous CC genotype, and the heterozygous CT genotype in *miR-196a2* were significantly higher in HBV-positive HCC patients than in controls. In contrast, the frequencies of

the T allele and the homozygous TT genotype were significantly lower in HBV-positive HCC patients than in controls. Our results showed that inheritance of the *miR-196a2* C allele and the CC and CT genotypes may increase the risk of HCC in HBV-positive patients by affecting the expression of mature *miR-196a2* and HOXC8, thereby enhancing cell migration/invasion. Our results provide evidence that the *miR-196a2* polymorphism may be an indicator of HCC susceptibility in HBV-positive patients. Xu *et al*. [25] 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. Polymorphisms in pre-*miR-196a-2* have been associated with HCC susceptibility in a Turkish population [26]. Other studies have also demonstrated the relationship between this SNP and other cancers. For example, Song *et al*. [27] showed that individuals who inherited the CC genotype were more susceptible to epithelial ovarian cancer than those who inherited the TT or CT genotype. With the finding of increased expression of mature *miR-196a* in patients with the C allele, they concluded that the *miR-196a2* CC genotype may increase the risk of ovarian cancer [27].

miR-146a functions as a pro-apoptotic molecule by inhibiting the nuclear factor kappa B pathway and blocking its influence on cell proliferation, angiogenesis, metastasis, and cancer cell survival. Loss of function of *miR-146a* promotes cancer cell migration and invasion *in vitro*, whereas reciprocally increased *miR-146a* expression inhibits cancer invasion [28, 29]. Functional polymorphisms in *miR-146a* have been associated with the risk of HCC [30], particularly in male patients in China [25]. Another study in China showed a significant association between *miR-146a* and susceptibility to HBV-related HCC [31]. In contrast, in our study and two others in a Turkish population [32, 33], *miR-146a* polymorphisms did not significantly affect susceptibility to HCC. The differences in results could be attributed to variations in genotypic and allelic frequencies of specific genes across ethnic groups. Further analyses of larger populations with different ethnic groups are needed to confirm the association of *miR-146a* polymorphisms with the risk of HCC.

The *SOX6* and *Rod1* genes are two direct targets of *miR-499*. The *SOX6* gene induces G_1/S cell cycle arrest by upregulating p53 and p21 and downregulating cyclin D1/CDK4, cyclin A, and β-catenin [34]. The association of the *miR-499* polymorphism with HCC susceptibility has been studied in Korean [20], Chinese [31, 35], and Turkish populations [32]. We also demonstrated a significant association between the *miR-499* polymorphism and HCC susceptibility in HBV-positive patients. We showed that the frequency of the G allele was significantly lower in HBV-positive HCC patients than in controls. In contrast, the frequency of the A allele was significantly higher in HBV-positive HCC patients than in controls. Similar to our results, Zou *et al*. [35] showed that the *miR-499* G allele was significantly associated with decreased risk of HCC among Chinese patients infected with HBV in a dominant model. Liu *et al*. [36] also demonstrated that *miR-499-5p* promotes cellular invasion and tumor metastasis in colorectal cancer by targeting FOXO4 and PDCD4. However, a study in a Turkish population reported that the *miR-499* polymorphism has no role in genetic susceptibility to HCC [32].

miR-149 is a pro-apoptotic miRNA that can repress the expression of the *Akt1* and *E2F1* genes. Silencing of *Akt1* and *E2F1* can induce apoptosis in human tumor cell lines [37]. We showed that the frequency of the CC genotype (mutant-type homozygote) in the *miR-149* polymorphism was significantly higher in female HCC patients than in female controls. In contrast, the frequency of the TC genotype was significantly lower in female HCC patients than in female controls. Similarly, two other studies reported that *miR-149* polymorphisms are associated with the development of HCC [38, 39]. However, other researchers found a significant decrease in HCC risk with the CT and CT+CC genotypes in *miR-149* in comparison with the TT genotype [20, 40].

Thus, a multicenter or nationwide study of the general Iranian population with a larger sample size is needed to confirm our results. Furthermore, it is still unclear whether the studied polymorphisms resulted in changes in the levels of mature *miR-149* and *miR-499* in HCC tissues. The specific mechanisms through which genetic polymorphisms affect the levels of pre-miRNAs and their target genes should be determined in future studies.

In conclusion, we found that polymorphisms in *miR-499, miR-149*, and *miR-196a2* were associated with the development of HCC in women and/or HBV-related HCC in the Iranian population. Additional studies on the relationships between these miRNA polymorphisms and HCC in diverse ethnic populations are warranted to clarify the importance of these miRNA SNPs as biomarkers for HCC susceptibility.

Authors' Disclosures of Potential Conflicts of Interest

The authors declare that they have no conflicts of interest.

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