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ORIGINAL ARTICLE

Association between single nucleotide polymorphism in miR-499, miR-196a2, miR-146a and miR-149 and prostate cancer risk in a sample of Iranian population

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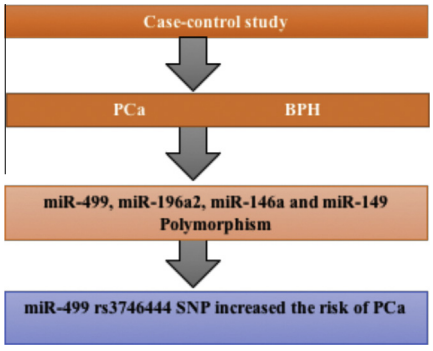
GRAPHICAL ABSTRACT



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ABSTRACT

MicroRNAs (miRNAs) play an important role in regulating gene expression at the post-transcriptional level and are involved in numerous physiological processes. Accumulating evidence suggests that single-nucleotide polymorphisms (SNPs) in human miRNA genes may affect miRNA biogenesis pathway and influence the susceptibility to several diseases such as cancer. The present study aimed to evaluate the impact of miR-499 rs3746444, miR-196a2 rs11614913, miR-149 rs2292832, and miR-146a rs2910164 polymorphisms on prostate cancer (PCa) risk in a sample of Iranian population. This case-control study was done on 169 patients with pathologically confirmed PCa and 182 benign prostatic hyperplasia (BPH). The genotyping assays were done using T-ARMS-PCR or PCR-RFLP methods. The findings indicated that CC genotype of miR-499 rs3746444 polymorphism increased the risk of PCa (OR = 1.76, 95% CI = 1.12–2.79, $P = 0.019$) compared to TT genotype. No statistically significant association was found between miR-196a2 rs11614913, miR-149 rs2292832, and miR-146a rs2910164 polymorphisms and PCa risk. In summary, the findings indicated that miR-499 rs3746444 polymorphism increased the risk of PCa in an Iranian population. Further studies with larger sample sizes and different ethnicities are necessary to verify the findings of the present study.

Introduction

Prostate cancer (PCa) is the most malignant tumor among men in the United States [1]. The lowest incidence rate of PCa is in the Asian population [2,3]. In Iran, the incidence rate of PCa is approximately 9.6 per 100,000 [4,5] which is comparable to Asia–Pacific region (9.9 per 100,000), but considerably lower than the world (32.8 per 100,000) [6].However, the exact mechanisms underlying the development and progression of PCa remain generally unknown. It has been proposed that both genetic and environmental factors contribute to the development and progression of PCa [7–9]. Genetic factors have been estimated to account for over 40% of PCa risk. Single nucleotide polymorphism (SNP) is the most common type of genetic variation in human genome and has been shown to be associated with PCa risk [10–12]. Genomewide association studies (GWAS) showed that more than 100 single nucleotide polymorphisms (SNPs) involved in prostate cancer (PCa) risk. However, the molecular mechanisms are unclear for most of these SNPs [13].

MicroRNAs (miRNAs) are a class of small single-stranded noncoding RNAs usually composed of about 17–25 nucleotides. They widely exist in human cells and regulate gene expression at the posttranscriptional level via either translational repression or mRNA degradation through binding to the 3′-untranslated region (3′-UTR) of target mRNAs [14–16]. miRNAs play an important regulating role in many biological processes, including cell proliferation, differentiation, and apoptosis, and also function as tumor suppressors and oncogenes [17–20].

SNPs residing within the miRNA genes could potentially alter various biological processes by influencing the miRNA biogenesis and altering target selection [21]. SNPs and mutations in miRNAs or miRNA target sites may affect the maturation process or target selection, respectively [22–25]. Several studies investigated the impact of mIR polymorphisms and risk of various cancers. In a meta-analysis performed by Fan et al. [26] revealed no significant association between miR-499 rs3746444 polymorphism and cancer risk. But in subgroup analysis by cancer type, this variant was associated with an increased risk of BC. The findings of a meta-analysis did not support an association between mIR-196a2 rs11614913, mIR-146a rs2910164, and mIR-423 rs6505162 polymorphism and esophageal cancer risk [27]. The findings of a meta-analysis revealed that miR-146a rs2910164 polymorphism is associated with increased risk for cervical and skin squamous cell carcinoma (SCC), while this variant decreased the risk of nasopharyngeal and oral SCC [28].

The miR-149 rs2292832 variant may decrease the risk of digestive cancer [29]. It has been reported that miR-146a rs2910164 polymorphism marginally decreased the risk of gastric cancer [30]. The rs3746444 variant of miR-499 has been reported to be associated with susceptibility to cancer [28].

There is little and inconsistent data regarding the impact of miRNA gene polymorphisms on risk/protection of PCa [31,32]. To the best of our knowledge, there is no report regarding the impact of miRs variants on PCa risk in Iranian population. Hence, the current study was aimed to find out the possible association between miR-499 rs3746444, miR-196a2 rs11614913, miR-146a rs2910164 and mir-149 rs2292832 variants polymorphisms and PCa in a sample of Iranian population.

Patients and methods

Patients

This case-control study was done on 169 unrelated men with histopathologically confirmed adenocarcinoma of prostate and 182 ages matched unrelated men with benign prostatic hyperplasia (BPH) with no history of any cancer. The study design and recruitment procedures were described previously [33]. The demographic and clinicopathological characteristics are shown in Table 1. Briefly, all subjects were registered from Department of Urology, Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. *The project was approved by local Ethics Committee of*

Table 1 Demographic and clinicopathological characteristics of prostate cancer (PCa) and control subjects.

Factors	Prostate Cancer	Control
Age, Mean ± SD (Years)	61.36 ± 6.61	62.51 ± 7.67
Post-void residual, mean ± SD (mL)	27.2 ± 25.2	–
PSA at diagnosis mean ± SD (ng/mL)	14.9 ± 14.3	–
Gealson score		
⩽6	57 (33.7)	–
7	73 (43.2)	–
>7	39 (23.1)	–
Stage		
PT1	8 (4.7)	–
PT2a	27 (16.0)	–
PT2b	11 (6.5)	
PT2c	76 (45.0)	–
PT3a	13 (7.7)	–
Perineural invasion		
Yes	106 (62.7)	–
No	63 (37.3)	–
Impotency		
Yes	26 (15.74)	–
No	143 (84.6)	–
Loss of Libido		
Yes	24 (14.2)	
No	145 (85.8)	
Addiction		
Yes	8 (4.7)	4 (2.2)
No	161 (95.3)	178 (97.8)
Any history of smoking		
Yes	27 (16.0)	6 (3.3)
No	142 (84.0)	176 (96.7)
Alcohol drinking		
Yes	7 (4.1)	0 (0.0)
No	162 (95.9)	182 (0.0)
Hypertension		
Yes	23 (13.6)	5 (2.7)
No	146 (86.4)	177 (97.3)

Zahedan University of Medical Sciences (#7081), and written informed consent was obtained from all cases and controls. Blood samples were collected in EDTA-containing tubes and genomic DNA was extracted using salting out method as described previously [34].

Genotyping

The primers used for detection of miRs polymorphisms are shown in Table 2. Genotyping of miR-146a rs2910164, and miR-196a2 rs11614913 was performed using T-ARMS-PCR assay as described previously [35,36]. Genotyping of miR-499 rs3746444 [37] and miR-149 rs2292832 was performed by PCR-RFLP method. PCR was done using commercially available Prime Taq premix (Genetbio, South Korea) according to the manufacturer's recommended protocol. In each 0.20 mL reaction PCR reaction tube, 1 µL of genomic DNA (~100 ng/mL), 1 µL of each primers (10 µM), 10 µL of 2X Prime Taq Premix and appropriate amount of ddH2O were added. The PCR conditions were set as follows: 5 min at 95 °C, followed by 30 cycles of 30 s at 95 °C, 30 s at 62 °C for rs2910164, 63 °C for rs11614913, 64 °C for rs3746444, 66 °C for rs2292832, and 72 °C for 30 s with a final extension step of 72 °C for 10 min. For detection of rs2292832 and rs3746444 variants, 10 µL of PCR product digested by restriction enzymes (Table 2). The PCR products were electrophoresed on agarose gel containing 0.5 µg/mL ethidium bromide and visualized on a UV transilluminator.

Statistical analysis

Statistical analysis was done using statistical package SPSS 20 software. Data were analyzed by independent sample *t*-test and χ^2 test. Association between polymorphisms and PCa was calculated by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. The statistical level of significance was defined as *P*-value less than 0.05.

Results

The study group consists of 169 Pca patients with an average age of 61.36 ± 6.61 years and 182 benign prostatic hyperplasia

Table 2 The primers used for miR polymorphisms genotyping.

Polymorphism	Sequence (5′ ⩾ 3′)	Restriction enzyme	Product size (bp)
miR-146a rs2910164 G > C	FO: GGCCTGGTCTCCTCCAGATGTTTAT	–	Contol: 364
	RO: ATACCTTCAGAGCCTGAGACTCTGCC		G allele: 249
	FI (C allele): ATGGGTTGTGTCAGTGTCAGACGTC		C allele: 169
	RI (G allele): GATATCCCAGCTGAAGAACTGAATTTGAC		
miR-196a2 rs11614913 C > T	FO: ACCCCCTTCCCTTCTCCTCCAGATAGAT	–	Control: 297
	RO: AAAGCAGGGTTCTCCAGACTTGTTCTGC		T allele: 199
	FI (T allele): AGTTTTGAACTCGGCAACAAGAAACGGT		C allele: 153
	RI (C allele): GACGAAAACCGACTGATGTAACTCCGG		
miR-149 rs2292832 C > T	F: CTCTGGCTCCGTGTCTTCACTC	PvuII	C allele: 225
	R: CCTGCAGGTTCTGAGGGGC		T allele: 154, 71
miR-499 rs3746444 T > C	F: CAAAGTCTTCACTTCCCTGCCA	BclI	C allele: 146
	R: GATGTTTAACTCCTCTCCACGTGATC		T allele: 122, 24

(BPH) with a mean age of 62.51 ± 7.67 years. No significant difference was found between the groups concerning age using independent sample *t*-test ($P = 0.135$).

The genotypes and allele frequencies of miR polymorphisms in PCa and control subjects are shown in Table 3. The results proposed that that TC genotype of miR-499 rs3746444 polymorphism increased the risk of PCa (OR = 1.76, 95% CI = 1.12–2.79, $P = 0.019$) compared to TT genotype, while the minor allele frequency (C allele) of rs3746444 was not associated with PCa.

As shown in Table 3, the miR-196a2 rs11614913, miR-146a rs2910164 and Mir-149 rs2292832 variants were not associated with PCa in any inheritance models tested (co-dominant, dominant and recessive). Regarding miR-149 rs2292832 C > T the result is not strong enough to attain a *P*-value < 0.05 but there is a tendency toward that.

As shown in Table 4, miR-146a rs2910164 variant was significantly associated with stage of disease [contingency coefficient (CC) = 0.333, $P = 0.021$]. The findings showed no significant association between miR-149 rs2292832, miR-196a2 rs11614913 and miR-499 rs3746444 polymorphism and clinicopathological characteristics of the PCa patients (Table 4).

Discussion

In the current study we examined the impact of miR-146a rs2910164, miR-149 rs2292832, miR-196a2 rs11614913 and miR-499 rs3746444 polymorphisms on PCa risk in a sample of Iranian population. We found that miR-499 rs3746444 variant significantly increased the risk of PCa in the population studied. The results did not support an association between miR-146a rs2910164, mir-149 rs2292832 and miR-196a2 rs11614913 polymorphism and PCa risk. Till now, 3 studies investigated the impact of miRNA polymorphisms on PCa susceptibility. Findings of George et al. [38] study showed that heterozygous genotype in miR196a2 and miR-499, heterozygotes confers the increased risk of developing PCa in North Indian population.

Nikolic et al. [31] have found no statistically significant association between miR-499 rs3746444 and miR-196a2 rs11614913 variant and PCa risk in Serbian population. Their findings proposed that rs3746444 variant is associated with PCa aggressiveness so that the rs3746444 minor allele G confers the decreased risk of PCa progression.

Xu et al. [32] found the subjects carrying miR-146a rs2910164 CC had a 0.65-fold reduced risk (95% CI = 0.43–0.99) than those carrying GG/GC genotypes ($P = 0.03$), and the C allele displayed a lower prevalence of PCa compared with the G allele (OR = 0.73, 95% CI = 0.57–0.94, $P = 0.01$).

Wang et al. [39] performed a meta-analysis and found that miR-146a rs2910164 polymorphism increased the risk of cancer risk in dominant model when all studies were pooled into the meta-analysis. Stratified analysis revealed that significant association between rs2910164 variant and cancer susceptibility was found in Asians but not in Caucasian populations. In the subgroup analysis by cancer types, no significantly increased risk of breast, gastric, prostate or bladder cancer was found in any of the genetic models. While, another meta-analysis indicated that miR-146a rs2910164 C allele decreased PCa risk among Chinese population [40,41].

It has been shown that the expression level of hsa-miR-155, hsa-miR-141 and hsa-miR-21 significantly elevated in PCa samples and negatively correlated with that of mismatch repair genes [42].

Previously we have investigated the impact of hsa-mir-146a rs2910164, has-miR-499 rs3746444 and Hsa-miRNA-196a2 (rs11614913 C > T and rs185070757 T > G) and risk of breast cancer. The results showed an association between miR-499 rs3746444 variant and risk of BC [43].

The miR-499 gene was mapped to 20q11.22. It lies within the 20th intron of the beta-myosin heavy chain 7B (Myh7b) gene. The miR-499 variant may influence the individual susceptibility to cancer risk by affecting *MYH7B* gene function as well functions of miR-499 [44,45]. It has been shown that MDM4 oncogene contributes to cancer susceptibility and progression through its capability to negatively regulate tumor suppressor genes [46]. The rs4245739 A > C variant located in the 3′-untranslated region (UTR) has been reported to create a target site for hsa-miR-191, resulting in decreased *MDM4* mRNA levels [47]. Computational calculations revealed that this variant is located within a predicted binding site for miR-191-5p, miR-887 and miR-3669. Thus the *MDM4* rs4245739 A allele may be associated with increased risk of PCa [47]. MiR-143 is one major tumor suppressor miRNA. A functional rs4705342 T > C variant in miR-143 promoter has shown to be associated with PCa risk [48]. Subjects with TC/CC genotypes had significantly decreased risk of PCa compared with those with TT genotype. It has been proposed that

Table 3 Genotypic and allelic frequencies of miR499 rs3746444, miR-196a2 rs11614913, miR-146a rs2910164 and miR-149 rs2292832 variants polymorphisms in prostate cancer (PCa) and control subjects.

Polymorphism	Prostate cancer (169 subjects) *n* (%)	Control (182 subjects) *n* (%)	OR (95% CI)	*P*-value
miR-499 rs3746444 T > C				
Codominant				
TT	62 (37.6)	85 (46.7)	1.00	–
TC	82 (48.5)	64 (35.2)	1.76 (1.12–2.79)	0.019
CC	25 (14.8)	33 (18.1)	1.04 (0.56–1.92)	0.897
Dominant				
TT	62 (37.6)	85 (46.7)	1.00	–
TC + CC	107 (62.4)	97 (53.3)	1.51 (0.99–2.32)	0.066
Recessive				
TT + TC	144 (85.2)	149 (81.9)	1.00	–
CC	25 (14.8)	33 (18.1)	0.78 (0.44–1.38)	0.472
Allele				
T	206 (60.9)	234 (64.3)	1.00	–
C	132 (39.1)	130 (35.7)	1.02 (0.85–1.57)	0.390
miR-196a2 rs11614913 C > T				
Codominant				
CC	64 (37.9)	77 (42.3)	1.00	–
CT	88 (52.1)	93 (51.1)	1.14 (0.73–1.77)	0.576
TT	17 (10.0)	12 (6.6)	1.70 (0.76–3.82)	0.224
Dominant				
CC	64 (37.9)	77 (42.3)	1.00	–
CT + TT	105 (62.1)	105 (57.7)	1.20 (0.78–1.85)	0.446
Recessive				
CC + CT	152 (90.0)	170 (93.4)	1.00	–
TT	17 (10.0)	12 (6.6)	1.58 (0.73–3.43)	0.251
Allele				
C	216 (63.9)	247 (67.9)	1.00	–
T	122 (36.1)	117 (32.1)	1.19 (0.87–1.63)	0.300
miR-149 rs2292832 C > T				
Codominant				
CC	77 (45.6)	101 (55.5)	1.00	–
CT	68 (40.2)	57 (31.3)	1.57 (0.99–2.480)	0.062
TT	24 (14.2)	24 (13.2)	1.31 (0.69–2.48)	0.418
Dominant				
CC	77 (45.6)	101 (55.5)	1.00	–
CT + TT	92 (54.4)	81 (44.5)	1.49 (0.98–2.27)	0.069
Recessive				
CC + CT	145 (85.8)	168 (86.8)	1.00	–
TT	24 (14.2)	24 (13.2)	1.16 (0.63–2.13)	0.645
Allele				
C	222 (65.7)	258 (70.9)	1.00	–
T	116 (34.3)	106 (29.1)	1.27 (0.92–1.75)	0.144
miR-146a rs2910164 G > C				
Codominant				
GG	25 (14.8)	24 (13.2)	1.00	–
GC	131 (77.5)	147 (80.8)	0.86 (0.47–1.57)	0.644
CC	13 (7.7)	11 (6.0)	1.14 (0.43–3.02)	0.917
Dominant				
GG	25 (14.8)	24 (13.2)	1.00	–
GC + CC	144 (85.2)	158 (86.8)	0.87 (0.48–1.60)	0.758
Recessive				
GG + GC	156 (92.3)	171 (94.0)	1.00	–
CC	13 (7.7)	11 (6.0)	1.29 (0.56–2.98)	0.673
Allele				
G	181 (53.6)	195 (53.6)	1.00	–
C	157 (46.4)	169 (46.4)	1.00 (0.74–1.35)	0.974

Table 4 Association of miR polymorphisms with clinicopathologic parameters in prostate cancer (PCa) patients.

Factors	miR-499 rs3746444			*P*	miR-196a2 rs11614913			*P*	miR-149 rs2292832			*P*	miR-146a rs2910164			*P*
	TT	TC	CC		CC	CT	TT		CC	CT	TT		GG	GC	CC	
Age at diagnosis Y, *n*				0.333				0.797				0.536				0.902
⩽65	47	59	15		46	64	11		56	46	19		18	93	10	
>65	15	23	10		18	24	6		21	22	5		7	38	3	
Stage				0.144				0.532				0.761				**0.021**
pT1	1	5	2		3	5	0		5	2	1		2	3	3	
pT2a	8	16	3		11	15	1		12	13	2		2	25	0	
pT2b	6	5	0		3	7	1		6	3	2		2	8	1	
pT2c	27	33	16		32	36	8		37	28	11		12	59	5	
pT3a	3	7	3		7	5	1		6	6	1		4	7	2	
pT3b	17	16	1		8	20	6		11	16	7		3	29	2	
PSA at diagnosis (ng/ml), *n*				0.094				0.061				0.722				0.545
⩽4	1	0	0		0	0	1		1	0	0		0	1	0	
4–10	24	43	17		32	44	8		41	32	11		14	61	9	
>10	37	39	8		32	44	8		35	36	13		11	69	4	
Gleason score, *n*				0.334				0.238				0.998				0.465
⩽6	18	26	13		26	28	3		26	23	8		8	42	7	
7	28	37	8		25	41	7		34	29	10		12	56	5	
>7	16	19	4		13	19	7		17	16	6		5	33	1	
Perineural invasion, *n*				0.728				0.781				0.431				0.122
Positive	41	49	16		38	57	11		52	41	13		18	83	5	
Negative	21	33	9		26	31	6		25	27	11		7	48	8	
Surgical margin, *n*				0.330				0.215				0.715				0.731
Positive	28	32	7		20	39	8		30	29	8		11	52	4	
Negative	34	50	18		44	49	9		47	39	16		14	79	9	

The bold indicate statistically significant.

genetic variants in miRs and miR target sites predict biochemical recurrence after radical prostatectomy in localized prostate cancer [25].

A meta-analysis performed by Ma et al. [49], investigated the association between miR-146a rs2910164, miR-196a2 rs11614913, miR-499 rs3746444, miR-149 rs2292832, and miR-27a rs895919 and the risk of cancer development. They found no significant association between rs2910164 and cancer risk in the overall group. However, in stratified analysis, they found that either the rs2910164 C allele or the CC genotype was protective against bladder cancer, prostate cancer, cervical cancer, and colorectal cancer, while it was a risk factor for papillary thyroid carcinoma and squamous cell carcinoma of the head and neck (SCCHN). In addition, rs11614913 was found to be significantly associated with decreased cancer risk, in particular, for bladder cancer, gastric cancer, and SCCHN. For miR-499, a significant association was found between the rs3746444 polymorphism and cancer risk in pooled analysis.

It has been reported that genetic variants in the miR machinery gene GEMIN4 are associated with risk of PCa in Chinese Han population [50]. It has been shown that rs1434536 variant in the 3′UTR of bone morphogenetic protein membrane receptor type IB (BMPR1B) gene affects the binding ability of miR-125b to *BMPR1B* mRNA and contributes to the genetic susceptibility to localized PCa as well as patients aged >70 years [51]. Prostate tumor invasion and hormone refractoriness may be caused by aberrant expression of miR-146a and miR-146b-5p [52].

The limitations of the present study are the following: (i) relatively small sample sizes, so replication with larger sample is needed. (ii) We did not determine gene-environment interactions. It has been proposed that both genetic and environmental factors may contribute to prostate cancer susceptibility.

Conclusions

In conclusion, the findings proposed that *miR-499* rs3746444 polymorphism increased the risk of PCa. The results did not support an association between genetic variant of miR-196a2, miR-149, and miR-146a and the risk of developing PCa. Larger sample sizes with diverse ethnicities are needed to confirm the findings.

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Conflict of Interest

The authors declare that there is no conflict of interest to disclose.

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