RESEARCH ARTICLE

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Association of *miR-126* and *miR-143/145* Gene Polymorphisms with the Risk and Clinicopathological Features of Prostate Cancer

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Abstract

Background: Prostate cancer (PCa) represents a significant cause of morbidity and mortality among men. Recently, several biomarkers have been introduced to address the shortcomings in PCa management, including screening and diagnosis. MicroRNAs (miRNAs) are relatively novel biomarkers that may be dysregulated in PCa. However, the knowledge regarding the association between miRNA dysregulations and PCa remains limited in Iran. **Methods:** We performed a case-control analysis, comparing *miR-126* rs4636297, *miR-143/145* rs353292, *miR-143/145* rs4705342, and *miR-143/145* rs4705343 polymorphisms in 185 PCa patients and 220 cancer-free men based on DNA extracted from blood samples of Iranian subjects. Moreover, the correlation between clinicopathological features of PCa and these polymorphisms was investigated. **Results:** *miR-126* rs4636297 A>G, *miR-143/145* rs353292 C>T, and *miR-143/145* rs4705343 T>C variants were associated with a reduced risk of PCa in codominant, dominant, recessive, and allelic inheritance models. With the exception of the recessive inheritance pattern, the *miR-143/145* rs4705342 T>C variant was also correlated with a reduced risk of PCa. Additionally, haplotype analysis of *miR-143/145* (rs353292, rs4705342, and rs4705343, respectively) polymorphisms revealed that the CTT haplotype was the most frequent in cases and controls. Moreover, CTC, TTT, TCC, and TCT were associated with decreased risk of PCa. Finally, no relationship was identified between certain clinicopathological aspects of PCa and the mentioned polymorphisms. **Conclusions:** We identified several miR polymorphisms and distinct haplotypes associated with reduced PCa risk in a sample of the Iranian population. These findings pave the way for optimized PCa management and provide a better understanding of its pathophysiology.

Keywords: MicroRNAs- *miR-126*- *miR-143/145*- Polymorphism- Prostatic Neoplasms

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Introduction

Prostate cancer (PCa) is considered a major health-related concern. Based on recent evidence, about 375,000 global deaths were attributed to PCa in 2020, supporting its role as a prominent cause of cancer-related deaths in males [1]. Moreover, about 1.4 million cases of newly diagnosed PCa were estimated for the year 2020, placing PCa in the second position of the most common cancers globally [2]. Accordingly, PCa burdens affected patients and healthcare systems significantly; therefore, numerous studies were conducted to improve the overall management of PCa.

Several biomarkers have been proposed, which may aid in screening, diagnosis, and/or representing the behavior of prostate tumors. These biomarkers can be collected from a variety of sources (e.g., prostate tissue, blood, and urine) and may allow for providing more customized therapeutic options for PCa patients [3]. Among these biomarkers, microRNAs (miRNAs) are relatively novel factors that have been in the spotlight recently. As a non-coding type of RNA, miRNAs were shown to regulate gene expression post-transcriptionally by interacting with messenger RNAs (mRNAs) [4]. The miRNA-oncogenesis interplay was studied in B-cell lymphocytic leukemia for the first time, in which two miRs were involved [5]. Recent experiments have shown that miRNAs play a role in tumor biology, exhibiting either oncogenic or tumor-suppressive effects. Consequently, the deregulation of miRNA function may result in oncogenesis via several mechanisms [6, 7]. Single nucleotide polymorphism (SNP) is a modulating process that can alter miRNA function, and its role in

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cancer biology has been evaluated [8, 9].

PCa is among the cancers that have been studied in light of distinct miRNA dysregulations. Notably, several miRNAs were shown to increase in the setting of PCa (e.g., miR-141, miR-375), while others tend to decline (including miR-205, let-7c) [3, 10]. miRNAs have the potential to replace the less precise biomarkers currently being used. Furthermore, their availability in circulation facilitates their use in clinical settings as a non-invasive method [11].

Nevertheless, there are discrepancies among the results of some studies, which limit miRNA clinical application in PCa management [3]. Furthermore, different ethnic groups have varied genetic predispositions to cancer, and the majority of experiments have been conducted in specific locales, limiting their generalizability [12, 13]. Notably, despite various investigations, little is known about the connection between miRNA dysregulation and PCa in Iranian patients [14-16]. This emphasizes the need for further region-specific studies to determine the eligible biomarkers for PCa management. Therefore, we aimed to assess the relationship of discrete miRNA gene polymorphisms with the risk of PCa in a sample from the Iranian population. For this purpose, using a case-control design, biomarkers were acquired from blood samples and compared among the subjects with and without PCa using specific primers. Moreover, the association of genetic variants with specific clinical characteristics was explored.

Materials and Methods

Patients

All patients and controls were drawn from the Department of Urology at Shahid Labbafinejad Medical Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. The research concept and enrollment technique were previously reported [14, 17, 18]. The study was approved by the Institutional Review Board and Ethics Committee of Zahedan University of Medical Sciences (approval ID: IR.ZAUMS.REC.1396.295). The written informed consent was obtained from all the participants. Whole blood samples were collected in ethylenediamine tetraacetic acid (EDTA) tubes, and genomic DNA was purified using the salting out method as described previously [19].

Genotyping

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method for genotyping was implemented for *miR-126* rs4636297, *miR-143/145* rs4705342, and *miR-143/145* rs4705343, whereas tetra-primer amplification refractory mutation system-polymerase chain reaction (T-ARMS-PCR) method was used to assess *miR-143/145* rs353292.

PCR was performed using commercially available prime Taq Premix (GeNet Bio, Nonsan, Korea) based on the manufacturer's instructions. Each 0.20 ml PCR reaction tube contained 1 µl genomic DNA (100 ng/ml), 1 µl of each primer (10 µM), 7 µl 2X master mix, and the appropriate amount of double-distilled H2O. Amplification was performed with an initial denaturation at 95˚C for 6 minutes, followed by 30 cycles of 30 seconds at 95˚C, 30 seconds at 59˚C (for *miR-126* rs4636297), 60˚C (for *miR-143/145* rs353292), 62˚C (for *miR-143/145* rs4705342), 54˚C (for *miR-143/145* rs4705343), and 30 seconds at 72˚C, with a final extension step of 72˚C for 5 minutes. The digested products were separated by 2.5% agarose gel electrophoresis (Figs 1-4). For genotyping, 10 µl of the PCR product was digested with appropriate restriction enzymes (8 µl for the Tetra-ARMS method). Table 1 displays the fragment sizes, restriction enzymes, and primer sequences.

Statistical analysis

Statistical analysis was conducted using IBM SPSS Statistics software for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Frequency/percentage was used to represent qualitative variables, while quantitative variables were presented as mean ± SD (standard deviation). Data were analyzed using independent samples t-test and $\chi 2$ test. Unconditional logistic regression analysis was used to examine the association between the *miR-126* and *miR-143/145* variants with PCa risk. A p-value < 0.05 was considered to indicate a statistically significant difference.

Table 1. The Primers Used for the Detection of the *miR-126* (rs4636297) and *miR-143/145* (rs353292, rs4705342 and rs4705343) Polymorphisms

Polymorphism	PCR primers (5'→3')	Restriction Enzyme	Fragment, bp
miR-126 rs4636297	F: CGCAGCATTCTGGAAGACGC	MwoI	A allele= 212
	R: CCTAAGTACGTCGGGGGG		G allele= 120+ 92
miR-143/145 rs353292	FO: TCATCGAGCAATCAATTGTCTCCAACCT	-	Control= 263
	RO: GCTCCCTAGGAGAATGGAAGGACATCT		C allele= 169
	FI: TAATATCCCAAAAGGGCTCCCCATGGT		T allele= 147
	RI: AAGGGCTTCAGAATTCAGCCTGATGG		
miR-143/145 rs4705342	F: GTAGATGCGGCAGACCCTC	Hin1II	T allele= 267
	R: ATGCCCCACCTTTATGCTTGG		C allele= 219+ 48
miR-143/145 rs4705343	F: TCCATGTATTGAAATATCCAGAAAGTA	AccI	T allele= 147
	R: TGTGCAAACAATGGCACAAT		C allele= 120+27

miR, MicroRNA; PCR, Polymerase; chain reaction; bp, Base pair

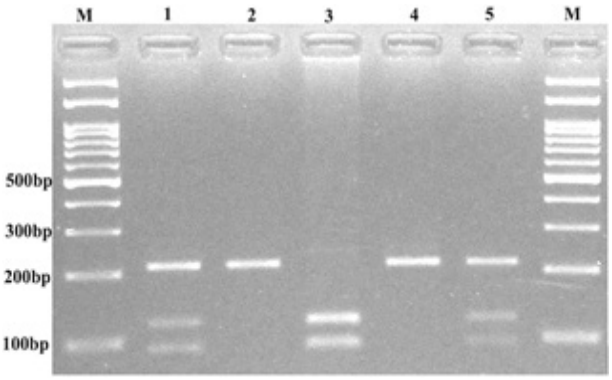

Figure 1. Photograph of the PCR-RFLP method for the detection of miR-126 rs4636297 polymorphism M: DNA marker; Lanes 1 and 5: AG; Lanes 2 and 4: AA; Lane 3: GG. PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism

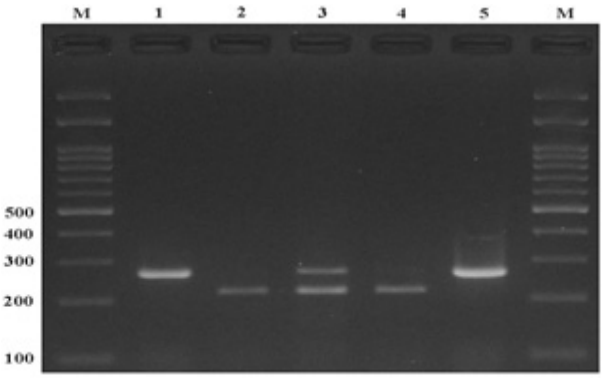

Figure 3. Photograph of the PCR-RFLP method for the detection of miR-143/145 rs4705342 polymorphism. M: DNA marker; Lanes 1 and 5: TT; Lanes 2 and 4: CC; Lane 3: TC. PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism

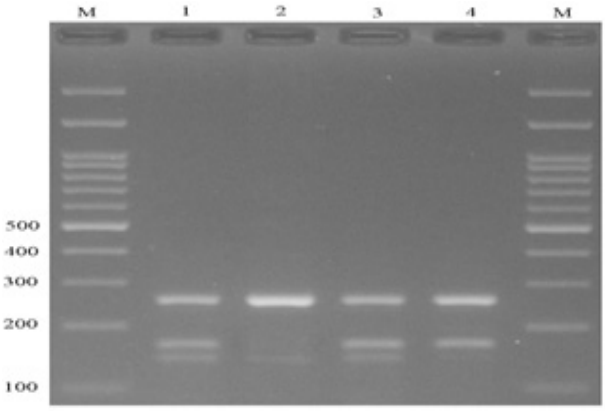

Figure 2. Photograph of the T-ARMS Method for the detection of miR-143/145 rs353292. Lane 1 and 3: CT; lane 2: TT; and lane 4: CC. T-ARMS: Tetra amplification-refractory mutation system

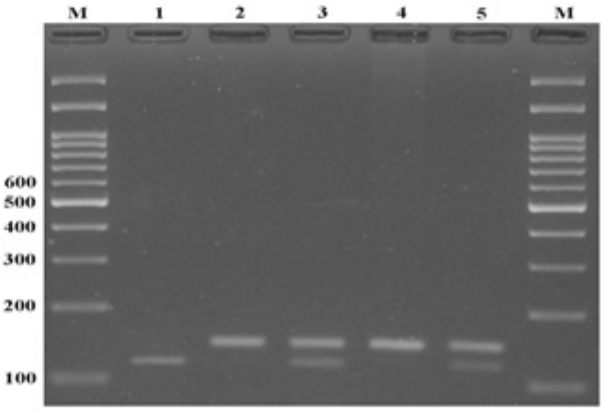

Figure 4. Photograph of the PCR-RFLP method for the detection of miR-143/145 4705343 polymorphism M: DNA marker; lane 1: CC; lane 2 and 4: TT, lanes 3 and 5: TC. PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism

Results

The present study involved 185 patients with PCa (mean age, 61.15 ± 6.51 years) and 220 healthy subjects (mean age, 62.39 ± 7.59 years). No significant age difference was detected between the two groups (p=0.086).

The genotypes and allele frequencies of *miR-126* rs4636297, *miR-143/145* rs353292, *miR-143/145* rs4705342, and *miR-143/145* rs4705343 polymorphisms in cases and controls are presented in Table 2. As regards *miR-126* rs4636297 A>G variant, our findings established that this variation was associated with a significantly decreased risk of the PCa in the codominant (odds ratio (OR)=0.351, 95% confidence interval (CI): 0.169-0.731, p=0.005, G/G vs. A/A), dominant (OR=0.632, 95% CI: 0.424-0.943, p=0.024, A/G+ G/G vs. A/A), recessive (OR=0.423, 95% CI: 0.211- 0.850, p=0.013, GG vs. AA+AG), and allelic (OR=0.661, 95% CI: 0.493-0.887, p=0.006, G vs. A) inheritance models.

Regarding *miR-143/145* rs353292 C>T variant, our findings showed that this variant was associated with a significantly decreased risk of the PCa in the codominant (OR=0.338, 95% CI: 0.138-0.830, p=0.018, T/T vs. C/C), dominant (OR=0.614, 95% CI: 0.412-0.915, p=0.016, C/T+ T/T vs. C/C), recessive (OR=0.393, 95% CI: 0.162-0.952, p=0.033, T/T vs. C/C+C/T), and allelic (OR=0.627, 95% CI: 0.452-0.869, p=0.005, T vs. C) inheritance models.

Concerning *miR-143/145* rs4705342 T>C variant, our findings demonstrated that this variant was associated with a significantly reduced risk of the PCa in the codominant (OR=0.652, 95% CI: 0.426-0.997, p=0.048, T/C vs. T/T), dominant (OR=0.627, 95% CI: 0.419-0.936, p=0.022, T/C+ C/C vs. T/T), and allelic (OR=0.670, 95% CI: 0.483-0.931, p=0.017, C vs. T) inheritance models.

Finally, our findings showed that *miR-143/145* rs4705343 T>C variant was associated with a significantly decreased risk of PCa in the codominant (OR=0.413, 95% CI: 0.266-0.643, p < 0.001, T/C vs. T/T; OR= 0.169, 95% CI: 0.072- 0.399, p < 0.001, C/C vs. T/T), dominant (OR=0.347, 95% CI: 0.229-0.525, p < 0.001, T/C+ C/C vs. T/T), recessive (OR=0.231, 95% CI: 0.099-0.537, p < 0.001, C/C vs. T/T+T/C) and allelic (OR=0.374, 95% CI: 0.267-0.526, p < 0.001, C vs. T) inheritance patterns.

The association between the clinicopathological

Table 2. Genotype and Allele Frequencies of *miR-126* and *miR-143/145* Gene Polymorphisms in Prostate Cancer Patients and Healthy Controls

Polymorphism	Case, n (%)	Control, n (%)	OR (95% CI)	p-value
miR-126 rs4636297				
Codominant				
A/A	86 (46.5)	78 (35.5)	1	-
A/G	87 (47.0)	111 (50.5)	0.711 (0.469- 1.077)	0.107
G/G	12 (6.5)	31 (14.0)	0.351 (0.169- 0.731)	0.005*
Dominant				
A/A	86 (46.5)	78 (35.5)	1	-
A/G+ G/G	99 (53.5)	142 (64.5)	0.632 (0.424- 0.943)	0.024*
Recessive				
A/A+ A/G	173 (93.5)	189 (86.0)	1	-
G/G	12 (6.5)	31 (14.0)	0.423 (0.211- 0.850)	0.013*
Allele				
A	259 (70.0)	267 (60.7)	1	-
G	111 (30.0)	173 (39.3)	0.661 (0.493- 0.887)	0.006*
miR-143/145 rs353292				
Codominant				
C/C	117 (63.2)	113 (51.4)	1	-
C/T	61 (33.0)	87 (39.5)	0.677 (0.446- 1.028)	0.067
T/T	7 (3.8)	20 (9.1)	0.338 (0.138- 0.830)	0.018*
Dominant				
C/C	117 (63.2)	113 (51.4)	1	-
C/T+ T/T	68 (36.8)	107 (48.6)	0.614 (0.412- 0.915)	0.016*
Recessive				
C/C+ C/T	178 (96.2)	200 (90.9)	1	-
T/T	7 (3.8)	20 (9.1)	0.393 (0.162- 0.952)	0.033*
Allele				
C	295 (79.7)	313 (71.1)	1	-
T	75 (20.3)	127 (28.9)	0.627 (0.452- 0.869)	0.005*
miR-143/145 rs4705342				
Codominant				
T/T	120 (64.9)	118 (53.6)	1	-
T/C	55 (29.7)	83 (37.7)	0.652 (0.426- 0.997)	0.048*
C/C	10 (5.4)	19 (8.7)	0.518 (0.231- 1.160)	0.11
Dominant				
T/T	120 (64.9)	118 (53.6)	1	-
T/C+ C/C	65 (35.1)	102 (46.4)	0.627 (0.419- 0.936)	0.022*
Recessive				
T/T+ T/C	175 (94.6)	201 (91.3)	1	-
C/C	10 (5.4)	19 (8.7)	0.605 (0.274- 1.335)	0.209
Allele				
T	295 (79.7)	319 (72.5)	1	-
C	75 (20.3)	121 (27.5)	0.670 (0.483- 0.931)	0.017*
miR-143/145 rs4705343				
Codominant				
T/T	132 (71.3)	102 (46.4)	1	-
T/C	46 (24.9)	86 (39.1)	0.413 (0.266- 0.643)	< 0.001*
C/C	7 (3.8)	32 (14.5)	0.169 (0.072- 0.399)	< 0.001*

*Statistically significant; miR, MicroRNA; OR, Odds ratio; CI, Confidence interval

Table 2. Continued

Polymorphism	Case, n (%)	Control, n (%)	OR (95% CI)	p-value
Dominant				
T/T	132 (71.3)	102 (46.4)	1	-
T/C+ C/C	53 (28.7)	118 (53.6)	0.347 (0.229- 0.525)	< 0.001*
Recessive				
T/T+ T/C	178 (96.2)	188 (85.5)	1	-
C/C	7 (3.8)	32 (14.5)	0.231 (0.099- 0.537)	< 0.001*
Allele				
T	310 (83.8)	290 (65.9)	1	-
C	60 (16.2)	150 (34.1)	0.374 (0.267- 0.526)	< 0.001*

*Statistically significant; miR, MicroRNA; OR, Odds ratio; CI, Confidence interval

Table 3. Association between *miR-126* and *miR-143/145* Variants and Clinical Characteristics of the Patients with Prostate Cancer

Characteristics of the patients		miR-126 rs4636297				miR-143/145 rs353292				miR-143/145 rs4705342				miR-143/145 rs4705343			
		A/A	A/G	G/G	p	C/C	C/T	T/T	p	T/T	T/C	C/C	p	T/T	T/C	C/C	p
					0.787				0.097				0.141				0.773
Age at diagnosis (years)																	
≤60	42	45	5			63	28	1		57	27	8		67	21	4	
>60	44	42	7			54	33	6		63	28	2		65	25	3	
Stage				0.226				0.673				0.457				0.275	
pT1	4	3	1			4	4	0		7	1	0		5	3	0	
pT2a	10	17	0			19	8	0		17	10	0		24	3	0	
pT2b	7	5	0			7	5	0		7	4	1		8	4	0	
pT2c	40	40	9			55	29	5		53	28	8		61	23	5	
pT3a	9	4	2			7	7	1		10	4	1		9	4	2	
pT3b	16	18	0			25	8	1		26	8	0		25	9	0	
PSA level at diagnosis (ng/ml)				0.313				0.32				0.071				0.866	
≤4	2	0	0			0	2	0		1	0	1		2	0	0	
4-10	39	48	8			63	29	3		64	27	4		69	22	4	
>10	45	39	4			54	30	4		55	28	5		61	24	3	
Gleason score				0.373				0.214				0.382				0.271	
≤7	63	71	10			95	45	4		90	45	9		106	34	4	
>7	23	16	2			22	16	3		30	10	1		26	12	3	
Perineural invasion				0.235				0.395				0.566				0.134	
Positive	61	51	8			77	37	6		76	36	8		83	30	7	
Negative	25	36	4			40	24	1		44	19	2		49	16	0	
Surgical margin				0.344				0.835				0.101				0.369	
Positive	32	33	2			44	21	2		50	15	2		47	19	1	
Negative	54	54	10			73	40	5		70	40	8		85	27	6	

miR, MicroRNA; PSA, Prostate-specific antigen

Table 4. Haplotype Analysis of the *miR-143/145* Polymorphisms and Prostate Cancer Risk

miR-143/145 rs353292	*miR-143/145* rs4705342	*miR-143/145* rs4705343	Case (frequency)	Control (frequency)	OR (95% CI)	p-value
C	T	T	0.5969	0.3866	1	-
C	T	C	0.0584	0.1745	0.18 (0.09- 0.35)	< 0.0001*
T	T	T	0.0976	0.1149	0.50 (0.28- 0.91)	0.023*
C	C	T	0.1052	0.1031	0.57 (0.31- 1.05)	0.074
T	C	C	0.0225	0.0702	0.26 (0.11- 0.60)	0.0017*
T	T	C	0.0445	0.049	0.56 (0.25- 1.26)	0.16
T	C	T	0.0381	0.0545	0.36 (0.15- 0.84)	0.018*
C	C	C	0.0368	0.0472	0.54 (0.26- 1.13)	0.1

*Statistically significant.; miR, MicroRNA; OR, Odds ratio; CI, Confidence interval

characteristics of PCa, including diagnosis age, tumor stage, prostate-specific antigen (PSA) level, Gleason score, perineural invasion, and tumor surgical margin with *miR-126* and *miR-143/145* polymorphisms is shown in Table 3. The findings revealed no significant connection between these SNPs and the clinicopathological parameters of PCa patients. The Hardy-Weinberg equilibrium (HWE) was implemented, and the results showed that the genotype distributions in both cases and controls were in HWE. Besides, the haplotype analysis of *miR-143/145* polymorphisms (rs353292, rs4705342, and rs4705343, respectively) was conducted, and the results were compared between PCa cases and healthy controls. The detected genotypes led to the development of eight haplotypes (Table 4). CTT haplotype was the most prevalent type in the patients and controls. Furthermore, the outcomes of the haplotype analysis showed that CTC, TTT, TCC, and TCT haplotypes were associated with a decreased risk of PCa ($p < 0.05$).

Discussion

The role of miRNAs in cancer biology has been in the spotlight as they may affect several mechanisms involved in carcinogenesis, including angiogenesis, cellular growth, apoptosis, migration, invasion, and metastasis [20]. In the present investigation, we chose four SNPs of the *miR-126* and *miR-143/145* genes to investigate their association with PCa risk and clinicopathological features. Furthermore, a haplotype analysis of the miR143/145 polymorphisms was performed to assess the connection between each haplotype and PCa risk.

miR-126 has been studied in the context of cancer, exhibiting both oncogenic and tumor-suppressive effects in various studies. Nevertheless, a systematic review of *miR-126*'s role in PCa revealed its downregulation, as well as its regulatory effects on specific biological mechanisms, such as the PI3K-Akt and epithelial-mesenchymal transition pathways [21]. *miR-126*-3p was significantly higher in the urinary extracellular vesicles of patients with high-risk PCa compared to those with low-risk PCa and benign prostatic hyperplasia (BPH). Moreover, among high-risk PCa patients, those with the invasion of lymph nodes had higher levels of this miR in their urine [22]. *miR-126* rs4636297 polymorphism, in particular, was studied in the setting of type-2 diabetes mellitus [23], diabetic retinopathy [24], and acute myocardial infarction [25]. Additionally, *miR-126* rs4636297 was reported to be correlated with polycystic ovary syndrome [26]. Our study indicates that the *miR-126* rs4636297 specific genotypes are associated with a decreased risk of prostate cancer across various inheritance models for the first time; however, different genotypes of this polymorphism have been correlated with an elevated risk of cervical cancer and cervical intraepithelial neoplasia [27]. This indicates that complex molecular mechanisms may be involved in *miR-126*'s impact on the process of oncogenesis. These mechanisms should be addressed in future studies.

Given the proximity of miR-143 and miR-145 genes on chromosome 5, they are considered to form a bicistronic unit [28, 29]. In general, miR143 and miR-145 have been thought to suppress oncogenesis separately or in a cluster. Kojima et al. revealed that *miR-143/145* inhibit the invasion and migration of PCa cells by targeting Golgi membrane protein 1 [30]. Based on a meta-analysis, the rs4705342 may have a protective effect against PCa [31]. This is in concordance with the results of our study. On the contrary, the *miR-143/145* rs4705342 CC genotype has been linked to an elevated risk of epithelial cancer of the ovaries and an unfavorable prognosis of this malignancy [32]. Furthermore, there is evidence suggesting that the carcinogenesis of hepatitis B virus in the context of hepatocellular carcinoma may be associated with a minor allele of rs4705342 that is located in the promoter region of miR-143 [33]. Similarly, an increased risk of oral squamous cell carcinoma in the presence of miR-143 rs4705342 was detected in a case-control study [34]. Regarding papillary thyroid carcinoma, rs4705342 was associated with a lower risk of this cancer; however, the distribution of the *miR-143/145* rs353292 genotypes did not differ significantly between those with this type of cancer and healthy controls [35]. In contrast to our study, the C allele of the rs4705342 was shown to be associated with an elevated PCa risk [36]. These findings suggest that a unique variant may be involved in both oncogenic and tumor-suppressing interactions. Consequently, additional unexplored components may be involved. To clarify the precise association between rs4705342 and PCa, further research should be conducted on a variety of ethnicities.

To the best of our knowledge, rs353292 and rs4705343

variants have not been investigated in the setting of PCa. Although based on our research, these variants were associated with a lower risk of PCa, it has been shown that rs353292 is involved in susceptibility to colorectal cancer [37]. Nevertheless, rs4705343 was linked to a reduced risk of colorectal cancer [38], and its T allele was proposed as a protector against non-small cell lung cancer [39]. On the other hand, rs4705343 may be involved in the increased risk of squamous cell carcinoma of the cervix [40]. *miR-143/145* rs353292, rs4705342, and rs4705343 polymorphisms in our study did not correlate with PSA level, tumor stage, and other clinical aspects of PCa. Altogether, it seems sensible to consider several polymorphisms jointly for PCa management until highly sensitive and specific variations are found.

The present study is the first to assess the mentioned SNPs in the PCa setting in an Iranian population. The results of such analyses can provide new insights into PCa pathogenesis and introduce novel biomarkers that are potentially useful in PCa screening, diagnosis, monitoring, and prognosis, as they can determine one's susceptibility to PCa development, aid in early detection of PCa in high-risk populations, or provide information regarding the behavior of the tumor. Moreover, by determining the molecular processes regulated by these variations, novel therapeutic avenues may be developed. However, one of the main drawbacks of this research is its small sample size; therefore, further studies with larger sample sizes are needed to validate the findings. Additionally, while the majority of the participants identified themselves as Persians (i.e., Fars), there is little information on the ethnic backgrounds and genetic predispositions of the participants, which might restrict how broadly the findings can be applied. Finally, possible biological mechanisms involved in the detected associations should be investigated by future studies to verify our findings.

In conclusion, we proposed distinct genetic variants of *miR-126* and *miR-143/145* associated with reduced PCa risk in a sample of Iranian subjects. Furthermore, the haplotype analysis of miR143/145 polymorphisms yielded haplotypes associated with lower PCa risk. The findings of the present study could be used to improve PCa management and provide novel insights regarding its pathophysiology.

Author Contribution Statement

Gholamreza Bahari: Conceptualization, Project administration, Writing – review & editing. Nahid Rahimi: Investigation, Data curation, Methodology, Formal analysis. Mohammad Hashemi: Conceptualization, Methodology. Mohammad Amin Siri: Writing – original draft, Writing – review & editing. Behzad Narouie: Supervision, Project administration, Writing – review & editing. All authors read and approved the final manuscript.

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None.

Data availability

The data supporting the current study's findings are available from the corresponding author upon reasonable request.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. The study was approved by the Institutional Review Board and Ethics Committee of Zahedan University of Medical Sciences (approval ID: IR.ZAUMS.REC.1396.295).

Consent to participate

The written informed consent was obtained from all the participants.

Competing interests

The authors declare that they have no competing interests.

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