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Influence of a single nucleotide polymorphism in miR-196a2 on idiopathic asthenozoospermia in Iranian Azeri males

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ABSTRACT

Introduction: Asthenozoospermia (AZS), sperm immobility, is one of the major cause of men infertility. Evidence suggested that microRNAs (miRNAs) play a critical role in spermatogenesis process. However, the association of miRNAs polymorphisms with idiopathic male infertility remains unknown. Therefore, we investigated correlation between miR-196a2 rs11614913 polymorphism and idiopathic AZS among Iranian Azeri men.

Materials and Methods: In this study, 50 men with idiopathic AZS (case group) as well as 50 age and ethnically matched healthy men (control group) were enrolled from East Azerbaijan, Iran. The proteinase K method was used to extract the genomic DNA from sperm samples. Finally, genotyping was conducted using tetra-primer amplification refractory mutation system-polymerase chain reaction (Tetra-ARMS PCR) method.

Results: The frequency of TT, TC, and CC genotypes were 12%, 54%, and 34%, respectively, in patients with AZS; whereas the figures were 8%, 40%, and 52% in healthy controls, respectively. We found a significant difference between case and control groups in term of CC genotype frequency (P = 0.016).

Conclusion: We found a significant correlation between miR-196a2 rs11614913 polymorphism and AZS in Iranian Azeri men.

Keywords: Asthenozoospermia, miR-196a2, Polymorphism, Tetra-ARMS PCR

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Introduction

Infertility is a common problem of human reproduction, which involves 10-15% of couples in worldwide. The factors of men infertility are responsible for 50% of infertility cases (1, 2). One of the important causes of the men infertility is dysfunction of sperm, which can occur in effects of environmental toxins or genetic variants (3, 4). Idiopathic asthenozoospermia (AZS) is a common male spermatogenesis disorders, and found in 18% of infertile men (5). Idiopathic AZS leads to male infertility due

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to reduction of sperm motility with unknown mechanisms (6, 7).

MicroRNAs (miRNAs), single stranded regulatory small RNAs, that play critical role in several biological process such as fat metabolism, cell death, cell proliferation, and stress resistance (8, 9, 10). Various polymorphisms in mature miRNAs can cause to diversity of genetic diseases and susceptibility to various genetic disease (11, 12).

In human, miR-196 encoded from intergenic regions in HOX family genes clusters (a gene family that encoded transcription factors during fetus development). This miRNA is involved in regulation of innate immune system, inflammation process, apoptosis, cell proliferation, as well as regulation of embryonic stemness (13, 14). Previous studies suggested that several polymorphisms on miRNAs are associated with sperm maturation and spermatogenesis process (15, 16).

To date, association of rs11614913 polymorphism on miR-196a2 and AZS was not evaluated in infertile Iranian Azeri men. In this study, we evaluated correlation of miR-196a2 rs11614913 polymorphism and idiopathic AZS among infertile Iranian Azeri men.

Materials and Methods

Study Subjects

The subjects in present case-control study is consisted of 100 men (25-50 years old), who were referred to ACECR Fertility Clinic, East Azerbaijan ART Center, Tabriz, Iran, during 2017-2019. Among these, 50 infertile men were considered as case group with confirmed idiopathic AZS using semen analysis. Also, 50 fertile men without abnormal sperm were considered as healthy controls. The infertile men with cryptorchidism, hypogonadism, ejaculatory duct obstruction, hypogonadotropic, orchitis, and microdeletions on Y chromosome or abnormal karyotype were excluded from the study. The demographic information of all patients and healthy controls, includes age, alcohol drinking, semen parameters, body mass index (BMI), family history of AZS, and tobacco smoking were collected and evaluated by interviews and questionnaires (Table 1). The consent form was signed by all individuals according to the Declaration of Helsinki ethical standards.

DNA Genotyping

Sperm sample (3 ml) received from all individuals, and genomic DNA extraction was conducted by proteinase K method. The quantity and quality of the extracted genomic DNA samples were evaluated using nanodrop instrument and electrophoresis on agarose gel, respectively. DNA genotyping was performed by tetra-primer amplification refractory mutation system-polymerase chain reaction (Tetra-ARMS PCR) method. The used primers were: Forward outer in: 5′-CTCGGCAACAAGAAACGGC-3′; Reverse outer in: 5′-GACGAAAACCGACTGATGTAA-3′; Forward inner: 5′-CACCCAGCAACCCAAAGTCTACTC-3′; Reverse inner: 5′-GCAGGGTTCTCCAGACTTGTTC-3′. PCR amplification was performed in 25 μL total volume (12.5 μL master mix, 1 μg template DNA, and 0.5 μL each primer) as followeng condition: 1 cycle for initial denaturation (in 94°C for 5 minutes), 30 cycles for denaturation (94°C for 5 minutes), annealing (60°C for 45 seconds), and extension (72°C for 45 seconds), and 1 cycle for final extension (72°C for 5 minutes). The size of amplified products was determined using electrophorese on 1% agarose gel was used in order to identification of PCR products sizes (381bp for the T allele and 240bp for the C allele). Statistical analysis was conducted by SPSS software. Correlation of miR-196a2 rs11614913 polymorphism and AZS was investigated by logistic regression analysis. Difference in demographic features of case and control groups were analyzed by

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independent sample t-test. We used chi-square (χ^2) test and Fisher's exact test in order to investigation of Hardy-Weinberg equilibrium (HWE). The statistically significant was considered as $P < 0.05$.

Results

The clinical features and demographic variables of infertile patients and healthy controls are presented in Table 2. We observed that alcohol drinking, family history, as well as semen parameters are significantly different between case ans control groups ($P < 0.05$). However, we found no significant difference between case and control groups in term of age, tobacco smoking, and body mass index (BMI).

Table 1. The clinical features and demographic variables of cases and controls.

Variables	Patients (n = 50)	Controls (n = 50)	P value
Age (year)	34.12 ± 3.33	36.23 ± 6.11	0.387
BMI (kg/m)	24.18 ± 4.09	23.76 ± 2.19	0.453
Tobacco smoking			
Never	29 (58%)	26 (52%)	-
Ever	21 (42%)	24 (48%)	0.122
Alcohol drinking			
Never	29 (68%)	38 (76%)	-
Ever	21 (42%)	12 (24%)	**0.001**
Family history			
Negative	41 (82%)	50 (100%)	-
Positive	9 (18%)	0 (0%)	**0.011**
Semen parameters			
Concentration ($\times 10^6$/ml)	45.90 ± 23.76	122.52 ± 41.56	**0.023**
Motility (%)	45.31 ± 21.24	79.83 ± 18.12	**0.016**
Volume (ml)	2.13 ±3.12	2.92 ± 1.77	0.832

BMI: Body Mass Index. Data are shown as mean ± SD.

We observed that polymorphism of miR-196a2 rs11614913 was in agreement with HWE in case and control groups ($P > 0.05$). The genotypes and alleles distribution of miR-196a2 rs11614913 polymorphism in case and control groups are presented in Table 2. The statistical analysis demonstrated a significant decrease in infertility risk in patients with CC ($P = 0.016$) genotype (Table 2).

Table 2. Genotype and allele distribution of miR-196a2 rs11614913 polymorphism.

Polymorphism	Genotype and Allele	Patients (n=50)	Controls (n=50)	P value	OR (95% CI)
miR-196a2 rs11614913	TT	6 (12%)	4 (8%)	Ref	Ref =1
	TC	27 (54%)	20 (40%)	0.071	1.29 (0.25 - 1.88)
	CC	17 (34%)	26 (52%)	**0.016**	1.25 (0.46 - 1.26)
	T normal	39 (39%)	28 (28%)	Ref	Ref =1
	C minor	61 (61%)	72 (72%)	0.102	1.12 (0.38 - 2.18)

OR: Odds Ratio, CI: Confidence Interval

Discussion

Infertility described as inability of pregnancy after one year unprotected sexual intercourse. Male infertility is approximately 50% of infertility cases (17). Various gene mutations or polymorphisms as well as chromosomal aberrations are known as important genetic basis of infertility (18). Idiopathic infertility is defined as infertilities with unidentified causes (19, 20). Therefore, in this study, we investigated associations of miR-196a2 rs11614913 polymorphism and AZS in Iranian male with idiopathic infertility. Evidence suggested that unregulated expression of several miRNAs are observed in men with idiopathic infertility, and expression of most of miRNAs are increased in infertile patients (21).

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To date, many studies have examined the effect of the miR-196a2 polymorphisms on human cancers (22, 23), but few have demonstrated this effect on idiopathic male infertility. In a study by Lu et al. reported that miR-196a2 rs11614913 polymorphism were associated with idiopathic infertility in Chinese men (24). In another study by Jeon et al. reported that miR-196a2 rs11614913 polymorphism are possible risk factors for idiopathic infertility in Korean women (25).

In the present study that was conducted on 50 infertile patients with idiopathic AZS and 50 healthy controls with previous successful fertility, we suggested a significant correlation between miR-196a2 rs11614913 polymorphism and AZS. However, many contradictory results have been reported (22, 23). The cause of difference between the results of previous studies can be due to function of various related genes, difference in geographical area, race, ethnicity, and samples size as well as environmental factors, (26-28).

Conclusion

In generally, our study determined a more details of idiopathic AZS, and demonstrated that miR-196a2 rs11614913 polymorphism may be play an important role in idiopathic AZS among Iranian Azeri infertile men. However, exact role of this polymorphism in idiopathic AZS are remain unknown. Thus, further studies are suggested on larger sample sizes as well as other populations and races.

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