Ilam University of Medical Sciences

Journal of Basic Research in Medical Sciences

Online ISSN: 2383-0972
Print ISSN: 2383-0506

Homepage: https://jbrms.medilam.ac.ir

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

Hamed Mohammadzadeh Zangalani[1], Seyed Babak Khalifehzadeh Kaleybar[2]✉

[1] Department of Molecular Biology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2] Department of Pathology, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Article Info

Article type:
Research Article

Article history:
Received: 14 November 2020
Revised: 28 December 2020
Accepted: 17 January 2021
Published online: 28 June 2023

✉**Correspondence to:**
Seyed Babak Khalifehzadeh Kaleybar, Department of Pathology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
Tel:+98 144101006
Fax: +98 -
Email: b_khalifehzadeh@yahoo.com

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

How to cite this article: Mohammadzadeh Zangalani M, Khalifehzadeh Kaleybar SB. Influence of single nucleotide polymorphism in miR-196a2 on idiopathic asthenozoospermia in Iranian Azeri males. J Bas Res Med Sci. 2023; 10(1):25-30.

© The Author(s).

Publisher: Ilam University of Medical Sciences

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

[Downloaded from demo.medilam.ac.ir on 2026-11-15]

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

[Downloaded from demo.medilam.ac.ir on 2026-11-15]

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).

[Downloaded from demo.medilam.ac.ir on 2026-11-15]

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.

Acknowledgments

This article was extracted from the M.Sc. project of Hamed Mohammadzadeh Zangalani where Seyed Babak Khalilzadeh Kaleybar supervised the project. We thank the whole staff of Biotechnology Research Center, Tabriz Branch, Islamic Azad University for assistance in the successful strategy of this research.

References

1. Coutton C, Satre V, Arnoult C, Ray P. Genetics of male infertility: the new players. Med Sci (Paris). 2012;28(5):497-502. doi: 10.1051/medsci/2012285014.
2. Soheilyfar S, Nikyar T, Fathi Maroufi N, Mohebi Chamkhorami F, Amini Z, Ahmadi M, et al. Association of IL-10, IL-18, and IL-33 genetic polymorphisms with recurrent pregnancy loss risk in Iranian women. Gynecol Endocrinol. 2019;35(4):342-5. doi: 10.1080/09513590.2018.1528220.
3. Nasirpour H, Azari Key Y, Kazemipur N, Majidpour M, Mahdavi S, Hajazimian S, et al. Association of rubella, cytomegalovirus, and toxoplasma infections with recurrent miscarriages in Bonab-Iran: a case-control study. Gene Cell Tissue. 2017;4(3): e60891. doi: 10.5812/gct.60891.
4. Hajizadeh YS, Emami E, Nottagh M, Amini Z, Maroufi NF, Azimian SH, et al. Effects of interleukin-1 receptor antagonist (IL-1Ra) gene 86 bp VNTR polymorphism on recurrent pregnancy loss: a case-control study. Horm Mol Biol Clin Investig. 2017;30(3):1-6. doi: 10.1515/hmbci-2017-0010.
5. Curi SM, Ariagno JI, Chenlo PH, Mendeluk GR, Pugliese MN, Sardi Segovia LM, et al. Asthenozoospermia: analysis of a large population. Arch Androl. 2003;49(5):343-9. doi: 10.1080/713828220.
6. Piomboni P, Focarelli R, Stendardi A, Ferramosca A, Zara V. The role of mitochondria in energy production for human sperm motility. Int J Androl. 2012;35(2):109-24. doi: 10.1111/j.1365-2605.2011.01218. x.
7. Poongothai JE, Gopenath TS,

[Downloaded from demo.medilam.ac.ir on 2026-11-15]

Manonayaki SW. Genetics of human male infertility. Singapore Med J. 2009;50(4):336-47. doi: 10.2174/1381612043453261.
9. Shademan B, Nourazarian A, Nikanfar M, Avci CB, Hasanpour M, Isazadeh A. Investigation the miRNA146a and miRNA155 gene expression levels in patients with multiple sclerosis. J Clin Neurosci. 2020; 87:189-193. doi: 10.1016/j.jocn.2020.04.071
10. Hajazimian S, Maleki M, Danaei Mehrabad S, Isazadeh A. Human Wharton's jelly stem cells inhibit endometriosis through apoptosis induction. Reproduction 2020;159 (4):549-58. doi: 10.1530/REP-19-0597.
11. Astamal RV, Maghoul A, Taefehshokr S, Bagheri T, Mikaeili E, Derakhshani A, Delashoub M, Taefehshokr N, Isazadeh A, Hajazimian S, Tran A. Regulatory role of microRNAs in cancer through Hippo signaling pathway. Pathol-Res Pract. 2020;216(12):153241. doi: 10.1016/j.prp.2020.153241.
12. Taefehshokr S, Taefehshokr N, Hemmat N, Hajazimian S, Isazadeh A, Dadebighlu P, Baradaran B. The Pivotal Role of MicroRNAs in Glucose Metabolism in Cancer. Pathol-Res Pract. 2020; 217:153314. doi: 10.1016/j.prp.2020.153314.
13. Soheilyfar S, Velashjerdi Z, Hajizadeh YS, Maroufi NF, Amini Z, Khorrami A, Azimian SH, Isazadeh A, Taefehshokr S, Taefehshokr N. In vivo and in vitro impact of miR-31 and miR-143 on the suppression of metastasis and invasion in breast cancer. J BUON. 2018;23(5):1290-6.
14. Fu JD, Rushing SN, Lieu DK, Chan CW, Kong CW, Geng L, Wilson KD, Chiamvimonvat N, Boheler KR, Wu JC, Keller G. Distinct roles of microRNA-1 and-499 in ventricular specification and functional maturation of human embryonic stem cell-derived cardiomyocytes. PloSone. 2011;6(11): e27417. doi: 10.1371/journal.pone.0027417.
15. Fathi Maroufi N, Taefehshokr S, Rashidi MR, Taefehshokr N, Khoshakhlagh M, Isazadeh A, Mokarizadeh N, Baradaran B, Nouri M. Vascular mimicry: changing the therapeutic paradigms in cancer. Mol Biol Rep. 2020; 47:1-7. doi: 10.1007/s11033-020-05515-2.
16. Wu J, Bao J, Kim M, Yuan S, Tang C, Zheng H, Mastick GS, Xu C, Yan W. Two miRNA clusters, miR-34b/c and miR-449, are essential for normal brain development, motile ciliogenesis, and spermatogenesis. Proc Natl Acad Sci USA. 2014;111(28): 2851-7. doi: 10.1073/pnas.1407777111.
17. Krawetz SA, Kruger A, Lalancette C, Tagett R, Anton E, Draghici S, Diamond MP. A survey of small RNAs in human sperm. Hum Reprod. 2011;26(12):3401-12. doi: 10.1093/humrep/der329.
18. Boivin J, Bunting L, Collins JA, Nygren KG. International estimates of infertility prevalence and treatment-seeking: potential need and demand for infertility medical care. Hum Reprod. 2007;22(6):1506-12. doi: 10.1093/humrep/dem046.
19. Isazadeh A, Hajazimian S, Rahmani SA, Mohammadoo-Khorasani M, Samanmanesh S, Karimkhanilouei S. The effects of Factor II (rs1799963) polymorphism on recurrent pregnancy loss in Iranian Azeri women. Riv Ital Med Lab. 2017;13(1):37-40. doi: 10.1007/s13631-017-0145-y.
20. Isazadeh A, Hajazimian S, Rahmani SA, Mohammadoo-Khorasani M, Moghtaran N, Maroufi NF. The effect of factor-xi (rs3756008) polymorphism on recurrent pregnancy loss in Iranian Azeri women. Gene Cell Tissue. 2017;4(1): e43717. doi: 10.17795/gct-43717.
21. Shiralizadeh J, Barmaki H, Haiaty S, Faridvand Y, Mostafazadeh M, Mokarizadeh N, et al. The effects of

[Downloaded from demo.medilam.ac.ir on 2026-11-15]

high and lowdoses of folic acid on oxidation of protein levels during pregnancy: a randomized double-blind clinical trial. Horm Mol Biol Clin Investig 2017; 33:20170039. doi: 10.1515/hmbci-2017-0039.

22. Wu W, Qin Y, Li Z, Dong J, Dai J, Lu C, Guo X, Zhao Y, Zhu Y, Zhang W, Hang B. Genome-wide microRNA expression profiling in idiopathic non-obstructive azoospermia: significant up-regulation of miR-141, miR-429 and miR-7-1-3p. Hum Reprod. 2013;28(7):1827-36.
23. Hu Z, Chen J, Tian T, Zhou X, Gu H, Xu L, Zeng Y, Miao R, Jin G, Ma H, Chen Y. Genetic variants of miRNA sequences and non–small cell lung cancer survival. J Clin Invest. 2008;118(7):2600-8. doi: 10.1172/JCI34934.
24. Liu Z, Li G, Wei S, Niu J, El-Naggar AK, Sturgis EM, Wei Q. Genetic variants in selected pre-microRNA genes and the risk of squamous cell carcinoma of the head and neck. Cancer. 2010;116(20):4753-60. doi: 10.1002/cncr.25323.
25. Lu J, Gu H, Tang Q, Wu W, Yuan B, Guo D, Wei Y, Sun H, Xia Y, Ding H, Hu L. Common SNP in hsa-miR-196a-2 increases hsa-miR-196a-5p expression and predisposes to idiopathic male infertility in Chinese Han population. Sci Rep. 2016; 6:19825. doi: 10.1038/srep19825.
26. Jeon YJ, Kim SY, Rah H, Choi DH, Cha SH, Yoon TK, Lee WS, Shim SH, Kim NK. Association of the mi R-146a C> G, mi R-149T> C, mi R-196a2 T> C, and mi R-499 A> G Polymorphisms with Risk of Spontaneously Aborted Fetuses. Am J Reprod Immunol. 2012;68(5):408-17. doi: 10.1111/aji.12005.
27. Fathi Maroufi N, Aghayi E, Garshsbi H, Matin MG, Bedoustani AB, Amoudizaj FF, et al. Association of rs1946518 C/A Polymorphism in Promoter Region of Interleukin 18 Gene and Breast Cancer Risk in Iranian Women: A Case-control Study. Iran J Allergy Asthma Immunol. 2019;18(6):671-678. doi: 10.18502/ijaai. v18i6.2180.
28. Fathi Maroufi N, Gholampour Matin M, Ghanbari N, Khorrami A, Amini Z, Haj Azimian S, et al. Influence of single nucleotide polymorphism in IL-27 and IL-33 genes on breast cancer. Br J Biomed Sci. 2019;76(2):89-91. doi: 10.1080/09674845.2018.1545554.
29. Isazadeh A, Azimian SH, Tariverdi N, Rahmani SA, Esmaeili M, Karimkhanilouei S, et al. Effects of coagulation factor XIII (Val34Leu) polymorphism on recurrent pregnancy loss in Iranian Azeri women. LaboratoriumsMedizin. 2017;41(2):89-92. doi: 10.1515/labmed-2017-0012.

[Downloaded from demo.medilam.ac.ir on 2026-11-15]