Original Article

Evaluating of HERV-K Genes Expression in Sperm Samples Collected from Infertile Men Compared to Fertile Individuals: A Case-Control Study

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Abstract

Background: Having detrimental effects on the health-care system, infertility can be related to some risk factors, especially different kinds of viruses. Human endogenous retrovirus-k (HERV-K) env, gag, np9, and rec can be considered as one of these viral agents that are likely to cause male infertility, and we attempted to evaluate it.

Materials and Methods: This case-control study was conducted on sperm samples of 96 participants in Imam Hossein Hospital, Tehran, Iran, from January 2020 to December 2021. After extracting the RNA form these samples, we evaluated the expression of HERV-K env, np9, rec, and gag using quantitative real-time polymerase chain reaction (qRT-PCR).

Results: Our data revealed that HERV-K, rec, np9, and env in abnormal samples were higher than normal ones. However, the opposite trend was true for gag expression since a meaningful reduction can be observed in abnormal samples.

Conclusions: The results of our study suggested that there is a plausible correlation between the expression level of this virus's genes and the progression of infertility. We proposed this marker as a promising biomarker to diagnose infertility. However, further studies are required to support these results.

Keywords: Biomarkers, human endogenous retrovirus (HERV), quantitative real-time polymerase chain reaction (qRT-PCR), sperm

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Introduction

Infertility is one of the reproductive system diseases all around the world, which can cause serious health problems in one-sixth of couples. It is defined as the inability of couples to get pregnant during a year; moreover, women who are not able to maintain their pregnancy are considered infertile.[1] It is reported that this disease can affect 8–12% of couples of childbearing age, which puts pressure on the health-care system. Infertility has been seen in more than 186 million individuals in numerous countries, especially developed ones. Indeed, from an infertility point of view, we are living in a marvelous age, as the prevalence of it has been reported at approximately 7.88% in Iran.[2,3] It is the reason why the outbreak of this disease is one out of every seven couples and three out of every ten couples in developed and developing countries, respectively.[4] Moreover, researchers in a study

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reported that 20–30% of infertility cases are attributed to males in more regions of the world.[5]

The primary cause of infertility is not clear, but there are several factors relating to this problem. Alcohol consumption, changing sexual behaviors, smoking, obesity, stress, and diet may be able to accelerate the prevalence of male infertility.[6–8] Microbial risk factors, such as chlamydia, gonorrhea, human papillomavirus (HPV), herpes simplex virus (HSV), and human endogenous retrovirus (HERV), are other items that can cause sexually transmitted disease (STD).[9,10]

Since the 1980s, studies have revealed that HERV are able to integrate into the host genome, stimulating some destructive mutations, ranging from frame-shifts to deletions.[9] These mutations can cause different types of cancer, and it is supposed that there are tangible relations between infertility and this virus as well.[11] Since 30–40 million years ago, HERV has compromised about 8% of the human genome, and it can be transmitted vertically.[12] By changing the expression of HERV genes, this virus can cause serious problems in numerous tissues, especially the placenta, epididymis, and testis.[9] This virus has been classified into three classes, such as class I (gammaretrovirus- and epsilonretrovirus-like), class II (betaretrovirus-like), and class III (spumaretrovirus-like). It is assumed that HERV-K has entered into the human genome more than other HERV families.[13] Some structural genes of this virus, especially HERV-K gag, env, np9, and rec genes, are regulated by two long-terminal repeat (LTR) sequences that may be related to infertility.[14]

Given the fact that the relation between HERV-K env, np9, rec, and gag gene expression and infertility has not been confirmed, in this study, we evaluated the expression of these genes in sperm samples.

Materials and Methods

Preparation of samples

This case-control study was conducted on sperm samples of 96 participants in the Imam Hossein Hospital, Tehran, Iran, from January 2020 to December 2021 to evaluate the expression level of the HERV-K env, np9, rec, and gag genes. We assessed samples after 3–5 days of sexual abstinence; furthermore, sperm motility, sperm concentration ($\times 10^6$ sperm/ml), and sperm morphology were analyzed according to the World Health Organization guidelines. In this study, samples were collected from participants with body mass index between 18 and 25 kg/m^2 who were not exposed to radiation therapy, or chemotherapy. Moreover, patients with varicocele, experiencing previous testicular surgery, endocrine disorders, or cancer, and infected samples with bacteria were excluded.[15]

We confirm that informed consent was obtained from all patients. We confirm that all methods were carried out in accordance with relevant guidelines and regulations. We confirm that all experimental protocols were approved by the Shahid Beheshti University of Medical Sciences.

Extraction of RNA

To remove protein, chloroform and 1 ml of RNX-plus solution reagent (CinnaGen, Tehran, Iran) were added to sperm samples. Then, RNA can be precipitated from the supernatant by adding isopropanol, and finally, the RNA was diluted with 50 ul of Diethyl pyrocarbonate (DEPC) -treated water. Agarose gel electrophoresis was utilized to observe 5S, 18S, and 28S.

Reverse transcription (cDNA synthesis)

To produce cDNA from an RNA template, we performed the reverse transcription reaction with Bio fact cDNA kit (Daejeon, South Korea). A 20-µl reaction volume contained random hexamer (1 µl), master mix (9 µl), and RNA samples (10 µl). Then the reaction was incubated for 40 min at 50°C and 10 min at 95°C in Bio Intellectica PCR. The cDNA was diluted by adding sterile water two times.

Quantitative real-time PCR

A real-time PCR technique was performed to evaluate HERV-K genes expression. We mixed BIOFACT™ 2× real-time PCR master mix (for SYBR Green I; BIOFACT, South Korea) (10 µl), sterile water (6 µl), forward primer 10 pmol (1 µl), reverse primer 10 pmol (1 µl) and cDNA of a final 20 µl volume (2 µl). The incubation was conducted in one cycle: an initial denaturation at 95°C for 10 min, forty cycles of 30-second denaturation at 95°C, the annealing step at 55°C for 30 s then at 72°C for 30 s in Rotor-gene 6000 (Corbett life sciences, Sydney, Australia) in 36-well Gene Discs. The temperature of the constructed melting curves ranged from 60°C to 95°C.

Commonly used GAPDH housekeeping gene as an internal control. For quantitative real-time PCR data, the 2-ΔΔCT method was used; furthermore, negative controls confirmed that there was no contamination. The list of primers used in the present study is in Table 1.

Ethical consideration

The school of medicine research department at Shahid Beheshti University of Medical Sciences supported the present study, financially"; (IR.SBMU.RETECH.REC.1400, 692), grant no. 29252.

Statistical analysis

To find the relation between HERV gene expressions and infertility, we employed GraphPad Prism software. Experimental data are expressed as mean ± standard deviation of three independent assays. Statistical significance was calculated using t-tests. The differences were determined based on a P value less than ($P < 0.05$), which means it is meaningful, or not.

Results

Samples

We were able to extract RNA and synthesize cDNA from 94 sperm samples from the Imam Hossein Hospital, Tehran,

Table 1: Primers used for genes in real-time PCR

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
GAPDH	ATGTTCGTCATGGGTGTGAA	GGTGCTAAGCAGTTGGTGGT
Rec	ATCGAGCACCGTTGACTCACAAGA	GGTACACCTGCAGACACCATTGAT
np9	AGATGTCTGCAGGTGTACCCA	CTCTTGCTTTTCCCCACATTTC
Env	TAACCCTGTCACTTGGATT	ATGTCACTGTCTCTTCGG
Gag	AGCAGGTCAGGTGACCGTAAC	GGTGCCATAGCATTGTCTCCT

Iran, from January 2020 to December 2021. Our samples (53 teratospermia samples and 41 normal ones) were analyzed to evaluate HERV-K env, gag, np9, and rec. All samples were confirmed through the World Health Organization guidelines, and we did not find a concrete relation between age, sex, and changes in HERV-K gene expressions. We will provide some of the same characteristics after collecting data from the university.

Evaluating the expression level of HERV-K rec gene in sperm samples

To assess the expression level of the HERV-K rec gene, we extracted RNA from the sperm samples. Quantitative real-time PCR analysis showed that there was no difference in the mRNA expression level of rec in infertile samples in comparison with the normal ones [Figure 1a].

Increase in the HERV-K np9 gene expression in sperm samples

Our results revealed that a climb in the mRNA expression level of np9 in abnormal samples can be observed; however, it was not statistically significant [Figure 1b].

Evaluating the expression level of HERV-K env genes in sperm samples

Similarly, env expressions illustrated a growth in abnormal samples, but with a higher intensive trend [Figure 1c].

Reduction in HERV-K gag in abnormal samples

Our data revealed that the HERV-K gag had a meaningful change in expression in abnormal samples in comparison with the normal ones [Figure 1d].

Discussion

According to previous reports, about 9% of men between the ages of 15 and 44 have suffered from infertility, which is the reason why this disease can be considered a significant problem all around the world. Scientists have published that many risk factors can play a crucial role in causing reproductive problems; however, further studies are required to approve their effects. Take, for example, HPV18, which is able to down-express p53.[16]

In this study, we evaluated the effects of the HERV on sperm infertility. This virus is likely to cause some mutations in the host genome, leading to a variety of health problems, ranging from different kinds of cancer to infertility. The molecular mechanisms of HERV activations in spermatogenesis have not been found, but DNA methylation and histone modification can be the most important items related to this virus.[16] In fact, changing the expression of numerous genes by some viral agents, especially HERV-K env, gag, rec, and np9, can cause infertility.[9] To illustrate, Chan and colleagues demonstrated that there is a concrete relation between HERV-K and testicular cancer.[17]

Due to these reasons, we evaluated the expression of HERV-K env, np9, rec, and gag using quantitative real-time polymerase chain reaction (qRT-PCR). Our data revealed that HERV-K, rec, np9, and env in abnormal samples were higher than normal ones. However, the opposite trend was true for gag expression since a meaningful reduction can be observed in abnormal samples. It is suggested that further studies should be performed to evaluate the role of this virus on infertility since the limitation of this study was the low number of samples.

Few researchers have evaluated the role of this virus on infertility, making it difficult to confirm it, for example, a high concentration of HERV-w env (syncytin 1) can accelerate the fusion process between gametes, increasing the chance of embryo development. However, HERV-H-pol, HERV-K-pol, HERV-W-pol, and Syn2 gene expressions were lower in spermatozoa in comparison with white blood cells (WBCs).[18] Herbst and colleagues reported that HERV-K gag and env are expressed in ovarian germ cell tumors and in their testicular precursor lesions (teratomas, mature and immature, and spermatocytic seminomas are exceptions). Moreover, gestational trophoblastic disease (GTD) shows the expression of this virus but not in benign GTD (noninvasive molar pregnancy). Our data revealed that there were no differences between embryonal and adult tissues' HERV-K expression.[19] Roelofs and colleagues assessed the expression of HERV-K gag, env, and prt genes in testicular germ cell tumors (TGCTs) of adolescents and adults with and without carcinoma by reverse transcription-polymerase chain reaction. All these samples illustrated the expressions of gag and prt, while env genes were detected in normal testicular parenchyma samples, but not in TGCTs.[20] In another study, Larsson reported that ERV3 (HERV-R) can be identified in certain cells in spermatogenesis and the placenta, but the opposite trend is true for Sertoli or Leydig cells.[21] Homologous recombination between many copies of HERV placed at different loci causes destructive mutations in the human genome. For example, the elimination of the azoospermia factor a (AZFa) region from male-specific regions of the Y chromosome (MSY)

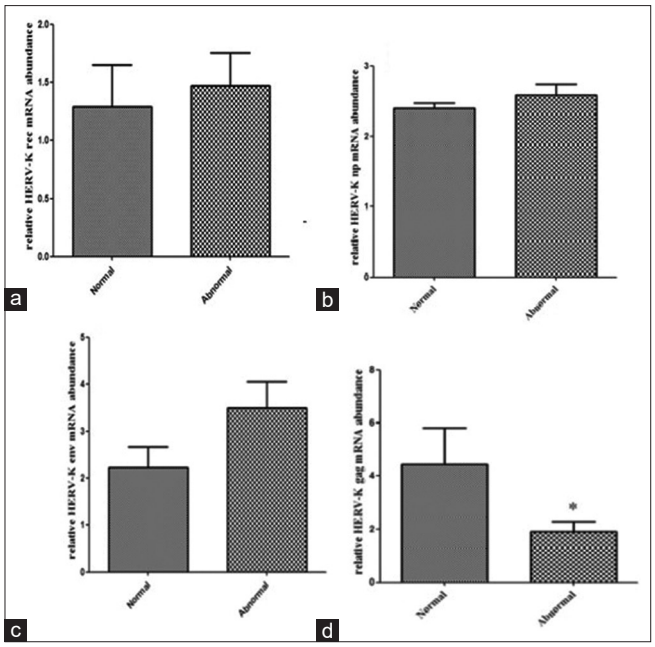

Figure 1: (a) Rec expression in the abnormal samples compared to normal ones had a slight rise. (b) Similarly, our results revealed that a climb in the mRNA expression level of np9 in abnormal samples can be observed; however, that was not statistically significant. (c) env expressions illustrated a growth in abnormal samples but with a higher intensive trend. (d) Moreover, our data revealed that HERV-K gag had meaningful changes in expression in abnormal samples in comparison with normal ones

disrupts spermatogenesis through recombination between HERV15 proviruses. The prior study suggested that HERVs are potentially diverse genetics integrated into the genome and have created maiden variants.[22] HERV-K14C transcripts were only found in the human testis and placed between the P1 and P4 regions. The omission of HERV-K14C causes disorder spermatogenesis. In other words, normal spermatogenesis was related to the expression of HERV-K14C transcripts.[23] Analyzing human oocytes showed that HERV-K10 L1 and SVA retrotransposons are transcriptionally expressed, resulting in the suggestion that HERV plays a role in oocyte development.[24] Scientists detected ERV3 env-like antigens on spermatogenic cells in mature baboon testes and epididymal spermatozoa. Moreover, ejaculate spermatozoa, seminal fluid, and epididymal spermatozoa had reverse transcriptase activities.[25] It seems that the expression of different kinds of HERV can be higher than in 18 other human tissues, concluding that these viruses can help develop the placenta. In fact, HERV-W (syncytin-1),[26–28] HERV-FRD (syncytin-2),[29] ERV-3, HERV-R, and HERV-W[30–32] have been reported in this tissue. In another study, L1 and HERV-W are assumed to progress human ovarian carcinomas.[33]

Conclusion

The results of our study suggested that there is a plausible correlation between the expression level of this virus's genes and the progression of infertility. We proposed this marker as a promising biomarker to diagnose infertility. However, it is suggested that further studies should be performed to evaluate the role of this virus on infertility since the limitation of this study was the low number of samples.

Ethics approval and consent to participate

The school of medicine research department at the Shahid Beheshti University of Medical Sciences supported the present study, financially"; (IR.SBMU.RETECH.REC.1400, 692), grant no. 29252

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Authors' contributions

Shaian Tavakolian analyzed the tests, Zahra Rafiei Atani wrote the text, and Amir Zarei, Hossein Goudarzi, Amir Reza Abedi, and Ebrahim Faghihloo analyzed the data.

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Conflicts of interest

There are no conflicts of interest.

References

1. Niakan S, Faghihloo E, Mofarahe ZS, Novin MG, Raee P, Karimi M, *et al*. Evaluation of human papillomavirus in the semen of infertile men and its relationship with semen quality. Arch Clin Infect Dis 2023;18.
2. Saei Ghare Naz M, Ozgoli G, Sayehmiri K. Prevalence of infertility in Iran: A systematic review and meta-analysis. Urol J 2020;17:338-45.
3. Cox CM, Thoma ME, Tchangalova N, Mburu G, Bornstein MJ, Johnson CL, *et al*. Infertility prevalence and the methods of estimation from 1990 to 2021: A systematic review and meta-analysis. Hum Reprod Open 2022;2022:hoac051.
4. Vander Borght M, Wyns C. Fertility and infertility: Definition and epidemiology. Clin Biochem 2018;62:2-10.
5. Agarwal A, Baskaran S, Parekh N, Cho CL, Henkel R, Vij S, *et al*. Male infertility. Lancet 2021;23:319-33.
6. Schuppe HC, Pilatz A, Hossain H, Diemer T, Wagenlehner F, Weidner W. Urogenital infection as a risk factor for male infertility. Dtsch Arztebl Int 2017;114:339-46.
7. Boeri L, Capogrosso P, Ventimiglia E, Pederzoli F, Cazzaniga W, Chierigo F, *et al*. Heavy cigarette smoking and alcohol consumption are associated with impaired sperm parameters in primary infertile men. Asian J Androl 2019;21:478-85.
8. Moridi A, Roozbeh N, Yaghoobi H, Soltani S, Dashti S, Shahrahmani N, Banaei M. Etiology and risk factors associated with infertility. Int J

Women's Health Reprod Sci 2019;7:346-53.
9. Akhigbe RE, Dutta S, Hamed MA, Ajayi AF, Sengupta P, Ahmad G. Viral infections and male infertility: A comprehensive review of the role of oxidative stress. Front Reprod Health 2022;4:782915.
10. Ramezani, A., Nikravesh, H., Faghihloo, E. The roles of FOX proteins in virus-associated cancers. J Cell Physiol 2019;234:3347-61.
11. Jiao, SY., Yang, YH., Chen, SR. Molecular genetics of infertility: Loss-of-function mutations in humans and corresponding knockout/mutated mice. Hum Reprod Update 2021;27:154-89.
12. Jia L, Liu M, Yang C, Li H, Liu Y, Han J, *et al*. Comprehensive identification and characterization of the HERV-K (HML-9) group in the human genome. Retrovirology 2022;19:11.
13. Rivas SR, Valdez MJM, Govindarajan V, Seetharam D, Doucet-O'Hare TT, Heiss JD, *et al*. The role of HERV-K in cancer stemness. Viruses 2022;14:2019.
14. Ragheb AM, Ibrahima RM, Elbatanouny AM, Moussa AS, Abdelbary AM, Sayed OM, *et al*. Role of sequential semen samples in infertile men candidates for assisted reproduction: A prospective study. AFJU 2018;24:363-7.
15. Björndahl, L. Methods for sperm concentration determination. Methods Mol Biol 2013;927:3-12.
16. Lyu Z, Feng X, Li N, Zhao W, Wei L, Chen Y, *et al*. Human papillomavirus in semen and the risk for male infertility: A systematic review and meta-analysis. BMC Infect Dis 2017;17:1-9.
17. Chan SM, Sapir T, Park SS, Rual JF, Contreras-Galindo R, Reiner O, *et al*. The HERV-K accessory protein Np9 controls viability and migration of teratocarcinoma cells. PLoS One 2019;14:e0212970.
18. Bergallo M, Canosa S, Galliano I, Dapra V, Montanari P, Sestero M, *et al*. Impaired transcription of human endogenous retroviruses in the sperm with exception of syncytin 1: Short communication. Mol Biol Rep 2021;48:5803-8.
19. Herbst H, Sauter M, Mueller-Lantzsch N. Expression of human endogenous retrovirus K elements in germ cell and trophoblastic tumors. Am J Pathol 1996;149:1727-35.
20. Roelofs H, van Gurp RJ, Oosterhuis JW, Looijenga LH. Detection of human endogenous retrovirus type K-specific transcripts in testicular parenchyma and testicular germ cell tumors of adolescents and adults: Clinical and biological implications. Am J Pathol 1998;153:1277-82.
21. Larsson E, Andersson AC, Nilsson BO. Expression of an endogenous retrovirus (ERV3 HERV-R) in human reproductive and embryonic tissues--evidence for a function for envelope gene products. Ups J Med Sci 1994;99:113-20.
22. Sun C, Skaletsky H, Rozen S, Gromoll J, Nieschlag E, Oates R, *et al*. Deletion of azoospermia factor a (AZFa) region of human Y chromosome caused by recombination between HERV15 proviruses. Hum Mol Genet 2000;9:2291-6.
23. Sin HS, Koh E, Kim DS, Murayama M, Sugimoto K, Maeda Y, *et al*. Human endogenous retrovirus K14C drove genomic diversification of the Y chromosome during primate evolution. J Hum Genet 2010;55:717-25.
24. Georgiou I, Noutsopoulos D, Dimitriadou E, Markopoulos G, Apergi A, Lazaros L, *et al*. Retrotransposon RNA expression and evidence for retrotransposition events in human oocytes. Hum Mol Genet 2009;18:1221-8.
25. Mwenda JM, Sichangi MW, Oduor-Okello D, Wango EO, Langat DK. Human endogenous retrovirus-R (ERV 3) env-like antigens expressed in baboon testes and epididymides. East Afr Med J 2003;80:264-70.
26. Blond L, Beseme F, Duret L, Bouton O, Bedin F, Perron H, *et al*. Molecular characterization and placental expression of HERV-W, a new human endogenous retrovirus family. J Virol 1999;73:1175-85.
27. Blond L, Lavillette D, Cheynet V, Bouton O, Oriol G, Chapel-Fernandes S, *et al*. An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. J Virol 2000;74:3321-9.
28. Mi S, Lee X, Li X, Veldman M, Finnerty H, Racie L, *et al*. Syncytin is a captive retroviral envelope protein involved in human placental morphogenesis. Nature 2000;403:785-9.
29. Malassiné A, Frendo L, Blaise S, Handschuh K, Gerbaud P, Tsatsaris V, *et al*. Human endogenous retrovirus-FRD envelope protein (syncytin 2) expression in normal and trisomy 21-affected placenta. Retrovirology 2008;5:6.
30. Kato N, Pfeiffer-Ohlson S, Kato M, Larsson E, Rydnert J, Ohlsson R, *et al*. Tissue specific expression of human provirus ERV3 mRNA in human placenta: Two of the three ERV3 mRNAs contain human cellular sequences J Virol 1987;61:2182-91.
31. Boyd T, Bax CMR, Bax BE, Bloxam DL, Weiss RA. The human endogenous retrovirus ERV-3 is upregulated in differentiating placental trophoblast cells. Virology 1993;196:905-90.
32. Yi M, Kim S. Molecular phylogenetic analysis of the human endogenous retrovirus E (HERV-E) family in human tissues and human cancers. Genes Genet Syst 2007;82:89-98.
33. Menendez L, Benigno BB, McDonald JF. L1 and HERV-W retrotransposons are hypomethylated in human ovarian carcinomas. Mol Cancer 2004;3:12.