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Original Article

Detection of HSV-1 DNA in the Semen of Infertile Men and Evaluation of its Correlation with Semen Parameters in Iran

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Abstract

Background and Aims: Sexually transmitted infections (STIs) are of major concern to clinicians and researchers in the field of reproductive medicine. Many STI pathogens cause incurable and often fatal diseases, and have been transmitted through insemination procedures. The role of herpes simplex virus in male infertility has been investigated using the sensitive methods. The aim of this study was to determine the prevalence of HSV-1DNA in the semen of an asymptomatic male group with infertility problems and its association with altered semen parameters.

Methods: A total of 70 semen samples from infertile men were collected in the Research and Clinical Center for Infertility Yazd, Iran. Semen analysis and diagnostic Real Time PCR using specific primers and probe for gpB gene of HSV-1, was performed for detection of HSV-1 DNA in the specimen.

Results: Semen analysis showed two groups of infertile men, including male factor group and unexplained group. HSV-1 DNA was detected in 16 (22.86%) of the70 semen samples. All HSV-1positive samples had abnormal semen parameters (male factor group).

Conclusion: Using a powerful molecular method, we detected a high prevalence of HSV-1 DNA in the semen of asymptomatic infertile patients. Although HSV-1 infection was not associated with motility and morphology defects of the sperms, it was related with decreased sperm count in the semen fluid.

Keywords: Infertility; Herpes simplex virus; Real Time Polymerase Chain Reaction; Semen fluid

Introduction

Herpes simplex virus 1 (HSV-1) is a double stranded enveloped DNA virus that is able to replicate in many types of cells. The HSV-1 is responsible for a spectrum of diseases, ranging from gingivostomatitis to keratoconjunctivitis, encephalitis, infections of newborns and genital diseases. After initial infection the virus will remain latent and may become reactivated throughout the lifetime of an individual, especially in immunocompromised patients. The first infection leads to antibody formation. However, even in the presence of antibodies, reactivation still occurs, so that the presence of antibodies does not ensure protection against reinfection (1).

Infertility is a major problem of modern medicine as it affects almost 20% of reproductive - aged couples. The cause of this

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problem is attributed to the male partner in nearly 40%– 50% of these cases (2, 3). The most common disorder associated with male infertility is varicocele, which accounts for 35% of the cases.

Endocrine disorders, spermatic duct obstruction, antisperm antibodies, gonado-toxins, drugs, cryptorchidism, infection, sexual dysfunction, and ejaculatory failure are less common causes, accounting for <5% of male infertility cases (3). In 50% of male infertility cases, the etiology remains unknown, and the infertility is classified as idiopathic. Idiopathic infertility represents a serious situation as it affects a high percentage of infertile men who cannot be successfully treated by the empirical therapeutic modalities currently applied (4, 5).

Human pathogens have been recognized as having a considerable possible effect on male factor infertility or low sperm counts in men. Bacterial infections may lead to male factor infertility with a prevalence of 6.6%–48% (6). Accumulating evidence indicates that viral infections contribute to male factor infertility, either directly through toxic effects on the cells of the male genital tract or indirectly, causing local infectious or immunological responses that in turn can negatively affect reproductive functions (7-9). The prevalence of herpes viruses in the male genital tract varies, particularly for HSV-1, which ranges between 3.1% and 49.5%, depending on the viral DNA detection method used (Polymerase chain reaction, in situ hybridization, etc.) and in several studies, it was also associated with infertility (10-17).

The aim of this study was to use Real Time PCR to determine the prevalence of herpes simplex virus type 1(HSV-1) in the semen of a randomized asymptomatic infertile male group attending an infertility clinic. Furthermore, the possibility of HSV-1 infection affecting semen parameters, and thus fertility was investigated.

Methods

Samples

We designed a cross-sectional study for Detection of HSV-1 DNA in the semen of infertile men and its correlation with semen parameters. Semen samples were collected from 70 men who attended the Research and Clinical Center for Infertility in Yazd, Iran. Informed consent was obtained from each patient for the purposes of the current study. None of the men studied or their spouses had reported any clinically confirmed genital herpetic infection in their medical history. In all cases, a complete semen analysis, including sperm count, motility and morphology of sperms was performed.

DNA Extraction from Semen Samples

After collection of the specimens each semen sample was centrifuged at 2,500 rpm for 10 minutes. The supernatant was removed and the pellet was transferred to an eppendorf tube. DNA extraction was performed using the protocol of High pure template PCR extraction kit (Roche, Germany). All DNA samples were subjected to spectrophotometer for the quantification of DNA at 260 nm.

Real Time PCR

All samples were tested for the presence of HSV-1 DNA by Real Time PCR method (Rotor Gene 6000, Corbett Research, Australia). PCR was performed using specific Primers and probe for detection HSV-1 DNA by Real Time PCR. Primers and probe were designed by Primer3 plus online tools (www.bioinformatics.nl/cgibin/primer3plus/primer3plus.cgi) from conserved domain of Glycoprotein B gene (Table 1). 10 µl of DNA was added to 10 µl of the reaction mixture containing 2 units TaqMan polymerase, 0.01% gelatin, 0.6 µM of each primer, 0.2 µM of probe, 200 µM of each deoxynucleotid triphosphate, 5 µl of reaction buffer (50 mM KC1, 10 mM tris-HCI, pH = 8.3) and 3 mM MgCl2. Polymerase chain reaction was performed at 95° C for 10 minutes, followed

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Table 1. Primers and probe sequence for HSV-1.

Forward primer	Reverse Primer	TaqMan probe
CGCATCAAGACCACCTCCTC	GCTCGCACCACGCGA	FAM-TGGCAACGCGGCCCAAC-BHQ1

by 45 cycles of 95°C for 15s, 60°C for 40s and fluorescence detection on the channels Fam (Green), for HSV-1 at 60°C. Before starting the runs, the tubes were kept at 50°C for 2 minutes for activation of UNG. Standard precautions were taken to avoid sample-to-sample contamination and PCR product carryover. As positive controls, we used DNA extracted from HSV-1 KOS Strain, infected Vero cells and a mixture without DNA template was used as negative control.

Statistical Analysis

Statistical analysis was performed using the SPSS V.13 statistical software package (SPSS Inc., Chicago, IL). Comparison of the mean sperm count, motility and morphology between virally infected and non infected samples was performed by T independent T-test and Mann-Whitney test. A P value of 0.05 was accepted as statistically significant.

Results

Table 2. Features for classification of samples to Male factor and unexplained groups.

Parameters	Male factor[1]	Unexplained[2]
Quick + Slow motility	<50	≥50
Sperm count(million/ml)	<20	≥20
Normal morphology	<30	≥30

1: infertile men with abnormal parameters of the semen analysis.
2: infertile men with normal parameters of the semen analysis.

Table 3. Percent of Positive and Negative samples by Real Time PCR.

No samples	Positive (%)	Negative (%)
70	16 (22.86)	54(77.14)

Semen analysis showed two groups of infertile men, including male factor group and unexplained group.HSV-1 DNA was detected by Real Time TaqMan PCR method in 16 (22.86%) of 70 semen samples. Semen analysis of positive and negative samples for HSV-1 DNA revealed that all positive samples had abnormal semen parameters (male factor group). Features for classification of samples to male factor and unexplained groups are shown in table 2.

Statistical analysis by T independent T test on HSV-1 positive and negative groups for semen parameters revealed no meaningful relation between HSV-1 infection and sperm motility or sperm morphology. However a significant relation between HSV-1 infection and reduction in sperm count was observed (Table 4).

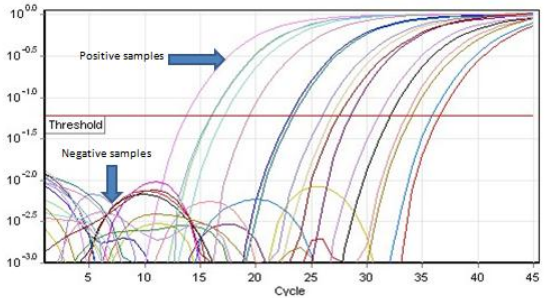

Fig. 1. Results of Real Time PCR

Discussion

HSV-1 mainly affects epithelial surfaces and causes oral and occasionally genital cold sores. Primary infection usually occurs through direct or indirect contact with herpetic lesions, (1).The demand for assisted reproduction has increased over the last few years because of infertility problems among young couples (18, 19). HSV-1 has been detected in semen and sperm cells (20). Male factor infertility in the majority cases remains asymptomatic with unknown causes. We designed our study to

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Table 4. Features for classification of samples to Male factor and unexplained groups.

Parameters	HSV-1	Mean	Std. Deviation	P. Value
Count (Million/ml)	Pos	27.6	16.7	<0.001
	Neg	60.3	48.2	
Quick progressive	Pos	3.5	5.4	0.776
Motility	Neg	4.01	7.05	
Slow progressive Motility	Pos	26.2	11.5	0.482
	Neg	23.4	14.5	
Non progressive Motile	Pos	16.06	3.2	0.418
	Neg	16.1	7.9	
Normal Morphology	Pos	18.6	8.6	0.423
	Neg	16.5	9.7	

investigate the prevalence of herpes simplex virus type 1, in the semen of infertile men, using a sensitive Real Time PCR TaqMan method that is a sensitive technique for diagnosis of infectious agents. Moreover, we aimed to study the relationship between the presence of HSV-1 DNA and semen parameters. In the current study, HSV-1was only detected in 16 (22.86%) of 70 semen samples of infertile men. In a study by El Borai *et al*, a significant association between HSV and infertility was observed. They detected HSV-1 DNA in 24% of semen samples from infertile men using a nested PCR technique (12). In another study HSV DNA was detected in 49.5% semen samples and its presence was significantly related to low sperm count and poor motility (13). In agreement with El Borai *et al* and other studies, a significant relation between HSV infection and low sperm count was shown in our investigation (13, 15). Neofytou *et al*. reported 2.1% HSV-1 DNA in the semen samples of infertile men but no association between the presence of HSV-1 DNA and semen parameters (21).

Experimental studies with transgenic mice revealed the relation between HSV infection and infertility. The expression of HSV thymidine kinase in testis of transgenic mice was associated with defects in spermatogenesis characterized by acrosomal aberrations, structural and developmental arrest (22-24). In one study to determine the prevalence of pathogens that cause sexually transmitted infections in semen from asymptomatic male infertile patients, 3.7% of cases harbored HSV DNA, and of all the pathogens studied, HSV infection had the most effects on both semen quality and levels of accessory gland/ epididymal function markers (20). Antiviral treatment of male infertile patients positive for HSV in semen resulted in successful pregnancies (12, 15). Thus, HSV infection of the male genital tract could explain some cases of male infertility, because of its association with decreased semen quality.

In conclusion, the findings of this study which is the first investigation regarding the correlation of HSV-1 infection and male infertility in Iran, together with other investigations is indicated that HSV, which affect the most important factors of semen quality. The early detection of this virus by the PCR technique will permit the suitable antiviral therapy to increase the possibility of fertility restoration and long-term protection of sperm quality. The detection of herpes viruses within semen will provide better control of the

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transmission of these viruses. Using sensitive Real Time TaqMan PCR assay, we detected a considerable prevalence of HSV-1 DNA in semen from asymptomatic infertile patients. HSV-1 infection was not associated with motility and morphology of sperms, but was associated with decreased sperm count in the semen fluid. These findings indicate that asymptomatic HSV-1 infections of the male genital tract are common and may contribute to male infertility. Because HSV-1 also endangers the health of partners and offspring, efforts to diagnose and treat subclinical HSV-1 genital-tract infection should be increased, and finally, we recommend for successful pregnancies in infertile couples with HSV infection, acyclovir treatment was don.

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