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Molecular detection and genotyping of herpes simplex virus (1 and 2) in some Iraqi infertile men

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In more than half of infertile men, the cause of their infertility is unknown. Several studies revealed the role of viral infections in male infertility. The aim of the present study was to determine the prevalence of herpes simplex virus-1 (HSV-1) and HSV-2 in semen from asymptomatic infertile male patients, and its association with altered semen parameters. A total of 100 semen samples were collected from Kamal AL-Samaree for fertilization and infertility Hospital and the Medical City Teaching Hospital, Iraq. Semen analysis and diagnostic real-time PCR for HSV-1 and HSV-2 DNA were performed. Comparison of semen parameters between patients and control samples were performed with. HSV-1, HSV-2 and HSV (1 and 2) DNA was detected in 9 (12.00%), 16 (21.33%) and 2(2.67) of 100 semen samples, respectively. All HSV-positive samples had abnormal semen parameters (the male factor group). Although HSV infection was not associated with morphological defects, it was correlated with lower sperm count and sperm motility in the seminal fluid. The findings suggest that asymptomatic seminal infection of HSV plays an important role in male infertility by adversely affecting sperm count and sperm motility.

Keywords: infertility, herpes simplex virus, real-time PCR, semen.

INTRODUCTION

Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse (Naina and Amit , 2015).Male infertility is a condition in which the male reproductive tract and sperm has diminished capacity to lead to the eventual fertilization of the egg to produce an embryo. Sometimes male infertility can refer to male's inability to cause pregnancy in a fertile female.(*Brugh and Lipshultz , 2004*) Infertility affects an estimated 15% of couples globally, amounting to 48.5 million couples. Males are found to be solely responsible for 20-30% of infertility cases and contribute to 50% of cases. (Ashok et al., 2015). Male infertility is a multifactorial syndrome encompassing a wide

variety of disorders and there are different causes of male infertility, including spermatogenesis disorders, chronic diseases, infection (Sexual transmitted diseases) and epigenetic (Nasser et al., 2014). In more than half of infertile men, the cause of their infertility is unknown (idiopathic) and could be congenital or acquired. (Poongothai et al., 2009). Chlamydia trachomatis, Ureaplasma spp., human papillomavirus, herpes simplex virus type 1 and type 2, hepatitis B and hepatitis C viruses, HIV-1 and human cytomegalovirus have all been detected in semen from symptomatic and asymptomatic men with testicular, accessory gland and urethral infections. These pathogens are associated with poor sperm quality and decreased sperm concentration and motility.(Schuppe et al., 2017)Viral infections can cause

male infertility, by specific and non-specific mechanism (Nasser et al., 2014). Herpes simplex viruses type-1 and 2 (HSV-1 and HSV-2) are human neurotropic viruses, (Nasser et al., 2014). HSV infects a large population (over 500 million worldwide) and is associated with a variety of diseases, including genital herpes, most of which cases are caused mostly by HSV-2 and partly by HSV-1. (Lilith et al., 2015). Sexual transmission is a major route for the spread of both herpes simplex virus-1 (HSV-1) and -2. Semen plays an important role in carrying the viral particle that invades the vaginal or rectal mucosa and, thereby, initiates viral replication (Chentoufi and Benmohamed , 2012). Different studies have demonstrated that the virus is associated with the occurrence of infertility in men and identified the presence of Herpes Simplex Virus (HSV) in sperm of men with genital infection (Amirjannati et al., 2014). According to a recent study, that, Herpes Simplex Virus infections impair male fertility, either by directly invading the male genital tract cells, or by indirectly causing local inflammatory or immunological responses that could deteriorate reproductive functions.(Johnson , 2015).The aim of the study was to detect the prevalence and genotyping of HSV (1 and 2) by multiplex real time PCR in some infertile Iraqi men .In addition find the impact of HSV infection on semen parameters and male infertility.

MATERIALS AND METHODS

Samples

This study has included 100 Iraqi men with different infertility complaints whose seminal fluid revealed abnormal parameter findings. The patients were examined in the Outpatient men infertility department of the Kamal AL, Samaree for fertilization and infertility Hospital and the Medical City Teaching Hospital. For each patient, a structured questionnaire containing different variables was completed , A Healthy Control Group (a sub group from the study population) was categorized to consist of 25 healthy Iraqi men; Examined specimens were collected seminal fluid from each men in both Patients and Healthy Control Groups. In all cases, a complete semen analysis, including sperm count, motility, and morphology was performed.

Semen analysis

Semen samples from infertile men and fertile controls were evaluated by standard semen

analysis using the (WHO, 2010) criteria. To get the best sample:

Avoid ejaculation for 24 to 72 hours before the test.

Avoid alcohol, caffeine, and drugs such as cocaine and marijuana two to five days before the test.

Avoid any hormone medications several days before the test.

Samples were collected by masturbation and ejaculation into sterile glass cups after 3–5 days of abstinence. After sperm fluid liquefaction at 37C for 30 min, sperm concentration and motility were evaluated in the Meckler chamber. Sperm morphology and leukocyte, white blood cell, round cell and epithelial cell counts were assessed with the use of pre-stained slides by using light microscope.

Genomic DNA isolation

Total DNA isolated from the seminal fluid sample for molecular studies was applied using genomic DNA purification kits of AmpliSens (RIBO-PREP DNA extraction kit / Russia), After genomic DNA extraction, agarose gel electrophoresis was adopted to confirm the presence and integrity of the extracted DNA (Sambrook et al., 1989) and the DNA concentration was measured by using Nano drop.

Real-time PCR

The PCR-based methods have been used successfully for the detection and typing of genital HSV genotypes in clinical specimens such as seminal fluid. Briefly, 10 µl of DNA obtained from clinical or control samples was add to the 15 µl of the prepared mixture which containing 10µl of PCR-mix-1 HSV-typing, 5.0µl of PCR-mix-2, and 0.5 µl of polymerase (Taq F). The control amplification reactions was prepared by :

NCA - 10 µl of DNA-buffer was add to the tube labeled NCA (Negative Control of Amplification).C+ - 10 µl of Positive Control complex (C+) was add to the tube labeled C+ (Positive control of amplification). C- - 10 µl of the sample extracted from the Negative Control (C-) reagent was add to the tube labeled C- (Negative control of Extraction). Tubes were closed and transferred them into the carousel of QPCR-Biometera PCR was performed at 95° for 15 min flowed by 45 cycles 95° for 20 sec , 60° for 20 sec and 72°for15 sec. fluorescence detection at 60°C on the channels Fam (Green), Joe (Yellow), and Rox (Orange) .

Statically analysis

The Statistical Analysis System- SAS (2012) program was used to effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage and LSD or T-Test was used to significant compare between means in this study.

RESULTS

This study was designed to investigate the prevalence of HSV-1 and HSV-2 DNA in the semen of infertile men using real-time PCR, a sensitive technique for detection of infectious agents. In addition, the association between the presence of HSV-1 and HSV-2 DNA and semen parameters was investigated.

HSV Real-Time Typing kit was an in vitro Real Time amplification test for qualitative detection and genotyping of Herpes simplex virus (1 and 2) in the seminal fluid samples (AmpliSens, Russia).They were three channel for read three dyes (FAM, JOE ,and ROX) to detect HSV and HSV genotyping , showed in table (4-1).

Table (4-1) : HSV genotypes detection dyes .

FAM	JOE	ROX
2	1	IC

IC: Internal Control

By analyzing the results of real time PCR for qualitative detection and genotyping of HSV it was revealed that there were three Quantitation data for cycling channels (Per dye figure) figure (4-1 , 4-2, and 4-3).

The results of this study indicated that the number of samples which were positive for HSV were (27/100) with percentage (36.00%) and number of samples which were negative for HSV were (73/100) with percentage (64.00%), Table (4-2) : Distribution of HSV in samples.

Among the HSV genotypes found (in 27 men), the HSV-1 was detected in (9) samples with percentage (12.00%) while HSV-2 was detected in (16) samples with percentage (21.33%) and HSV(1and 2) was detected in (2) samples with percentage (2.67%) .Table(4-3) Distribution of HSV type in samples.

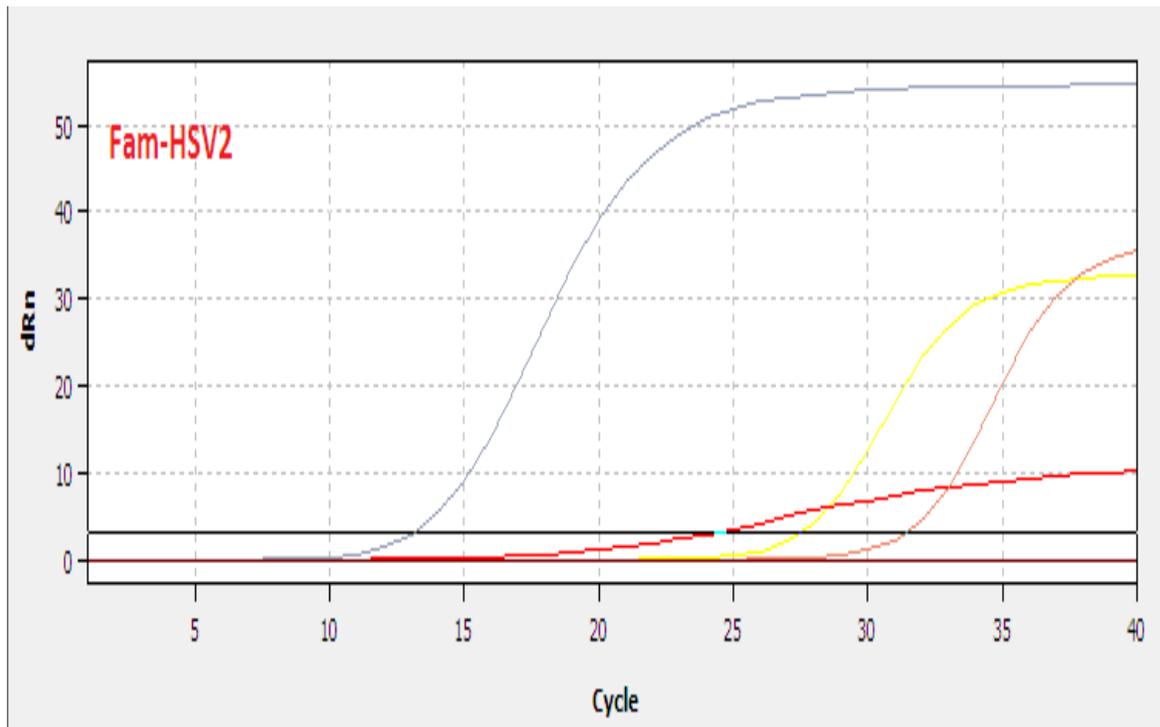


Figure (4-1) : Quantitation data for cycling Green channel (FAM)

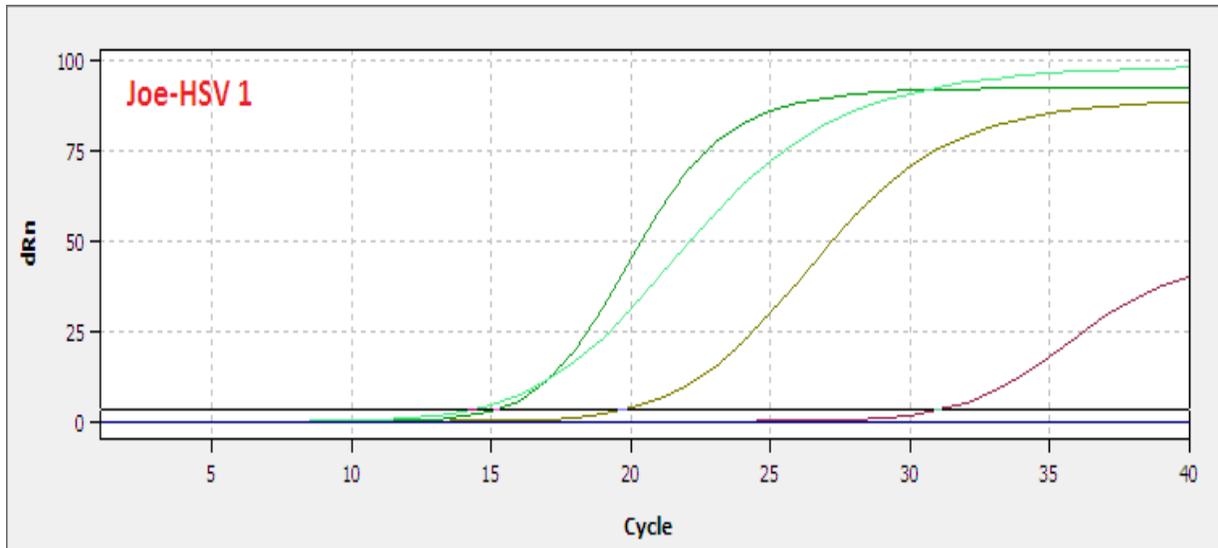


Figure (4-2) : Quantitation data for cycling Yellow channel (JOE).

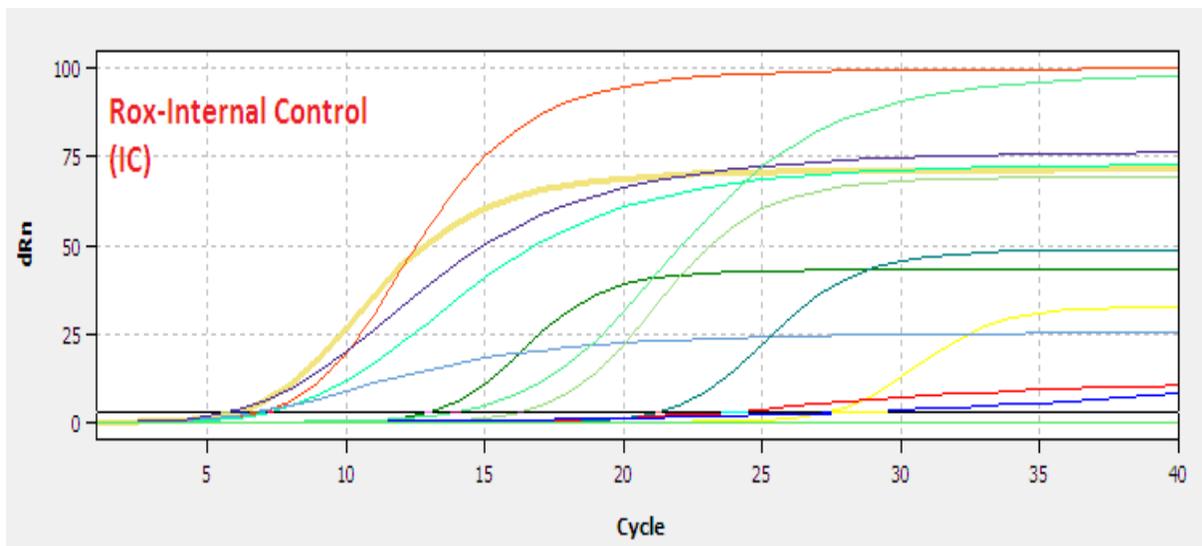


Figure (4-3) : Quantitation data for cycling Orange channel (ROX).

Table (4-2) : Distribution of HSV in samples.

HSV	Total No.	Patients (No = 75)		Control (No = 25)	
		No	%	No	%
Positive	27	27	36.00	0	0
Negative	73	48	64.00	25	100

Table (4-3). Distribution of HSV type in samples.

HSV type	Total No.	Patients (No = 75)		Control (No = 25)	
		No	%	No	%
1	9	9	12.00	0	00.0
2	16	16	21.33	0	0.00
1 & 2	2	2	2.67	0	0.00
Null	73	48	64.00	25	100.00

Table (4-4). Effect of HSV in semen parameters.

HSV	Mean \pm SE				
	Sperm count	Motility progressive	Motility non-progressive	Motility immotile	Morphology
Positive	5.85 \pm 0.14	2.41 \pm 0.08	7.96 \pm 0.25	18.33 \pm 1.55	31.11 \pm 2.09
Negative	20.27 \pm 1.36	3.12 \pm 0.11	16.88 \pm 0.61	23.65 \pm 1.20	40.83 \pm 2.44
P-value	0.0001 **	0.538 NS	0.004 **	0.021 *	0.067 NS

* (P<0.05), ** (P<0.01), NS: Non-Significant.

Table (4-5). Effect of HSV type in semen parameters .

HSV type	Mean \pm SE				
	Sperm count	Motility progressive	Motility non-progressive	Motility immotile	Morphology
1	6.11 \pm 0.35	0.00 \pm 0.00	7.22 \pm 0.37	20.55 \pm 0.84	30.55 \pm 1.53
2	6.25 \pm 0.41	3.75 \pm 0.12	8.75 \pm 0.50	18.12 \pm 1.07	31.87 \pm 2.27
1 & 2	1.50 \pm 0.07	2.50 \pm 0.08	5.00 \pm 0.08	10.00 \pm 0.42	27.50 \pm 1.46
Null	20.27 \pm 1.15	3.12 \pm 0.15	16.87 \pm 1.14	23.62 \pm 1.30	40.83 \pm 2.62
P-value	0.0067 **	0.042 *	0.044 *	0.025 *	0.899 NS

* (P<0.05), ** (P<0.01), NS: Non-Significant.

In our study is the first to investigate the correlation between HSV infection and infertility among Iraqi men, and indicates that HSV, by affecting the most important semen parameters (sperm count , sperm motility and sperm morphology), plays an important role in male infertility.

In general, the result of the study shows that in samples which were positive for HSV, the sperm count was (5.85 \pm 0.14) while motility were [progressive (2.41 \pm 0.08), non-progressive (7.96 \pm 0.25) and immotile (18.33 \pm 1.55)] and morphology was (31.11 \pm 2.09).also , the result of the study shows that in samples which were negative for HSV, the sperm count was (20.27 \pm 1.36) while motility were [progressive (3.12 \pm 0.11), non-progressive (16.88 \pm 0.61) and immotile (23.65 \pm 1.20)] and morphology was (40.83 \pm 2.44).table (4-4) show the effect of HSV in semen parameters .

In specificity, the result of the study shows that the samples were positive for HSV-1, the sperm count was (6.11 \pm 0.35) while motility were [progressive (0.00 \pm 0.00), non-progressive (7.22 \pm 0.37) and immotile (20.55 \pm 0.84)] and morphology (30.55 \pm 1.53) , also, the result of the

study shows that the samples were positive for HSV-2 , the sperm count was (6.25 \pm 0.41) while motility were [progressive (3.75 \pm 0.12), non-progressive (8.75 \pm 0.50) and immotile (18.12 \pm 1.07)] and morphology was (31.87 \pm 2.27), and also, the result of the study shows that the samples were positive for HSV(1 and 2) , the sperm count was (1.50 \pm 0.07) while motility were [progressive (2.50 \pm 0.08), non-progressive (5.00 \pm 0.08) and immotile (23.62 \pm 1.30)] and morphology was (27.50 \pm 1.46) , Table (4-5). Effect of HSV type in semen parameters

DISCUSSION

About 50% of infertility cases are due to male factor. In the majority of male infertility cases, the cause of infertility remains unknown. The role of viral infections in male infertility has been investigated, and many previous studies revealed that HSV infections were related with abnormal sperm parameters. This study was designed to investigate the prevalence of HSV-1 and HSV-2 DNA in the semen of infer-tile men using real-time PCR, a sensitive technique for detection of infectious agents. In addition, the association between the presence of HSV-1 and HSV-2 DNA and semen parameters was investigated.The

results showed that 9 (12.00%), 16 (21.33%) and 2 (2.67%) of the semen samples were positive for HSV-1, HSV-2 and HSV(1and2) DNA, respectively. el Borai et al.,(1997) were detected HSV-1 DNA in 24% of semen samples from infertile men using a nested PCR technique. In another study by Kapranos et al., (2003) HSV DNA was detected in 49.5% of semen samples, while Kotronias et al., (1998) detected HSV-1 and HSV-2 infections in the semen of 21% and 20% of infertile men, respectively. Statistical analysis of the result shows that there is a relationship between the infection with herpes simplex virus and decrease in quality of semen parameters especially on sperm count, sperm motility and sperm morphology, the result found that HSV infection had significant effect at level ($P < 0.01$) on counts and non-progressive motility of sperm and also had significant effect at level ($P < 0.05$) on immotile sperm and had no effect on sperm morphology. The statistical analysis of the result shows that HSV play a vital role in male infertility by effecting on semen parameters in passive manner. The found that the HSV (type-1, type2, type 1 and 2) had significant effect on sperm count at level ($P < 0.01$) and the HSV (type-1, type2, type 1 and 2) had significant effect on sperm motility (progressive, non-progressive, immotile) at level ($P < 0.05$) and had no effect on sperm morphology. The result of the study agreed with previous studies of WHO ,(1999) which revealed that the viral infections have play a strong role in male infertility and other studies which found that HSV infections were related with abnormal sperm parameters. The result of the study agreed with el Borai et al., (1997) which revealed that there is a significant association between HSV infection and infertility, also agreed with the study by Kapranos et al., (2003) which found that HSV infection was significantly related to low sperm count as well as poor motility. Also, our result support the finding of Klimova et al., (2010) whose observed that HSV infection was directly correlated with the reduced amount of actively motile sperm and decrease sperm count. The result of the study agreed with Kotronias et al., (1998) which found that HSV infection was related with reduced sperm count and progressive motility. The result of the study agreed with a study of Leruez-Ville et al., (2005) to determine the prevalence of pathogens that caused sexually transmitted infections in semen from asymptomatic male infertility patients, 3.7% of cases harbored HSV DNA, and among all the pathogens studied, the most robust adverse effect

on both quality and levels of accessory gland markers was associated with HSV.

CONCLUSION

In conclusion, the present study is the first to investigate the correlation between HSV infection and infertility among Iraqi men, and indicates that HSV, by affecting the most important semen parameter sperm count, plays an important role in male infertility. Treatment with approved anti-HSV drugs such as acyclovir, controls HSV lytic infection. Therefore, early detection of this virus using sensitive and specific methods like PCR enables us to reduce the abnormal semen parameters and the possibility of infertility as well as to control the transmission HSV infection. Using real-time PCR assay, we detected a considerable prevalence of HSV DNA in semen from asymptomatic infertile males. HSV can be easily transmitted to the partner and cause genital lesions in mothers as well as severe problems such as encephalitis in newborns. Thus, early diagnosis and appropriate antiviral therapy of asymp-tomatic genital HSV infection should be purposed.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

HMR, MIN designed and performed the experiments and also wrote the manuscript. HMR and MIM performed the RT-PCR analysis. MIN reviewed the manuscript. All authors read and approved the final version.

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