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The association of Human Herpes virus simplex 1 and 2 infection of male genital tract and male infertility

Ahmed Sahib Abdulamir [1] Salah Mahdi hassen[2]

[1]Al-Nahrain University/College of Medicine - Microbiology department
[2]Medical technical university- College of health and medical Technology/ Baghdad-Department of medical lab.Technology

Abstract

Objectives: To investigate the association of viral infections present in the lower genital tract of males and relationship among male infertility.

Methods: ELISA and RT-PCR technique of 100 semen and serum specimens, collected over 8 months from males investigated for infertility, were retrospectively assessed, by screening anti-human Herpes Simplex Virus (HSV) IgM and IgG in the serum and detection of HSV DNA in seminal fluid.

Results: One hundred seminal fluid and blood samples were taken from men and investigated for infertility over a period of 8 months from July 2016 to February 2017. The seminal fluids and serum of patients mentioned to the laboratory from the fertility clinics of Kamal AL-samarai hospital in Baghdad and outer clinics. The investigations were conducted to find the HSV detection rate in the seminal fluid of infertile men and to evaluate the impact of the viral infection on the major sperm parameters. Blood and seminal fluid were collected from 100 infertile male. The results were shown that 20/100 (20.0%) were positive for IgM and 77/100 (77.0%) were positive for IgG of HSV-1; for HSV-2, positive IgM and IgG antibodies were 9/100 (9.0%) and 54/100 (54.0%), respectively. The PCR showed that out of 100 samples 13 (13.0%) gave positive results for HSV-1 while 12 (12.0%) for HSV-2, In case of control group, results were (3.0%),(4.0%) positive for anti-HSV-1, HSV-2 IgM antibodies respectively, and (27.0%), (10.0%) positive for anti-HSV-1, HSV-2 IgG antibodies respectively in control group, using ELISA.

Conclusions: Using sensitive procedures for assays, seminal samples of asymptomatic infertile patients showed unexpectedly high incidence of the sexually transmitted pathogens.

Introduction

Certain microorganisms such as viruses, bacteria, and protozoa, which colonize male and female genital tract cause sexually transmitted diseases or (STDs), usually causing only mild symptoms (1). STDs affect human's health and cause economic and social problems in the world (2). Most STD pathogens have been found in the seminal fluid of symptomatic and asymptomatic men (3).The most important global cause of genital ulcer is Herpes simplex virus type- 2 (4), but either HSV-1 or HSV-2 can be the cause (5).

Furthermore, during primary infection and reactivation, HSV leads to lifelong infections by replicating in the epithelial cells and establishing latency in the host sensory neurons (6). These viruses develop and are transmitted between human-beings by physical contact, and they often cause localized muco-cutaneous lesions (7).

Male factors are the causes of nearly 50% of infertility cases. The etiology behind male infertility commonly remains unknown (8). Investigations of the viral infection function in male infertility revealed a possible linkage between these viruses and abnormal sperm parameters (9).

At the time of initial, primary, or recurrent infection, both types of Herpes virus may cause genital infection, which can be asymptomatic (10).

Seminal fluid can play a major role in viral particle transmission, which then invade the vaginal or rectal mucosa to initiate viral replication (11).

Materials and methods

One hundred seminal fluid and serum specimens from infertile men attending Kamal AL–Samarai hospital investigated for infertility and 100 fertile men as control group were collected over a period from July 2016 to February 2017 were analyzed. ELISA technique was used to measure anti-HSV IgM and IgG antibodies (Human, Gesellschaft for Biochemical and diagnostical mbH, Germany).

After (3-5) days of sexual abstinence, semen samples were collected in the laboratory by masturbation. The ejaculate was deposited in s sterile wide mouth, screw capped , and plastic container. The seminal fluid was examined according to world health organization (WHO) 2010 criteria of seminal fluid. The ejaculate was deposited in s sterile wide mouth , screw capped , and plastic container. The seminal fluid examination included ejaculate pH, volume, viscosity and sperm concentration in (millions / ml), which have been determined by using the Makler Chamber, while sperm motility has been determined as motile spermatozoa percentage, and morphology as percentage of sperms with normal shapes. Measuring of the ejaculate volume was performed by placing the whole specimen in a graduated pipette, and measurement of the total sperm count was done by multiplying the ejaculate volume by the sperm concentration. To study the morphology of sperms, seminal smears were put on slides, and allowed for drying at room temperature, then stained with eosin and in accordance with WHO criteria, they were examined for normal or abnormal sperm morphology (13). Using a light microscope, motility of sperms was examined after putting a drop of semen on a slide and evaluation of 100 sperms in each specimen. Sperm motility was defined pursuant to the World Health Organization as the following: type 1 (fast sperms with straight forward motility); type 2 (fast sluggish moving sperms); type 3 (locally shaking sperms); and type 4 (immotile or non-motile sperms) (13).

The reference points of a normal seminal fluid were reported in accordance with the WHO criteria as: Sperm concentration (≥20 millions sperms/ml), morphology (≥30% sperms), motility (≥50% sperms with forward motility category a and b or ≥25% sperms with category (a) (13).

The seminal fluid samples with abnormal results were examined on two different occasions at eight week intervals. The average of the two readings was estimated. Immediately after receiving semen samples, they were analyzed. All samples were tested for the presence of HSV-1 and HSV-2 DNA by real-time PCR method (genetic PCR solutions^{TM} ,spain). Real time PCR instrument used in this work was STRATAGENE MxPro QPCR (Agilent Technologies, USA). The thermal protocol for HSV-1 dtec- qPCR Test F-100/F300 format and HSV-2 dtec- qPCR Test F-100/F300 format Kits is composed of an initial denaturation for activation the

HotStarTaq DNA Polymerase, amplification cycle and a terminal hold (Table 1).The real-time data is collected at the second step of the amplification cycle. Before starting a Real-Time PCR reaction, the following steps were completed:

Choosing the correct filters (FAM).-
·Identifying and labeling of unknown samples, -
-Standards, positive and negative controls, and assigning the quantitative values for the standards
·Selected the correct thermal protocol-

Table (1): HSV-1 1nd -2 real time PCR amplification profile

Step		Time	Temperature
Activation[1]		15 min	95^0c
40 cycles	Denaturation	15sec	95^0c
	Hybridization / Extension and data collection[2]	60 sec	60^0c

In order to avoid sample to sample contamination and PCR product carryover, standard precautions were taken. DNA was extracted from HSV-1 strain and HSV-2 G strain, and infected Vero cells were used as positive controls, while a mixture without DNA template served as a negative control.

Results

In the present study, age ranged between 20-50 years. The highest prevalence of infection was at age 26-30 year. The lowest prevalence of infection was at age 45-50 year, statistical analysis showed there was significant difference (P value=0.043) in distribution of genital tract infections among age groups as shown in table (2).
In the current study, results revealed that there was non-significant difference between HSV-1 and HSV-2 in seminal fluid parameters (P >0.05). The sero-prevalence of HSV-1 was 20.0% versus 3% for IgM and 77.0% versus 27% for IgG in infertile and fertile groups, respectively (P<0.05), as shown in tables (3),(4). Regarding anti-HSV-2, the result revealed that 9.0% versus 4% were positive for IgM (P>0.05) and 54.0% versus 10% for IgG antibodies (P<0.05), as shown in tables (5),(6).
The PCR technique revealed 13.0% versus only 5% gave positive HSV-1 DNA and 12.0% versus only 4% for positive HSV-2 DNA in seminal fluid(P<0.05). Statistical analysis by independent t-test on HSV-1 positive and negative groups for semen parameters revealed no significant relation between HSV-1 infection and sperm motility or sperm morphology and sperm concentration (P>0.05). However a significant relation between HSV-1 infection and reduction in sperm count was observed, as shown in tables (7),(8).

Table (2) Age descriptive data among study groups.

		Group	
		Patients	Control
	<=25 years	30	26
	%	30.0%	26.0%

Age groups	26-30 years	39	50
	%	39.0%	50.0%
	31-40 years	19	22
	%	19.0%	22.0%
	41-45 years	6	2
	%	6.0%	2.0%
	>45 years	6	0
	%	6.0%	0.0%
Total		100	100
		100.0%	100.0%
p value		0.043	

Table (3): Identification of HSV-1 (IgM) Ab from infertile and fertile control groups by ELISA technique.

			Group		p value	RR (CI)
			Patients	Control		
HSV1 IgM	Positive	Count	20	3	0.001	1.92(1.53- 2.41)
		%	20.0%	3.0%		
	Negative	Count	80	97		
		%	80.0%	97.0%		
Total		Count	100	100		
		%	100.0%	100.0%		

Table (4):Identification of HSV-1 (IgG) Ab from infertile and control groups by ELISA

			Group		p value	RR (CI)
			Patients	Control		
HSV1 IgG	Positive	Count	77	27	0.001	3.09 (2.13-4.49)
		%	77.0%	27.0%		
	Negative	Count	23	73		
		%	23.0%	73.0%		
Total		Count	100	100		
		%	100.0%	100.0%		

Table (5): Identification of HSV-2 (IgM) Ab from infertile and control groups by ELISA

			Group		p value	RR (CI)
			Patients	Control		
HSV2 IgM	Positive	Count	9	4	0.125	1.42 (0.96-2.1)
		%	9.0%	4.0%		
	Negative	Count	91	96		
		%	91.0%	96.0%		
Total		Count	100	100		
		%	100.0%	100.0%		

Table (6): Identification of HSV-2 (IgG) Ab from infertile and control groups by ELISA

			Group		p value	RR (CI)
			Patients	Control		
HSV2 IgG	Positive	Count	54	10	0.001	2.5 (1.93-3.23)
		%	54.0%	10.0%		
	Negative	Count	46	90		
		%	46.0%	90.0%		
Total		Count	100	100		
		%	100.0%	100.0%		

Table (7): The percentage of positive HSV-1 DNA in the seminal fluid of the fertile and infertile groups by RT-PCR.

			Group		p value	RR (CI)
			Patients	Control		
HSV1 PCR	Positive	Count	13	5	0.041	1.51 (1.09-2.09)
		%	13.0%	5.0%		
	Negative	Count	87	95		
		%	87.0%	95.0%		
Total		Count	100	100		
		%	100.0%	100.0%		

Table (8): The percentage of positive HSV-2 DNA in the seminal fluid of the fertile and infertile groups by RT-PCR

			Group		p value	RR (CI)
			Patients	Control		
HSV2 PCR	Positive	Count	12	4	0.033	1.57 (1.14-2.16)

		%	12.0%	4.0%		
	Negative	Count	88	96		
		%	88.0%	96.0%		
Total		Count	100	100		
		%	100.0%	100.0%		

Discussion

HSV-1 usually affects epithelial surfaces and results in oral and less frequently genital cold sore. Primary infection often happens when there is a direct or indirect contact with herpetic lesions (14). Over the last few years, there was a greater demand for assisted reproduction due to infertility problems among younger couples (15). HSV-1 virus was detected in the seminal fluid specimens and spermatozoa (16). In most cases, male infertility remains asymptomatic with unknown reasons.

The quality of seminal fluid has been generally regarded as a male fecundity measurement, and changes in this quality may happen prior to toxic agent exposure or from host factors such as age (17, 18). Several studies indicate an association between age and reduced seminal volume, sperm morphology and / or sperm motility, while sperm concentration is less affected (19-20). Several studies recently found that sperm quality in men is decreased with age (21, 22-23). The idea of sperm motility decrease with advancing age has been supported by the results of several study results (24, 25).Our study also noticed a significant reduction in sperm motility with aging.

Formerly, the study of Huttner *et al* on transgenic mice, demonstrated a more and more association between HSV and infertility (26). In agreement with El Borai *et al* and other studies, a significant relationship was found between HSV infection and decreased sperm count indicating a linkage between the virus and infertility (27). Many studies revealed the connection between this virus and men infertility ; Aynaud *et al* detected the presence of Herpes Simplex Virus in sperm of men who have genital infection by using PCR technique (28), and the study by Foresta *et al* based on data obtained from in situ hybridization technique, found that HSV was detected in sperm cells and a defined a relationship with infertility (29).

These results agree with the study of In Bezold et al which was performed on 241 infertile men, and reported about 18.7% of herpes prevalence (30). The prevalence of 22.86% herpes simplex virus type I was also recorded in Salehi-vaziri *et al* study(31).Klimova et al (32) observed a more frequent presence of seminal HSV infection in infertile male patients than the controls, and showed a direct connection between HSV infection and the low amount of actively motile sperms, so many Studies suggested genitourinary tract infections (GTIs) many cause male infertility (43).

The findings of our study disagreed with Neofytou et al (33) who detected 2.1% of HSV-1 DNA, but no HSV-2 DNA was detected in the semen samples of infertile males.

Wald et al observed by using the PCR technique that 47% of sero-negative samples had herpes simplex virus DNA (34). In the study of Kapranous et al, the prevalence of

HSV was about 56.6% in 113 samples (35). Borai et al (36) found a significant connection between infertility and HSV infection. They detected HSV-1 DNA in 24% of seminal specimens from infertile men by applying the nested PCR. In another study conducted by Kapranos et al (37), HSV DNA was detected in 49.5% of semen samples and HSV infection was significantly associated with reduced sperm count as well as weak motility. Klimova et al (38) noticed that seminal HSV infection is more often present in infertile men than the controls, and revealed that HSV infection is directly connected with the low amount of the actively motile sperms. Furthermore, Abdulmedzhidova et al (39) reported a detection of HSV in 25% of infertile men and an association of HSV infection with oligospermia and sperm structure abnormality. Kotronias et al (40) demonstrated HSV-1 and HSV-2 infections in the seminal samples of 21% and 20% of infertile males respectively. In addition, HSV infection was related to reduced sperm count and progressive motility. In an agreement with these findings, our current study found a significant association between HSV-1 infection and low sperm count.

In this study, due to the partial number and age sharing of studied subjects, age groups have not been classified so the probablity of more exact survey has not been found, which may explain the statistically significant relationship between age and prevalence of herpes simplex. In concordance with Smith et al in a study conducted in Poland, reported that the HSV type 2 prevalence rises with age (41). The relationship between PCR results of seminal fluid specimens and serological survey of infected patients are positive and significant.

The prevalence of HSV-2 and HSV-1 infections differs markedly by country, region & ethnics (42). These discrepancies could be because of variety in Para-clinical methods used, sample size, the population studied, the frequency of sampling and sample storage conditions. Moreover, the cause of this variation can be associated with the type of PCR method used .Given the low number of studies to verify relationship between infertility and infections, the current survey was performed to study the herpes simplex prevalence in the seminal fluid of males with idiopathic infertility, to be determined by PCR, then compared with prevalence of herpes serology tests. In our study, some difference between ELISA and PCR results were seen. It seems that this difference is attributed to the fact that sampling was done among symptomatic patients.

Interestingly in this study , there was a correlation between HSV-1 DNA presence and abnormal semen parameters. In a previous study determining the prevalence of pathogens that cause sexually transmitted infections in semen from asymptomatic male infertility patients, among all the pathogens studied, the most robust adverse effect on both quality and levels of accessory gland markers was associated with HSV (43).

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