

The Association of Human Cytomegalovirus Infection of Male Genital Tract and Male Infertility

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Abstract

Background: The human cytomegalovirus (HCMV) is a widely distributed virus among humans and is included within the sexually transmitted viruses. Because its low detection rate in semen, the impact of HCMV on sperm formation and its vertical transmission within the sperm cells has not been studied sufficiently.

Objectives: To investigate the association of viral infections present in the lower genital tract of males and relationship among males 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 (HCMV) 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 and outer clinics. The investigations were conducted to find the HCMV 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 males. The results were shown that 24/100 (24.0%) were positive for IgM and 86/100 (86.0%) were positive for IgG of HCMV. In case of control group, results were (1.0%) positive for anti-HCMV IgM antibodies, while (20.0%) positive for anti-HCMV IgG antibodies in control group, using ELISA test.

The PCR showed that out of 100 samples 24 (24.0%) gave positive results for HCMV patients, while 7 (7.0%) positive for HCMV in control group.

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

1- Introduction

In the reproductive medicine field, the sexually transmitted infections (STIs) are of major concern to researchers and doctors. Many STI pathogens usually cause incurable and even fatal diseases, and have been shown to be transmitted via insemination procedures (1). Furthermore, many of these pathogenic microorganisms can be transmitted from infected mothers to their fetuses or children (2). Over time, men can also harbor the subclinical infection in their genital tract. Many viruses, such as cytomegalovirus (CMV) and herpes simplex virus type 2 (HSV-2) were detected in the seminal fluid of asymptomatic men (3). The importance of genital tract pathogens as causative factors of male infertility is still a controversial topics. This study aims to determine the prevalence of many commonly sexually transmitted pathogens in healthy and infertile male persons (4). Like other herpesviruses, after primary infection HCMV has the ability to establish latency in various different types of cells (5). HCMV infection may occur in individual without prior exposure to the virus (primary infection), or in those with previous exposure (recurrent infection) (6). Primary or recurrent infection (active infection) may be asymptomatic or cause mild or severe illness (7). HCMV circulate worldwide without seasonal variation and commonly infects people of all ages, races, and those from a variety of socioeconomic, cultural, and geographic

backgrounds (8). Seroprevalence of HCMV in developing countries is higher than seroprevalence in developed countries (9). HCMV viruses have been isolated from oropharyngeal secretions, stool, urine, seminal fluid, blood, cervical & vaginal secretions as well as milk (10). The virus can be discharged in the seminal fluid before coming into contact with the sperms during its presence in the accessory glands. The presence of these viruses in the seminal fluid indicates a genital tract infection, and the seminal fluid can be a viral propagation vector (11, 12). CMV is the leading cause (up to 10%) of neonatal deaths in Iraq (13). In the current study, HCMV was the target of investigation in order to elucidate any relationship between this virus infection of genital tract and idiopathic male infertility.

2- Materials and methods

One hundred seminal fluid and serum specimens from infertile men attending Kamal AL–Samarai hospital were investigated for infertility and 100 fertile men as a control group were collected over a period from July 2016 to February 2017 were analyzed. ELISA technique was used to measure anti-HCMV 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 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 a 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(14). 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)(14).

The reference points of a normal seminal fluid were reported in accordance with the WHO criteria as: Sperm concentration (≥ 20 millions sperms/ml), morphology ($\geq 30\%$ sperms), motility ($\geq 50\%$ sperms with forward motility category a and b or $\geq 25\%$ sperms with category a)(14).

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. All samples were tested for the presence of HCMV DNA by real-time PCR method (genetic PCR solutions™, Spain). Real time PCR instrument used in this work was STRATAGENE MxPro QPCR (Agilent Technologies, USA). The thermal protocol for HCMV dtcc- qPCR Test F-100/F300 format Kit is composed of an initial denaturation for activation the HotStarTaq DNA Polymerase, amplification cycle and a terminal hold as shown in 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. The correct thermal protocol selected.

Table (1): Human cytomegalovirus real time PCR amplification profile

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

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

3- Results

In the present study, the principal finding was the age, which ranged between 20-50 years. The highest prevalence of infection was age 26-30 years than other age classes. The lowest prevalence of infection was at age 45-50 year, statistical analysis showed there were significant difference (P value=0.043) in distribution of genital tract infections among age groups as shown in table (2).

Table (2) Age descriptive data among study groups

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

No similar seropositive results regarding anti-CMV IgM and IgG antibodies were found in both patient men and controls. There was a significant association ($p < 0.05$) between infertility and the high levels of anti-CMV IgG and IgM antibodies, as shown in tables (3) and (4).

The current study revealed that the seroprevalence of anti-CMV IgM and IgG was 24 (24.0%) and 86 (86%) antibodies in infertile patients respectively, tables 1 and 2, and was compared with fertile control antibody 1 (1.0%) for IgM and 20 (20.0%) for IgG. The relative risk (RR) for having male infertility was 2.21 and 5.45 for positive HCMV IgM and IgG, respectively ($P < 0.05$).

Table (3): Identification of HCMV (IgM) Ab from infertile and control groups by ELISA technique.

			Group		p value	RR (CI)
			Patients	Control		
CMV IgM	Positive	Count	24	1	0.001	2.21 (1.83-2.67)
		%	24.0%	1.0%		
	Negative	Count	76	99		
		%	76.0%	99.0%		
Total	Count	100	100			
	%	100.0%	100.0%			

Table (4): Identification of HCMV (IgG) Ab from infertile and control groups by ELISA technique

			Group		p value	RR (CI)
			Patients	Control		
CMV IgG	Positive	Count	86	20	0.001	5.45 (3.33-8.91)
		%	86.0%	20.0%		
	Negative	Count	14	80		
		%	14.0%	80.0%		
Total		Count	100	100		
		%	100.0%	100.0%		

HCMV DNA was analyzed by using the Real-Time PCR method in 24 (24.0%) of 100 semen samples of infertile males versus only 7% in fertile control group ($P < 0.05$). Seminal fluid analysis for positive and negative specimens for HCMV DNA demonstrated that all positive specimens had abnormal semen parameters as seen in table (5).

Table (5): The detection of positive HCMV DNA in infertile males compared to fertile control group by RT-PCR.

			Group		p value	RR (CI)
			Patients	Control		
CMV PCR	Positive	Count	24	7	0.001	1.72 (1.34-2.22)
		%	24.0%	7.0%		
	Negative	Count	76	93		
		%	76.0%	93.0%		
Total		Count	100	100		
		%	100.0%	100.0%		

4- Discussion

Studies suggest that reduced semen volume, sperm motility and/or sperm morphology are all associated with individual's age, while sperm concentration is less affected (15-16). Many recent studies also reveal that sperm quality in men decreases with age (17, 18, 19). Several study results support the finding that sperm motility decreases with advancing age (20, 21). Our study also found a significant reduction in sperm motility with aging.

The presence and persistence of Cytomegalo virus (CMV) in seminal fluid samples, which is a herpesvirus family member, has been always reported previously (22). In this study, HCMV seroprevalence and HCMV infection of semen was all significantly associated with male infertility. However, a lower level of CMV IgM was detected in serum than IgG reflecting a logical data showing recent infections of the tested individuals. No significant relationship between CMV DNA and seminal parameters was revealed in this study, although a trend for low motile sperms and α -glucosidase levels was seen in the CMV-infected study group, which may indicate a modest impact of CMV infection on seminal quality, probably by affecting the function of epididymis. No association between CMV infection and a reduction in the seminal fluid parameters was reported in the previous studies (23). Our study is consistent with (24). HCMV may be infectious in the seminal fluid (25), and the virus has also been isolated from endometrial cells of humans (26), indicating a probable direct infection mechanism of endometrial cells by HCMV which is carried by sperms. Moreover, active HCMV infections may be seen in human endometrial stromal cells, (27). The anti-CMV IgG antibody prevalence, which is reported in this study, has been also found consistent with other studies reported in Turkey and other developing countries. CMV

seroprevalence has been recorded to be (84.3%) in patients from Nigeria, Afyon and Lagos. (28) found a 84.2% seroprevalence in infertile men.

Results of the current study disagreed with (29) who revealed 96.0% of seroprevalence of CMV IgG. In Northern Turkey, (30) reported a 1.0% and 97.3% seropositivity for anti-CMV IgM and anti-CMV IgG antibodies respectively. Seroprevalence of CMV has been also demonstrated to be age dependent in consistency with the study of (31), he recorded that 58.7% of persons aged ≥ 80 years had positive CMV infection. An association was detected between the high CMV concentrations in ejaculate and a transient decrease in the sperm motility, and a reduction in the sperm cell concentration in seminal fluid samples of CMV infected men has been also found (32). However, no effect of CMV infection on the quality of sperms has been detected by most researchers (33).

Previously, different and contradictory studies reported the following results: 0% (culture and hybridization) among 60 males, including 30 CMV positive versus 32.7% (PCR) among 217 males from a 92% seropositive population who were referred for infertility(34).

Unlike (35), who found no positive specimens in seronegative patients, our results found that about 1.4% of initially seronegative people were HCMV DNA positive when the ejaculate PCR was used. This result was in agreement with that of (36), who indicated that several PCR positive results were reported in the seminal samples of seronegative males, some of whom had probable seroconversion, while other men showed persistent seronegative results for several months. Similar results have been detected in other types of tissues (37). On the other hand, it has been shown by Eggert *et al* that the genital tract CMV infection in sub-fertile people is considerable, and seems to have a little role in infertility (38).

Another study by (22) reported the detection of DNA of sexually transmitted pathogen in the semen of large numbers of asymptomatic infertile men, which has been correlated with poor quality of seminal fluid (39). We may attribute this high prevalence of HCMV to the poor socioeconomic condition, poor hygienic practices and poor living conditions (40).

CONFLICT OF INTERESTS

There are no conflicts of interest.

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العلاقة بين الفيروسات المضخمة للخلايا التي تصيب القناة التناسلية عند الرجال مع الذكور العقيمين

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الخلاصة

الخلفية: الفيروس المضخم للخلايا واسع الانتشار بين الناس وله القدرة على الانتقال جنسياً. لها تأثير على عملية تكوين الحيامن والانتقال بواسطة الخلايا المنوية، إذ لا توجد تفاصيل كافية لتشخيصها لكون نسبة وجودها في السائل المنوي قليلة.

الهدف: للتحري عن الاصابات الفيروسية المشتركة في الجهاز التناسلي الذكري للذكور العقيمين وعلاقتها مع حالات العقم في الذكور

طرق العمل: تقنية الاليزا وتفاعل البلمرة المتسلسل استخدمت لتشخيص ١٠٠ عينة مصل وسائل منوي، جمعت على طول فترة ٨ شهرا من الذكور المشخصين والمصابين بالعقم. عن طريق التحري عن مضادات HCMV (IgM, IgG) في المصل وكذلك تحديد HHSV (DNA) في السائل المنوي.

النتائج: ١٠٠ عينة سائل منوي ومصل استحصلت من الذكور المصابين بحالات عقم بالاضافة الى مجموعة السيطره خلال فترة ٨ اشهر من تموز ٢٠١٦ الى شباط ٢٠١٧. عينات السائل المنوي والمصل اخذت وشخصت في مختبرات مستشفى كمال السامرائي في بغداد للعقم ومن العيادات الخارجية. الفحوصات الحديثة اشركت في دراستنا لتحديد نسبة HCMV في السائل المنوي للذكور العقيمين وتقييم اثر الفيروس على معلمات السائل المنوي. اظهرت النتائج ان من بين ١٠٠ اصابة فيروسية فان ٢٤/١٠٠ (٢٤.٠%) تعود الى IgM، ٨٦/١٠٠ (٨٦.٠%) الى IgG موجب بالنسبة لفيروس HCMV. اما مجاميع السيطره فان النتائج كانت (١.٠%) موجب بالنسبة لاضداد HCMV IgM بينما (٢٠.٠%) لاضداد HSMV IgG باستعمال طريقة الاليزا.

اظهرت نتائج RT-PCR ان من بين ١٠٠ عينة هناك ٢٤/١٠٠ (٢٤.٠%) موجب الى HCMV، اما مجاميع السيطره فان النتائج كانت (٧.٠%) موجب بالنسبة HCMV.

الاستنتاجات: باستخدام تقنيات حساسه للتشخيص، تمكنا من تحديد نسب عاليه للاصابات الفيروسية المساهمه بالامراض المنقلبه جنسيا في السائل المنوي والمصل للذكور العقيمين.

الكلمات الدالة: الفيروسات، الذكور العقيمين، الجهاز التناسلي الذكري.