Detection of Human Cytomegalovirus (HCMV) among Infertile Male in AL-Najaf governorate.

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الخلاصة:

هدفت هذه الدراسة إلى التحري عن الفيروس المضخم للخلايا البشرية بين الذكور الذين يعانون من العقم .تم جمع عينات الدم و السائل المنوي بصورة عشوائية من ثلاثمائة مريض، لمعرفة نمط العدوى فيما بينها.

تم استخدام تقنية ELISA لمعرفة نشاط المناعة الخلطية للازواج من خلال قياس الاجسام المضادة للفايروس البشري المضخم للخلايا ال IgM و IgG ، أظهرت أن 31 (11 %) كانت النتيجة موجبة لأضداد الـ IgM و 234 (78 %) كانت موجبة لأضداد الـ IgG . أظهرت تقنية PCR أن من أصل 300 عينة 22 فقط (7.4 %) اعطت نتيجة ايجابية.

اما في حالة مجموعة السيطرة، كانت النتائج سالبة بالنسبة للاجسام المضادة للفايروس المضخم للخلايا البشري باستخدام اختبار ELISA، وتقنية .PCR, في حين اعطت الاجسام المضادة IgG نتائج موجبة ضعيفة.

Abstract:

The current study aimed to detect human cytomegalovirus among infertile individual. Blood and seminal fluid collected from 300 infertile male randomly , ELISA technique was used to know the activity of humoral immunity among them through measuring anti-HCMV IgM and IgG antibodies , the result revealed that 31 (11%) were positive for IgM result and 234 (78%) were positive for IgG. The PCR technique showed that out of 300 samples only 22 (7.4%) gave positive results,

In case of control group, results were negative for anti- HCMV IgM antibodies in ELISA test and PCR technique. While IgG gave a weak positive results.

Aim of the study:

The present study was to study the relationship between the virus and infertility through detection of human cytomegalovirus (HCMV) infection in infertile male in AL-Najaf governorate by screening of anti-human cytomegalovirus IgM and IgG in the serum and detection of human cytomegalovirus DNA in seminal fluid, using PCR technique.

Keywords: HCMV, Infertile males, ELISA, PCR.

INTRODUCTION:

Hunan cytomegalovirus (HCMV) belongs to the herpes virus family, DNA virus. CMV infection is most commonly sub clinical. In the immunocompromised host, primary CMV infection, reactivation and re-infection are all associated with significant morbidity and mortality **(1,2)**. In the immunocompetent adult, primary CMV infection is usually asymptomatic but can result in a mononucleosis syndrome **(1,3)**. CMV infection in immunocompetent hosts may rarely be able to lead to severe organ specific complications. But some serious complications have been reported. Severe hepatitis is a frequent presentation **(4, 5)**. Human CMV has been isolated from

saliva, urine, blood, human milk, cervical secretions, various tissue specimens, and even from human semen **(6)**. Human cytomegalovirus (HCMV) is the most common pathogen in uterus during pregnancy, which may lead to some serious results such as miscarriage, stillbirth, cerebellar malformation, fetus developmental retardation, but its pathogenesis has not been fully explained **(7).** Tests for IgM antibody to CMV often lack specificity for primary infection because of false positive test results or because patients with past infection may have IgM antibody to CMV. The avidity of IgG antibody increases with time after initial infection and demonstration of low CMV-IgG avidity can improve the accuracy of identification of recent primary infection **(8,9).**

Infection is spread by intimate contact with infected body fluids including semen. The virus generally remains in a latent form and causes a lifelong infection, but it may be activated either by a primary or recurrent infection. The demonstration of cytomegalovirus in semen has led to speculation that certain infections caused by this virus may be sexually transmitted.

MATERIALS, SUBJECTS AND METHODS:

The present study was conducted in Al-Sader teaching Hospitals and infertility center in Al-Najaf governorate .The study period was from June 2010 to December 2011.

1. Three hundred serum samples were obtained from infertile male patients aged 15-44 years were chosen randomly for the detection of anti- HCMV IgG and IgM antibodies.

2. Three hundred seminal fluid samples were obtained from the same infertile males in order to detect DNA of HCMV by PCR technique.

3. Fifty individual aged 15-44 years as a control group with male infertile individuals.

Prospective study was conducted on infertile out patient randomly selected from 300 infertile male. Screening for CMV were done by using ELISA and PCR technique according manufacturers**.**

RESULTS:

The results of ELISA test for anti-HCMV IgM and IgG antibodies among different age groups of infertile males

It was found that the highest percentage of anti-HCMV IgG antibodies seropositivity was 234 (78%) out of the total infertile males involved (300) and it was found that the age group (27-32) years ranked the highest seropositivity.

In case of anti-HCMV IgM antibodies seropositivity , it was found that the highest rate was seen in (15-20) age group where the rate was (25%) , whereas the lowest rate was 4 (8.4 %) out of the total sample (48) examined among (39-44) year group. Figure (1)

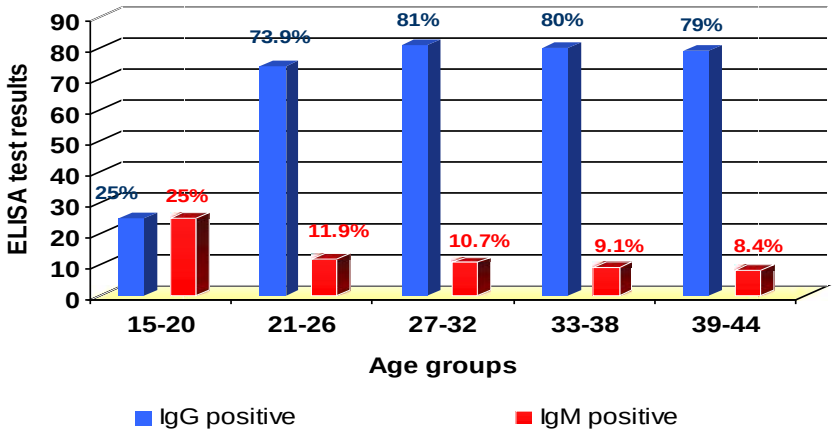

Figure (1): The results of ELISA test for anti-HCMV IgM and IgG antibodies among different age groups of infertile males

The results of ELISA test for anti-HCMV IgM and IgG antibodies among different age groups of healthy males (control group)

It was found that the results for anti-HCMV IgM antibodies in ELISA test and PCR technique. percentage of anti-HCMV IgG antibodies seropositivity was 14 (28 %) out of the total healthy males involved (50) and it was found that the age group (33-38) years ranked the highest seropositivity. Figure (2)

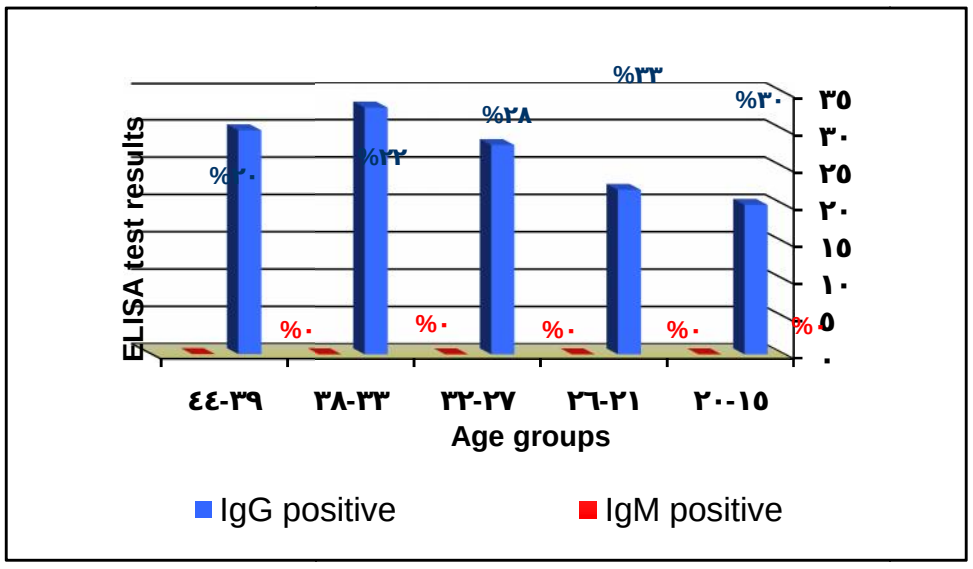

Figure (2): The results of ELISA test for anti-HCMV IgM and IgG antibodies among different age groups of healthy males (control group)

Correlation between seroprevalence detected by anti-CMV IgM antibodies and viral shedding detected by PCR technique among infertile male

It was found that there were 31 (10.33) seropositive anti-CMV IgM out of 300 infertile males, and among those 31 cases, only 5 (16.13) showed positive PCR test results for HCMV.

Table (1): Correlation between seroprevalence detected by anti-CMV IgM antibodies and viral shedding detected by PCR technique among infertile individuals (male and female)

	Seroprevalence by anti-CMV IgM (%)	**Viral shedding by PCR**
Infertile male	31(10.4%) / 300	5 (16.13%)
Healthy male	0 / 50	0

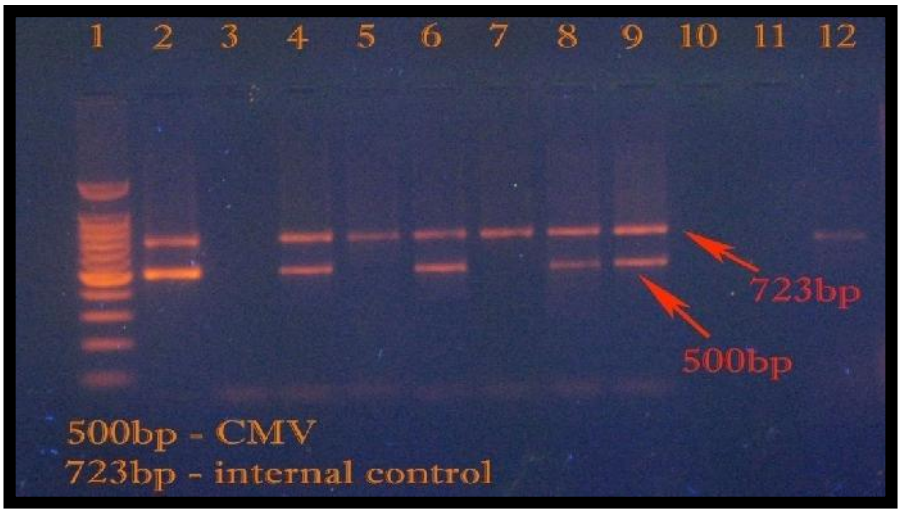

Figure (3): Polymerase chain reaction detection of *HCMV* in Ethidium bromide-stained agarose gel using specific primers MIE gene in seminal fluid sample of infertile male.

Lane (1): DNA molecular size marker (1000-bp ladder).

Lane (2): Control positive of HCMV includes 2 band (500 bp) refers to CMV and (723 bp) refers to internal control.

Lane (3): Negative control.

Lane (10 and 11): Samples show negative result, there is no band detected.

Lane (5 , 7 and 12): Samples show only internal control bands that is mean negative results.

Lanes (4 , 6 , 8 and 9) : Samples show two bands (I.C and CMV) refers to positive results.

DISCUSSION :

Figure (1) showed that anti-HCMV IgG antibodies seropositivity among infertile men, occurred mostly among those who were present within the age of more than 25 years and less than 40, and it was less common among the younger age group and the older one. This can showed that through the light on an important fact in the epidemiology of infection with this virus and the role of cumulative effect of the chronicity of infection which needs time for anti-HCMV IgG antibodies to be built up. So we could find more and more anti-HCMV IgG antibodies positive serum samples for anti-HCMV IgG antibodies among males with an older age group. However, the lower incidence of anti-HCMV IgG antibodies seropositivity among those who are around 40 years of age and older, could be due to the low number of cases studied at this age group; otherwise , no clear explanation could be found for the decrease incidence of anti-HCMV IgG antibodies at this age group.

Table (1) studied the rate of viral antigen detection by PCR in relation to anti-HCMV IgM antibodies in both males and females with infertility and it showed that out of 31 male who was positive for anti-HCMV IgM antibodies 5 of them gave positive detection of viral; antigen by PCR and out of 24 females 4 were positive.

These rates of detection of viral antigen by PCR technique showed an equal detection rate in both males and females who were infertile, and these rates reflected the number of individuals who are acutely infected at the time of study. However, this would not reflect the actual rate of those who were actually infected, as they would not be equivalent to those who were positive for anti-HCMV IgM antibodies whom were the actual number of those who were acutely infected.

The difference was attributed to the fact that most viruses went to latent stage at certain cells, quickly and the PCR which was adapted and used depended on the major immediate early gene , which is only shows for a short period of time during the infective cycle, this is in agreement with what was shown by **(10,11). 12** mentioned that CMV was identified in the semen of patients who were positive for IgG antibodies to CMV.

He revealed that IgG antibodies to CMV were detected in 146 (58.4%) of the 250 serum samples of infertile couples. Two of them (2.85%) exhibit PCR positive for HCMV DNA. These results corroborate serologic data concerning the adult immunocompetent population in central Europe or North America **(13)**.

Further studies showed that the anti-CMV IgG was detected in 249 (99.6 %) of the 250 male serum samples and in 247 (98.9 %) of the 250 female serum samples. Viral shedding was detectable in 83 (33.5 %) of 248 semen samples and 83 (33.7 %)

of 246 cervical mucus samples by dot-blot DNA hybridization assay. Semen quality was not apparently affected by the existence of viral shedding. The co-shedding rate in semen and cervical mucus was high (15.9 %) **(14).**

Sexual transmission of CMV is a major route of virus transmission in adults **.** CMV has been recovered from the semen and uterine cervix of sexually active individuals **(15).**

Though the results of PCR from seminal fluids and vaginal swabs in infertile couples were not significant statistically in regards to the infertility state. Yet , its presence as an acute infections in both males and females who were infertile couples may point a probable effect of the presence of acute viral infection on the infertility state**.**

Many studies pointed out the effect of acute viral infection by HCMV on the infertility state both in males and females . **(16)** stated that HCMV could be isolated from the extracellular fluid of human sperms in infertile group and they put a probable causation for infertility in their study group. **(15)** in their dot-blot assay of viral shedding rate in semen and cervical mucus in infertile group found 33.5% in semen and 33.7% in cervical mucus which were a higher rates in comparison to other studies as they stated. Also, it is much higher that which could be isolated in the present study.

(17) concluded that HCMV was present in a considerable number in subfertile patient genital tract but the finding did not suggested a causal relationship between that and the sexual transmission as a rout for the viral infection and hence the causes of infertility.

CONCLUSION:

1. There were high percentage of IgG positive sera in infertile male enrolled in the present study, while they were less than this in control group.

2. The were IgM positivity in sera , while it was no antibody detected in control group.

3. Human cytomegalovirus was present in the semen of infertile male, whereas in control group no virus detected.

4. The PCR is a reliable and applicable tool for detection of HVMV in semen of infertile male.

5. The results suggest that human cytomegalovirus is sexually transmitted among infertile couples.

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