DETECTION OF CYTOMEGALOVIRUS IN IRAQI RECURRENT MISCARRIAGE WOMEN

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ABSTRACT

This study was aimed to investigate the existence of HCMV in recurrent miscarriage women by using Real-time PCR and compare it with enzyme-linked immunosorbent assay (ELISA). Seventy blood samples were examined from recurrent miscarriage women and forty from women with normal pregnant (aged between 18 and 45 years old) which the patients were suspected to be infected with HCMV. Serological technique included Enzyme-linked Immune sorbent Assay (ELISA) was done to detect the presence of anti - Human Cytomegalovirus antibody IgG and IgM against the virus in the patient’s serum. The serology test showed that all samples of recurrent miscarriage women 70(100%) seropositive to IgG and 28(40%) seropositive to IgM. Compared with control women 35 out of 40 seropositive to IgG and no positive results to IgM. Molecular analysis using Real-time PCR for detection the presence of HCMV DNA in the samples. The results for Real-time PCR showed that; 16 out of 70 (22.85%) patients were found to be HCMV positive while 54 out of 70 (45.65%) patients were found HCMV negative. According to the results of this study, Real Time – PCR technique was used for the detection of Human Cytomegalovirus in whole blood specimens had high sensitivity, effective and more specificity than serological methods (ELISA).

KEYWORD: CMV; ELISA; PCR.

INTRODUCTION

Human cytomegalovirus is a species of virus that belongs to the viral family known as Herpesviridae or herpesviruses. It is typical abbreviated as HCMV and is alternatively known as Human herpesvirus 5 (HHV-5). Within Herpesviridae, HCMV belong to the
Betaherpesvirina subfamily, which also includes cytomegaloviruses from other mammals.\cite{23, 30, 36} Human cytomegalovirus (HCMV) is the most common source of congenital malformation resulting from viral intrauterine infection in developed countries.\cite{16}

HCMV affects 1% of all infants born in the USA and less than 1% of all infants born worldwide. HCMV is large, with a genome of 235 kb encoding 165 genes.\cite{11} The virion consists of a double-stranded linear DNA core in an icosahedral nucleocapsid, enveloped by a proteinaceous matrix.\cite{10}

These components are enclosed in a lipid bilayer envelope that contains a number of viral glycoproteins.\cite{22} Cytomegalovirus (CMV) infection during pregnancy can be transmitted to the fetus, resulting in a congenital infection and is a leading cause of hearing loss, vision loss and mental retardation.\cite{32} About 58.9% of individuals aged 6 and above are infected with CMV.\cite{36}

Women infected for the first time during pregnancy are likely to transmit HCMV to their fetuses. More children suffer serious disabilities caused by congenital HCMV than by several better-known childhood maladies such as Down syndrome or fetal alcohol syndrome.\cite{21} Although they may be found throughout the body, CMV infections are frequently associated with the salivary glands.\cite{17}

Cytomegalovirus infection is typically unnoticed in healthy people, but can be life-threatening for the immunocompromised, such as HIV-infected persons, organ transplant recipients, or new born infants, after infection, HCMV has an ability to remain latent within the body over long periods.\cite{30}

Cytomegalovirus infection is more widespread in developing countries and in communities with lower socioeconomic status and represents the most significant viral cause of birth defects in industrialized countries.\cite{9}

Transmission can occur during pregnancy or after birth, from breast milk, cord blood, saliva, urine, fomites and other sources, Some of infants are infected during delivery.\cite{18}

The development of RT-PCR technology has simplified nucleic acid quantification and is coming into more widespread use. Real-time, quantitative PCR has several advantages over older forms of quantitative PCR assays. Experience to date has reported comparable
sensitivity but superior reproducibility and precision compared to previous methods, with a wide dynamic range. [31]

MATERIALS AND METHODS

Blood sample collection
Whole blood (5ml) was obtained under aseptic conditions from each subject by a vein puncture using a disposable syringe. Blood samples were divided into two tubes.

First tube: EDTA tubes for viral DNA isolation, second tube: The serum was obtained by putting the blood samples in a clean dry plain plastic tube and was allowed to clot at 37°C for 30 minutes before centrifugation. The tubes centrifuged at 6000 rpm for 5 minutes for serology test. The blood sample and serum were placed in a cool box and were then transferred to the laboratory to be kept at -20°C and processed within 24h.

Molecular detection of HCMV By Real-time PCR

a) Genomic viral DNA Isolation
The method was used for the isolation of genomic DNA from whole blood patients and control. Involved isolating the genomic DNA from a human whole blood using full automated nucleic acid extraction system (ExiPrep™ 16 Plus Cat. No.: A-5030) Bioneer/Korea and the ExiPrep™ Plus Viral DNA Kit (Cat. No.: K-4272 Bioneer/ Korea).

b) RT-PCR assay
RT-PCR assay procedure according to AccuPower® CMV Quantitative PCR Kit. The AccuPower® CMV Quantitative PCR Kit (Bioneer, south Korea, Cat. No: 1111) is an in vitro diagnostic kit designed for the quantification of cytomegalovirus (CMV) DNA in human samples such as whole blood, EDTA-plasma, urine and breast milk through real-time PCR using ExiStation™ Molecular Diagnostic System or Exicycler™96.

Enzyme-linked Immunosorbent Assay (ELISA) for the detection of human Cytomegalovirus IgM /IgG
ELISA method was done according to manufacturing company protocol (BioCheck, USA).

RESULTS AND DISCUSSION
Miscarriage is the spontaneous loss of pregnancy between conception and 24 weeks into pregnancy.[14] Serum specimens were tested for the presence of IgG and IgM using enzymatic immunoassay technique. These included (70) women with previous history of recurrent
abortions, intrauterine death (IUD), and premature deliveries. Compared with 60 women with normal delivery. The results obtained from the serological test were (52.9%) 37 patients were positive for IgG and negative for IgM, while the (40%) 28 patients were positive for both IgG and IgM and (7.1%) 5 patients were negative for both IgG and IgM. While the results obtained from the control were (87%) 35 patient positive for IgG and negative for IgM and (12.5%) 5 patient negative for IgG and negative for IgM. (Fig. 1) showed distribution of IgG and IgM in patients and Control group according to serological test.

Fig1: Disruption of the positive and negative samples to HCMV in the miscarriage women and the Control Group detected by ELISA technique.

Khudhur et al., (2014) detected HCMV in 151(70%) of the 214 abortion women by IgG and IgM antibody. According to the age of patients, No difference were seen in results. Also, Pouria et al., (1998); Munro et al., (2005) and Arabpour et al., (2007) and Tawfeq, (2009) got same results. While Shams et al., (2011) noticed that there is a difference between results according to patients age. In study by Al-Kazaz et al., (2014) HCMV detection by ELISA and found 46 positive to IgG out of 46 and 40 positive to IgM out of 46. Ramadhan and Jihad, (2015) reported that miscarriage women were highest percentage of seropositive to HCMV for IgG (40%) and (25%) for IgM out of 40 samples. Yasir, (2012) showed higher of IgG positive at age (27-32) also Sotoodeh, (2009) showed 94% of positive IgG at age (25-34). Shams et al., (2011) found from 327 pregnant women 54 were anti HCMV positive. In Iran, anti HCMV was detected in 94% of the women who had abortion history. Jalal, (2010) conducted their study on 130 (aborted women, suspected men and pregnant women) could detect positive IgM to 70 patient and positive IgG to 97 patient. HCMV infection is endemic in Iraq in (2002); the prevalence rates of human cytomegalovirus IgM and IgG in non-pregnant women have been reported to be 1% and 84% respectively, and 2.5% and 90%
in pregnant women.\cite{4} Khalif et al., (2012) who detected positive IgM to HCMV in 15.7 of 108 pregnant women. Also Mahdi et al.,(2011) reported that an increase in seropositive HCMV IgG in relation with abortion and infection. The presence of a specific IgG antibody means that previous infection or acute infection with HCMV. This is considered true when there is no PCR product because IgG level over time is an uncertain approach for distinguishing primary from non-primary HCMV infection.\cite{28} In this study, high positive results for IgG could be attributable to many factors one of these factors, in Iraq the majority of women of childbearing age are seropositive for HCMV and that they contract the infection either through prenatal or postnatal transmission during early childhood.\cite{3} Another factor the poor of living conditions and hygienic practices, which facilitates that HCMV is easily transmitted by many routes; sexual and non-sexual routes.\cite{16} The presence of a specific IgM antibody means that the women are in primary infection, It is also produced during reactivation and reinfection. The prevalence rates of IgM antibody have been associated with different causes such as women in pregnant state because the primary HCMV infection has been found to be more rate in pregnant women than non-pregnant women this difference may be attributed to women with seronegative to HCMV more susceptibility at beginning of pregnancy to the first infection with HCMV\cite{35}, that may because suppressed immune systems in pregnant women. Risk factors for HCMV infection have been correlated with the socioeconomic status within community.\cite{13} Real time PCR was rapid, sensitive and useful technique for diagnosis active disease and monitoring response to therapy.\cite{27} In this study Real-time PCR was run out on all samples of extracted DNA (Fig.2) from both patient and control group belong to women that have no medical history of hypertension, diabetes and renal disease.

Fig 2: The gel electrophoresis of viral DNA from whole blood of miscarriage women on 1% agarose gel at 100 volt for 30min. Lanes (1-8) =Viral DNA samples
The results for the RT-PCR showed that 16 out of 70 (22.85%) miscarriage women were found to be HCMV positive while 54 out of 70 (77.14%) patients were found HCMV negative. 40 out of 40 control women did not detect HCMV (0%). Both results for the serological test and RT-PCR showed in (Table 1).

Table (1): showed the results for both serological, Real-time tests.

<table>
<thead>
<tr>
<th>No. of Specimens</th>
<th>ELISA</th>
<th>Real-Time PCR</th>
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<tbody>
<tr>
<td></td>
<td>IgG</td>
<td>IgM</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>28</td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td>Total 70</td>
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</tbody>
</table>

These results suggested the best method to detection HCMV was Q-PCR. Al-Kazaz et al., (2014) found that 25(54.34%) out of 46 patients were positively identified to HCMV carrier as 21(45.65%) samples of the serum was free from nucleic acid of the virus when detected by Real-time PCR. And the results for ELISA tests 46(100%) had positive for anti HCMV (IgG) and 40(86.95%) had positive anti HCMV (IgM). When diagnosis using q-PCR, the results for one run showed in (Figure 2). The positive specimens will appear in red color and internal positive control will appear in yellow color. Many factor may be contributed to make q-PCR to be the best technique to detect HCMV. Quantitation can be performed in different fractions of the blood[7], It used to measure the quantity of a PCR product, Indicators of virus load in the blood, such as quantitative PCR product, provide important insights into pathogenesis and confirm the persistent nature of HCMV infections[6], q-PCR considerably faster and highly sensitive than conventional PCR and others detection methods.[2]

The variation between results in this study might be attributable to many factors, such as a result of immune selection and that the allelic clusters have a long history, perhaps having emerged during the evolution of populations of early humans or their predecessors ,a recombination has occurred during HCMV evolution[8], and the same factor that caused variation in PCR results. These rates of detection viral antigen by q-PCR technique showed an equal detection rate in patient women, 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. Another reason that tests for IgM antibody to HCMV often
lack specificity for primary infection because patients with previous infection may have IgM antibody to HCMV. This explains HCMV DNA was not detected in 13 patients who were IgM positive. This might be due to the persistence of IgM antibodies for an extended period of time after primary infection.\[^{15}\] By IgG avidity test can determination primary or chronic infection because antibody bind to antigen with less avidity during acute infection than past infection or chronic infection.\[^{39}\] Therefore, HCMV DNA was not detected in 13 patients who were IgM positive. This might be due to the persistence of IgM antibodies for an extended period of time after primary infection.\[^{15}\]

The Real Time - PCR run for detect HCMV as appeared in the computer attached to the RT-PCR machine. Positive specimens in red color and IPC in yellow color.

**CONCLUSION**

Sever life-threatening complication of HCMV infection in pregnant women may not be as rare as previously thought. The detection of HCMV in women before pregnant should reduce miscarriage rate and also the number of congenital infect infant. Real-time PCR is the best technique and more sensitive and effect than PCR and ELISA The molecular techniques.

**REFERENCES**


