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Real Time Polymerase Chain Reaction versus Enzyme Linked Immunosorbent Assay in the Diagnosis of *Cytomegalovirus* Infection in Pregnant Women

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MSAA, SMES, AABSM and ZAO designed the study and wrote the protocol. Author MSAA interpreted the data, anchored the field study, gathered the initial data and performed preliminary data analysis. Authors MSAA, YHB and MHB managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Cytomegalovirus* infection is endemic worldwide. Most frequently used methods for antibodies detection in developing world are enzyme linked immunosorbent assay. The polymerase chain reaction induces production of large amounts of specific deoxyribonucleic acid fragments from very low concentrations of complex substrates allowing detection of very low amounts of viral particles.
Objectives: To assess the accuracy of ELISA test in comparison with the polymerase chain reaction in maternal blood to detect pregnant woman at high risk of CMV infection and transmission to the fetus.
Study Design: Three hundred blood samples prospectively tested for CMV-specific IgG and IgM antibodies by using ELISA and for CMV DNA using real time PCR.
Results: CMV IgG and IgM were present in 274(91.3) and 17(5.7%) sample respectively. However, CMV DNA was detected in 89 (29.7%) samples. Eighty-four tested samples exhibited both IgG by ELISA and DNA by Real-time PCR. Likewise, IgM was detectable by ELISA from 10 subjects with DNA concomitantly demonstrable by Real-time PCR. By comparison, IgG detected from 190 subjects, with no DNA detectable by Real-time PCR. Similarly, IgM present in seven samples tested by ELISA, but no DNA detected by Real-time PCR.
Conclusion: This study demonstrated that the real time polymerase chain reaction test is more helpful for detection among pregnant woman who are at high risk of CMV infection and transmission to the fetus.

Keywords: Accuracy; latent; acute; chronic; congenital.

1. BACKGROUND

Cytomegalovirus (CMV) is a member of the Betaherpesvirinae subfamily, which belongs to the Herpesviridae family [1]. Immunoglobulin G (IgG) class, indicating previous infection, are found in approximately 60% of adults in developed countries and 100% in developing countries. Infection acquired as a fetus, a neonate, a toddler, a child or an adult. In poor socio-economic circumstances, people acquire CMV earlier than do others [2,3]. In healthy individuals, infections with CMV are usually asymptomatic. However, individuals with impaired or underdeveloped immune systems as in organ transplant recipients, human immunodeficiency virus (HIV) infected patients, and the fetus or newborn infants, infections with CMV often result in life threatening conditions. After infection, CMV has an ability to remain latent within the body over long periods [4]. By the time of delivery, approximately 2% of seronegative pregnant women seroconverted. Young children, especially toddlers with high levels of CMV in their saliva and urine are the major source of CMV for such women [2,3].

Cumulative evidence suggests that 32% of pregnant women had primary infection during pregnancy and transmitted virus across the placenta to the fetus producing intrauterine infection [5]. The fetus can also be infected by reactivation of latent maternal infection or by maternal reinfection with a new strain of CMV [6,7]. Approximately 1% of women who are seropositive prior to pregnancy deliver babies with congenital CMV infection [8]. The risk of severe disease caused by intrauterine infection is about 20% [9,10]. The risk of transmission from mother to fetus increases throughout the stages of pregnancy [11]. There is an increased risk of sequelae when the fetus is infected earlier in gestation [12]. In general, the clinical outcome for the infant or later the child that result out of maternal primary infection is poorer than that result from reactivation or reinfection [7].

Diagnosis of CMV disease is based on clinical symptoms, but the symptoms of CMV can be confused with those due to Epstein-Barr virus (EBV) resulting in difficulties for diagnosis. Laboratory confirmation can be achieved using serological and molecular techniques [13]. The most frequently used serological method for detecting immunoglobulin M and immunoglobulin G antibodies are the enzyme linked immunosorbent assay (ELISA). The polymerase chain reaction is a molecular biology technique in which the production of large amounts of specific DNA fragments is induced from very low concentrations of complex substrates [14]. The high sensitivity of the polymerase chain reaction allows the detection of very low amounts of viral particles (DNA or RNA) [15] and several studies

have reported the utility of this technique for the quantification of CMV DNA in blood or urine [16,17,18]. Therefore, the purpose of this study was to assess the accuracy of ELISA serological test in comparison with the polymerase chain reaction in maternal blood to detect whether pregnant women are at high risk of CMV infection and transmission to the fetus.

2. MATERIALS AND METHODS

2.1 Study Design

Prospective analytical study to assess the accuracy of the ELISA serological test in comparison with the real time polymerase chain reaction.

2.2 Sample Size

Three hundred blood samples, one from every pregnant woman visiting different hospitals, medical clinics and health care centers.

2.3 Samples Collection

Blood samples were collected from consenting pregnant women visiting different hospitals, medical care centers and medical clinics throughout Sana'a city during the period of sample collection. Blood was collected in two sterile tubes, one without anticoagulant to obtain serum and other using EDTA as the anticoagulant and mixed adequately, then centrifuged to separate plasma. Each sample was identified at the referring laboratory by the age or date of birth of the subject, date of collection, and place of collection. The samples were coded by date of collection, sample number and referring laboratory. All samples were stored at -20℃ until use.

2.4 Study Population

Blood samples were collected from pregnant women between 16 and 45 years of age. The samples were categorized according to their stage of pregnancy into the following groups: first trimester group, second trimester group and third trimester group.

2.5 Sampling Techniques

Blood samples were tested for CMV IgG and IgM antibodies using enzyme linked immunosorbent assay (ELISA) and for CMV DNA using COBAS® AmpliPrep/COBAS® TaqMan® CMV (real time PCR) Test.

2.5.1 ELISA

Serum samples were tested for CMV-specific immunoglobulin G and M using enzyme-linked immunosorbent assay (ELISA) technique using DRG kit for IgG detection (DRG International, Inc., USA) and NovaLisa kit for IgM detection (Dietzenbach, Germany).

2.5.1.1 Principle

Microplate wells were pre-coated with CMV antigen to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material horseradish peroxidase labeled anti-human, conjugate was added. This conjugate binds to the captured CMV specific antibodies. The immune complex formed by the bound conjugate was visualized by adding tetramethylbenzidine (TMB) substrate, which gives a blue reaction product. The intensity of this product was proportional to the amount of CMV specific antibodies in the specimen. Sulphuric acid was added to stop the reaction. This produces a yellow color. Absorbance at 450 nm was read using microwell plate reader.

2.5.2 Molecular testing

Plasma samples were tested for CMV DNA using COBAS® AmpliPrep/COBAS® TaqMan® CMV Test using Roche kit (Roche Diagnostic Gmbh, Mannhein, Germany).

2.5.2.1 Principle

The COBAS® AmpliPrep/COBAS® TaqMan® CMV Test is a nucleic acid amplification test for the quantitation of *Cytomegalovirus* (CMV) DNA in human plasma. The COBAS® AmpliPrep/ COBAS® TaqMan® CMV Test is based on two major processes: (1) specimen preparation to isolate CMV DNA and (2) simultaneous PCR amplification of target DNA and detection of cleaved dual-labeled oligonucleotide probe specific to the target. The COBAS® AmpliPrep/ COBAS® TaqMan® CMV Test permits automated specimen preparation followed by PCR amplification and detection of CMV target DNA and CMV Quantitation Standard (QS) DNA. The Master Mix reagent contains primers and probes specific for both CMV DNA and CMV QS DNA. Detection of amplified DNA was performed using target-specific and QS-specific dual-labeled oligonucleotide probes that permit independent identification of CMV amplicon and CMV QS amplicon. The quantitation of CMV viral DNA was performed using the CMV QS. The COBAS®

TaqMan® 48 Analyzer calculates the CMV DNA concentration in the test specimens by comparing the CMV signal to the CMV QS signal for each specimen and control.

2.6 Data Analysis

The percentages of pregnant women with positive, negative and equivocal results was determined using SPSS software version 15 (IBM-SPSS Inc, Armonk, NY).

3. RESULTS

In total, 300 pregnant women were included for analysis. CMV IgG antibodies were present in 274 (91.3%) of 300 serum samples, 57 (20.8%) was first trimester, 118 (43.1%) was second trimester and 99 (36.1%) was third trimester (Table 1). However, CMV IgM antibodies were demonstrable in 17(5.7%) serum samples. Of these positive samples, 1 (5.9%) was first trimester, 5 (29.4%) were second trimester and 11 (64.7%) were third trimester (Table 2). CMV DNA detected in 89 (29.7%) plasma samples. In terms of trimester: 21 (23.6%) were first trimester, 36 (40.4%) were second trimester, while 32 (36%) were third trimester (Table 3). Eighty-four tested samples exhibited both IgG by ELISA and DNA by Real-time PCR. Likewise, IgM was detectable by ELISA from 10 subjects with DNA concomitantly demonstrable by Real-time PCR. By comparison, IgG was detected from 190 subjects with no DNA detectable by Real-time PCR. Similarly, IgM was present in seven samples tested by ELISA, but Real-time PCR (Table 3) detected no DNA.

4. DISCUSSION

In this study, the enzyme linked immunosorbent (ELISA) assay used for the detection of CMV IgG and IgM and real time PCR for detection of CMV DNA. The real time PCR used as the gold standard for CMV infection diagnosis. This was because a positive real time polymerase chain reaction test signifies viral replication and detects pregnant woman at high risk of CMV infection and transmission to the fetus [19]. The serological tests using the immunoglobulin G reagent are helpful in determining CMV previous infections. However, the specific immunoglobulin M is helpful in determining active infection either due to primary or recurrent infections. A positive polymerase chain reaction result during pregnancy identifies patients who are undergoing viral replication within the cell [20].

Table 1. CMV IgG antibodies in studied population

Stage of pregnancy	No. of samples	IgG positive	
		No.	%
1st trimester	60	57	20.8
2nd trimester	133	118	43.1
3rd trimester	107	99	36.1
Total	300	274	91.3

Table 2. CMV IgM antibodies in studied population

Stage of pregnancy	No. of samples	IgM positive	
		No.	%
1st trimester	60	1	5.9
2nd trimester	133	5	29.4
3rd trimester	107	11	64.7
Total	300	17	5.7

Table 3. CMV DNA in studied population

Stage of pregnancy	No. of samples	DNA positive	
		No.	%
1st trimester	60	21	23.6
2nd trimester	133	36	40.4
3rd trimester	107	32	36
Total	300	89	29.7

Table 4. Cross-tabulation between real-time PCR and ELISA (IgM and IgG)

ELISA		Real time PCR		
		Positive	Negative	Total
IgG	Positive (274)	84	190	274
	Negative (26)	5	21	26
Total	(300)	89	211	300
IgM	Positive (17)	10	7	17
	Negative (283)	79	204	283
Total	(300)	89	211	300

The first type of antibody that develops in response to CMV is IgM, which develops within a few days following primary infection and remains detectable for six to nine months. Medium to high levels of CMV IgM can be detected during the first three months of a primary infection. IgM can also be detected during some secondary infections, both reactivation and reinfection, and is therefore not a valid marker of primary infection. The second antibody type to respond to CMV infection is IgG. This antibody develops within 1 to 2 weeks after infection and, once developed, can be detected throughout life [21].

In the present study, IgG was detected from 190 subjects by ELISA with no DNA detectable by Real-time PCR. This attributed to the fact that *Cytomegalovirus* went to latent stage in certain cells quickly; when PCR adapted and used the major immediate early gene that only shows for a short period during the infective cycle [22]. IgG was not detected from five subjects, with DNA detectable by Real-time PCR, this attributed to time lag between primary infection and IgG antibody production. Similarly, IgM was present in seven samples tested by ELISA, but no DNA was detected by Real-time PCR. This attributed to positive results in ELISA IgM due to rheumatoid factor interference and some blood diseases [23]. Furthermore, IgM antibodies can persist for a long time after infection in some healthy individuals [24].

Similarly, Parmigiani et al. [19] reported that the accuracy of the serological tests for the diagnosis of CMV infection was lower than that of the polymerase chain reaction. Also in agreement with our data, Shams and his colleagues [25] concluded that PCR was a more sensitive, reliable and accurate method for the detection of CMV infection in pregnant women. Also Hameed and Aziz [26] reported that the Real Time – PCR technique for the detection of *Cytomegalovirus* in whole blood specimens had high sensitivity, effective and more specificity than serological methods (ELISA). Finally, when comparing serology with the polymerase chain reaction for CMV diagnosis, it is important to remember that serology is a diagnostic test that detects circulating antibodies and identifies the history of previous infections through immunoglobulin G and active infections using immunoglobulin M. The polymerase chain reaction, on the other hand, is a diagnostic test that detects the presence of the DNA virus within the cell.

5. CONCLUSION

This study demonstrated that the real time polymerase chain reaction test is more helpful for detection among pregnant woman at high risk of CMV infection and transmission to the fetus.

ETHICAL APPROVAL

Ethical approval was obtained from the Research Ethics Committee of Faculty of Medical Laboratory Sciences. The objectives of the study were explained to participants and they assured about voluntary participation and confidentiality of the taken information. Written informed consent was obtained from each participant who agreed to participate in this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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