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Comparison of Real-time PCR versus ELISA in the diagnosis of cytomegalovirus infection in pregnant women

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Abstract

Background: Cytomegalovirus infections are endemic worldwide. The most frequently used methods for detecting antibodies in developing world are the enzyme linked immunosorbent assay. The polymerase chain reaction is a molecular biology technique in which the production of large amounts of specific deoxyribonucleic acid fragments is induced from very low concentrations of complex substrates allowing the detection of very low amounts of viral particles.

Objectives: To assess the accuracy of ELISA tests in comparison with the polymerase chain reaction in maternal blood to diagnose cytomegalovirus infection.

Study design: 300 blood samples were prospectively tested for CMV-specific IgG and IgM antibodies by using ELISA and for CMV DNA using real time PCR.

Result: 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%) sample. A total of 84 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

Conclusion: This study demonstrated that the real time polymerase chain reaction test is more accurate than serological ELISA test in the diagnosis of cytomegalovirus infection among pregnant women.

Background

Cytomegalovirus (CMV) is a member of the Betaherpesvirinae subfamily which belongs to the family Herpesviridae [1]. 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 [2]. 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), and this may lead to difficulties in diagnosis. Laboratory confirmation can be achieved using serological and molecular techniques [3]. The most frequently serological used 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 [4]. The high sensitivity of the polymerase chain reaction allows the detection of very low amounts of viral particles (DNA or RNA) and several studies have reported the utility of this technique for the quantification of CMV DNA in blood or urine [5,6].

Objectives

The purpose of this study was to assess the accuracy of the ELISA serological test in comparison with the polymerase chain reaction in maternal blood to diagnose cytomegalovirus infection in pregnant women.

Materials and methods

Three hundred blood samples were collected from pregnant women. Blood samples were collected in two sterile tubes, one without anticoagulant to obtain serum and other with EDTA as the anticoagulant to separate plasma. The samples were categorized according to their stage of pregnancy. Serum samples were tested for CMV-specific IgG and IgM antibodies using DRG kits (DRG International Inc) and NovaLisa kit (Dietzenbach, Germany), respectively, by using enzyme-linked immunosorbent assay. Plasma samples were tested for CMV DNA using COBAS® AmpliPrep/COBAS® TaqMan® CMV Test using Roch kit (Roch Diagnostic Gmbh, Mannhein, Germany). The percentages of pregnant women with positive, negative, and equivocal results were determined. SPSS software version 15 (IBM-SPSS Inc, Armonk, NY) was used for analysis.

Result

In total, 300 pregnant woman 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

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(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 was 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). A total of 84 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 subject, with no DNA detectable by Real-time PCR. Similarly, IgM was present in 7 samples tested by ELISA, but no DNA was detected by Real-time PCR (Table 4).

Discussion

In this study, the enzyme linked immunosorbent (ELISA) assay was used for the detection of CMV IgG and IgM and real time PCR for detection of CMV DNA. The real time PCR was used as the gold standard for 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. The difference between the results obtained by immunological method and real time PCR is attributed to the fact that cytomegalovirus 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. The serological tests using the immunoglobulin G reagent were helpful in determining CMV and antecedents of previous infections. However, the specific immunoglobulin M showed very little relationship with viral replication regarding active and recurrent infections, since it was positive in only 10 of the 89 cases of positive real time polymerase chain reaction for CMV. A positive polymerase chain reaction result during pregnancy identifies patients who are undergoing viral replication within the cell [7]. There are limitations to the interpretation of the test results for immunoglobulin M, and these should be kept in mind. Disadvantages include false negative results due to abundant IgG and false positive results due to rheumatoid factor interference and in immunosuppressed patient, e.g. chronic renal failure, end stage renal disease and blood diseases [8]. Furthermore, there is a time lag between primary infection and IgM antibody production (IgM level can remain undetectable because of delayed seroconversion owing to immunosupressive agents). IgM antibodies can also persist for a long time after infection in some healthy individuals [9]. Similarly to our result Parmigiani *et al.* [10] 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 (2011) concluded that PCR was a more sensitive, reliable and accurate method for the detection of CMV infection in pregnant women.

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	IgG 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	IgG 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

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Competing interests

No conflict of interest.

Ethical approval

Ethical approval was not required.

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