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Original Research Article

A comparative study between PCR and immunoglobulin level in the diagnosis and monitoring of CMV infection in women in Babylon province

Milal Mohammad* and Hadi M.A.Almosawi

Medical College /Babylon University-Iraq
**Corresponding author*

Keywords

CMV, VIDAS equipment PCR immuno-globulin level

A B S T R A C T

To evaluate the accuracy of PCR in the diagnosis of active CMV infection and monitoring response to treatment, and compare it with the traditional immunoglobulin test. CMV is the most common cause of congenital infection and complicates approximately 1% of all live births. Primary maternal CMV infection carries a 30% to 40% risk of vertical transmission to the fetus. In cases where maternal CMV infection is suspected, it is important to evaluate the risk to the fetus to provide appropriate counseling and guidance to parents. A prospective study involved 76 women from the period of 4th of November 2011 till 16th of September 2012. They were screened for CMV infection by immunoglobulin IgM and IgG titer measured by VIDAS equipment. When the immunoglobulin was positive, they subsequently tested by PCR (real- time). PCR regarded undetectable when the viral load less than 50 copies/ml. Follow up after treatment by a second PCR viral load measurement done in positive cases. A prospective study involve 76 women; 41 out of 76(54%) were non pregnant in the periconceptional period. 35 out of 76 (46%) were pregnant in first or second trimesters of pregnancy. Their ages range between 16-47 years and parity 0-9, and their gestational age range between 6-16 weeks. IgM was positive in 18 out of 76 (24%), and negative in 58 women (76%). IgG was positive in 68 out of 76 (89%), and negative in 8 women (11%). While PCR was positive in 17 out of 76 (22%), and negative in 59 women (78%).

Introduction

Cytomegalovirus (CMV) is a double-stranded DNA virus and is a member of the Herpesviridie family. CMV has the largest genome of the herpes viruses, which also includes herpes simplex type 1 and 2, Epistein-Barr virus, and Varicella-Zoster.

The prevailing age of infection varies worldwide. In developing countries, most infections are acquired during childhood, whereas in developed countries, up to 50% of young adults are CMV seronegative.

CMV usually causes an asymptomatic infection; afterward, it remains latent throught life and may reactivate. Infection is defined as isolation of CMV, its viral proteins, or its nucleic acid from any tissue sample or body fluid. In immune-competent individuals, symptomatic disease usually manifests as a mononucleosis syndrome.

Individuals at an increased risk for CMV infection includes individuals who attend or work at daycare centers, patient who undergo blood transfusions, persons who have multiple sex partners, and recipients of CMV mismatched organ or bone marrow transplants (1).

CMV is the most common cause of congenital infection (2). Moreover, congenital CMV is the most frequently identified viral cause of mental retardation and is the leading nongenetic cause of neurosensory hearing loss (3, 4).

In developed countries, congenital CMV infection occurs in 0.3% to 2.4% of all live births(5). Infection in the newborn can be acquired through close contact (via contaminated blood, urine, and secretions), vertically through transplacental transmission, and postnatal through breast milk(6). Most symptomatic neonatal CMV infections occur when a woman is newly infected just prior to or during pregnancy (7, 8).

The probability of intrauterine transmission following primary infection is 30% to 40%, but only 1% after secondary infection. About 10% to 15% of congenitally infected infants will have symptoms at birth, and 20% to 30% of them will die, whereas 5% to 15% of the asymptomatic infected neonates will develop squeal later. Children with congenital CMV infection following first trimester infection are more likely to have central nervous system sequel, whereas infection acquired in the third trimester has a high rate of intrauterine transmission but a favorable outcome(9).

Maternal CMV tends to be asymptomatic and patients will rarely be diagnosed by clinical symptoms alone. For most infections, evidence of maternal seroconversion (defined as a seroconversion from a negative to a positive IgM or a 4-fold increase in IgG antibody titer over 4 to 6 weeks period) is sufficient to confirm the diagnosis of a primary infection(6).

Another method of determining the timing of maternal CMV infection is to measure antibody avidity, which refers to the strength of antibody binding to a target antigen. As the immune response to a particular antigen matures overtime, avidity increases. Thus detection of low-avidity anti CMV IgG early in pregnancy suggests a recent acute infection and can be used to identify pregnant women at increased risk of having an infected fetus(10).In contrast, the presence of high-avidity antibodies at 12 to 16 weeks of gestation indicates a past infection (10, 11).

Improvement in CMV igM testing has been reported by performing gel electrophoresis Western blotting of CMV viral polypeptides and may provide the most accurate way to diagnosing a primary maternal CMV infection (12, 13).

On average, detection of CMV-specific igM antibody by ELISA has a sensitivity of only 75% for primary infection; this leaves 25% of women with primary CMV without detectable levels of IgM and a false-negative result (14).

Nucleic acid amplification by polymerase chain reaction PCR is the newest and most promising rapid method to detect virus. This method requires specific primers complementary to DNA sequence on either side of the target CMV DNA segment. A single gene copy may be amplified up to 1 million fold (15). PCR has a sensitivity of 80% to 100% when compared with that of culture; however, this high sensitivity can lead to false-positive results based on the

amplification of extraneous viral contaminants or defective, non infectious viral particle. PCR also can be used quantify levels of virus (16).

Negative results on a DNA test do not rule out CMV infection, the virus may be present in very low numbers or may not be present in the body sample tested (17, 18, 19).

Recently, quantitative PCR has also proven its utility in the monitoring of response to antiviral treatment; decreasing viral loads reflect a response to antiviral treatment. Levels do not drop in response to antiviral treatment might reflect a resistance to the therapy being used (20).

The three antiviral drugs that are currently licensed for the treatment of CMV infection are ganciclovir, phosohonoformate or foscarnet and cidofovir. A promising new agent, valganciclovir, a prodrug of ganciclovir.

Long-term and/or suboptimal antiviral therapy may induce resistant CMV strains(21, 22).

Oral ganciclovir is a specific FDA approved anti-CMV medication administered to mothers with positive CMV who are non-pregnant at a dose of 1000 mg t.d.s for 14 days (23, 24).

Intravenous hyper immunoglobulin therapy seems to be promising, but its efficacy needs further investigation (25).

Recently, a vaccine targeted towards CMV envelops glycoprotein B, an antigen that typically induces a serum antibody response, entered phase 2 clinical trials. The observed efficacy of the vaccine was 50%. Further studies are necessary to demonstrate the safety and efficacy of this vaccine before it can be used for primary prevention of congenital CMV (26).

Materials and Methods

A prospective study done at Babylon Hospital For Maternity and Children and privet clinic from the period of 4th of November 2011 till 16th of September 2012, and involves 76 women sawn during the periconceptional period and in the first two trimesters of pregnancy.

These women presented because of bad obstetric history (in form of repeated abortions or intrauterine death, or neonatal death), or they gave history of previous congenital abnormal babies.

They were tested for CMV infection by immunoglobulin IgM and IgG, and when these antibodies are positive, they subsequently tested for CMV by PCR test.
When PCR was positive, they were given antiviral treatment in form of ganciclovir, and then re-tested by PCR again to monitor the response to treatment.

The blood was collected in EDTA tube, the plasma separated and freeze to -8 C, also serum separated in plain tube and CMV IgM and IgG antibody titer was measured by VIDAS equipment.

The DNA of CMV was extracted from plasma by Max Well full automated method within 42 minutes, and then the real-time PCR (Smart cycle type) used for amplification to detect quantities the viral load by Sacacce kit.

The interpretation of the results was as follows:
CMV IgM was regarded positive if the titer 0.7 IU/ml.

CMV IgG was regarded positive if the titer 8 IU/ml. PCR was regarded undetectable when the viral load <50 copies/ml. The follow-up after treatment was by a second PCR viral load measurement done in positive cases.

Results and Discussion

A total of 76 women participated in this study, 41 of them were non pregnant (54%) and 35 women were pregnant in first or second trimesters of pregnancy (46%).

Pregnant women were mostly in the first trimester, 32 out 35 (91%) and only 3 women were in the second trimester (9%).
Their mean ages was …28.36……., mode of parity was ……..
The mean gestational age according to the last menstrual period was 11 weeks.

These women presented because of bad obstetrical history in form of repeated miscarriages in 31 women out of 76 (41%), or IUD in 7 women (9.2%), or neonatal death in 2 women only (2.6%). History of delivery of congenital abnormal babies was reported in 20 women (26.3%).

The IgM was positive in 18 women out of 76 (24%), and negative in 58 women (76%). The IgG was positive in 68 out of 76 (89%), and negative in 8 women only (11%).

While PCR test was positive in 17 women out of 76 (22%) and negative in 59 women (78%).

The PCR was less than 1000 copies/ml in 2 out of 17 women (11.82%) and between 1ooo-4000 copies/ml in 14 women out of 17 (82.3%) and > 6000 copies/ml in one women only (5.88%). The PCR was regarded undetectable when it was less than 50 copies/ml.

The 17 women with positive PCR were treated by oral ganciclovir and followed up. 16 women out of 17 (94%) showed complete recovery, and only one woman not responding to treatment (6%). Efficacy of this medical intervention response was monitored by real-time PCR before and after treatment.

Congenital CMV infection is an important cause of hearing impairment, mental retardation and cerebral palsy. Principal sources of infection during pregnancy are young children and intimate contacts. Prevention of maternal and congenital CMV infection depends on counseling women regarding the sources of infection and hygienic measures that might prevent infection. There is currently insufficient evidence to support use of antiviral treatment or passive immunization for post exposure prophylaxis of pregnant women or as maternal treatment aimed at preventing fetal infection. Vaccines for CMV are under development but it will be a number of years before one is licensed (27).

The rate of CMV infection in study group did not increase with age, but instead was consistently high in women of less than 30 years of age (11 out of 17 women 64%). This is in agreement with Australian study in which the rate was 66% (13).

Risk factors for CMV infection have been correlated with the socioeconomic status within a community (28, 29).

Most of the women in our study were of middle or low socioeconomic status. This is similar to published data from the United States and Western Europe, in which women of childbearing age, of upper class socioeconomic status, have a lower seroprevalence rate of CMV (30).

Regarding maternal findings in the present study, 41% of cases presented by history of repeated abortions, followed by history of delivery of congenital abnormal babies (26.3%), then repeated intrauterine deaths IUFD (9.2%) and neonatal deaths (2.6%).

While in the study of Meguid N.A 40% of cases presented by delivery of an affected infant, followed by repeated abortions (30%), then repeated neonatal deaths (20%) and repeated IUFDS (10%) (24).

The above results indicate that repeated abortions is the most common antenatal presentation of CMV infection, which is consistent with that stated by Van Lijnschoten et al (31).

The results demonstrated that the serological tests had a low diagnostic performance in identifying CMV infection in pregnant and non pregnant women. The fact that the serological tests showed a reduced diagnostic performance when compared to the PCR test is important because it means that a pregnant woman who is nonreactive to immunoglobulin M for CMV may still be undergoing viral replication through recurrent infections or viral reactivation.

There are limitations to the interpretation of the test results for immunoglobulin M, and these should be kept in mind. The presence of CMV IgM is not solely indicative of primary infection. CMV IgM is detectable when re-infection or reactivation of CMV infection occurs. Other disadvantages include false negative results and false positive results due to low titers from cross-reaction to rheumatoid factor (32). In this study, the PCR was used as the gold standard for infection diagnosis; this was because a positive PCR test signifies viral replication and detects pregnant women at high risk of CMV infection and transmission to the fetus.

The VIDAS test for specific immunoglobulin M, used for detecting viremia, demonstrated lower sensitivity in comparison with the PCR. The sensitivity was 58% which is comparable with the results found in the studies of Stagno and Whitley (33), Griffths et al. (34) and Donner et al. (35). These authors obtained sensitivity levels that ranged 20% to 80% at different gestational ages.

The sensitivity was higher than was found in the study of Silvanna Varella in which the sensitivity of IgM was 4% (36).

The confirmation of CMV infection by PCR was obtained in only 10 of 18 women with positive IgM (55.5%), while 8 women (44.5%) were positive five for CMV-IgM and negative for CMV-DNA. While in a study of Naumnik et al. in 2007, the PCR was positive in 11.5%he PCR was positive in 11.5% and 73.and 73.1% was positive for CMV-IgM and negative for CMV-DNA (37).

While in a study of Meguid N.A., the CMV-IgM was positive in 12 mothers out of 50(24%), and CMV-IgG were highly positive in all mothers (100%) (24).

The IgM showed a moderate relationship with viral replication regarding active and recurrent infections, it was positive in only 10 of the 17 cases of positive PCR for CMV (58%). This is in disagreement with study of Silvana et al. where it was positive in only 2 of the 49 cases of positive PCR (4%) (36).

According to the a study conducted by Stagno et al., 73% of the pregnancies with primary infections and 11% of the pregnancies with secondary infections were diagnosed using IgM on a group of patients with clinical suspicion of CMV infection (38).

The serological tests using the IgG reagent were helpful in determining CMV seroprevalence and antecedents of previous infections. In this study the sensitivity of IgG was 94%, this is in agreement with study of Silvana et al. who report the sensitivity of IgG was 93.8% (36).

When comparing serology with the PCR 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 acute infections using immunoglobulin M. The PCR, on the other hand, is a diagnostic test that detects the presence of the DNA virus within the cell (39). A positive PCR result during pregnancy identifies patients who are undergoing viral replication within the cell but does not clarify the risk for disease development and fetal transmission.

Aitken et al. conducted a study showed that viral loads found in patients with primary infections were higher than those in patients with recurrent infections; since recurrent infections are more common during pregnancy the viral load may be enough to detect the risk of vertical transmission (40).

Therefore, pregnant woman with a risk of CMV fetal transmission could be identified using the PCR test for viral replication and, consequently, adequate follow-up could be established in order to monitor the fetus for infections and sequel.

Real-time PCR is a promising method for detection of CMV and helps in discrimination of viral load from viral replication and differentiation between latent from active infection, with the more advantage of avoiding post PCR handling that can be the source of DNA carryover (41).

Several studies have reported the utility of this technique for quantification of CMV load in blood or urine (42, 43, 44, 45, and 46). The most significant advance of the real-time PCR comes from its rapid thermo cycling and simultaneous detection characteristics (24).

In general, there is good evidence that high CMV load is associated with a higher risk pf progression to CMV disease especially in immunocompromized patients; as well it can be used to track response to therapy. Moreover, a high viral load is correlated with symptomatic congenital CMV infections at birth (47).

Our results indicated that the clinical manifestations of the mothers do not depend on viral quantity.

The viral load was < 100 copies/ml in 4 women out of 17 (23.5%), and it was 6ooo copies/ml in one woman only (6%). In the remaining 12 women (70.5%), the viral load was between 1000-4000 copies/ml. The response to oral ganciclovir in the present study was monitored in 17 women by real-time PCR before and after treatment.

Of the treated group, 16 cases were responding to treatment, and only one case not responds initially, the viral load was very high in this woman (6225 copies/ml). This may be due to the appearance of drug-resistant CMV strains to this antiviral drug (ganciclovir) with specific mutations in the UL 97 and UL 54 genes of CMV (48).

In a study of Meguid 12 mothers were treated by ganciclovir and there was 5 cases were not responding to therapy, 2 of them had very high viral load, and delivered severely affected outcome with CMV infection, thus suggesting the presence of significant correlation between CMV load in mothers and disease severity in their newborns (24).

Table.1 Demographic criteria of study group

No.	age	parity	Gestational age(weeks)	miscarriage	Congenital abn.	IUD	Neonatal death
1	28	1	Not preg.	1	1	o	o
2	37	1	Not preg.	1	o	o	o
3	16	o	9	2	o	o	o
4	25	o	Not preg.	1	o	o	o
5	39	3	Not preg.	2	o	o	o
6	27	o	Not preg.	1	o	o	O
7	18	o	6	1	1	o	O
8	31	o	8	1	o	o	O
9	29	o	Not preg.	3	o	o	O
10	38	1	8	2	o	o	O
11	24	o	6	1	o	o	O
12	24	2	6	1	2	o	O
13	33	5	Not preg.	2	2	o	O
14	30	2	Not preg.	1	1	o	O
15	47	5	6	3	o	0	0
16	21	1	6	1	0	0	0
17	23	1	Not preg.	1	0	0	0
18	43	0	5	2	0	0	0
19	35	2	Not preg.	3	0	0	0
20	43	4	7	3	0	0	0
21	29	9	Not preg.	2	0	0	0
22	30	3	Not preg.	2	0	0	0
23	32	7	Not preg.	2	2	0	0
24	35	3	8	3	0	0	0
25	23	2	Not preg	1	1	0	0
26	25	1	Not preg.	2	1	0	0
27	24	0	Not preg.	2	0	0	0
28	28	0	Not preg.	3	0	0	0
29	19	0	Not preg.	1	1	0	0
30	34	3	Not preg.	0	2	0	0
31	22	1	7	0	0	1	0
32	18	0	Not preg.	1	1	0	0
33	29	3	Not preg.	3	1	0	0
34	35	4	8	3	0	0	0
35	40	1	12	4	0	0	0
36	27	1	Not preg.	1	0	0	0
37	24	2	Not preg.	4	0	0	0
38	26	4	Not preg.	0	2	0	0
39	42	2	Not preg.	2	0	0	0
40	17	0	6	1	0	0	0
41	32	1	Not preg.	1	1	0	0
42	24	1	Not preg.	0	1	0	0

43	38	3	6	0	1	0	0
44	27	2	6	0	1	0	0
45	20	1	Not preg.	1	0	0	1
46	18	0	6	3	0	0	0
47	35	0	6	2	0	0	0
48	40	3	Not preg.	3	1	0	0
49	27	2	Not preg.	1	0	0	0
50	22	1	Not preg.	3	1	0	0
51	30	5	Not preg.	0	0	4	0
52	20	0	Not preg.	3	0	0	0
53	26	2	14	0	2	0	0
54	36	4	16	2	0	0	0
55	35	3	15	2	0	0	0
56	20	1	Not preg.	1	0	0	1
57	18	0	Not preg.	1	0	0	0
58	20	3	Not preg.	1	0	2	0
59	26	1	6	1	0	0	0
60	18	0	Not preg.	1	0	0	0
61	19	1	Not preg.	0	0	1	0
62	36	3	12	1	0	0	0
63	20	0	8	2	0	0	0
64	35	1	10	1	0	1	0
65	22	0	Not preg.	1	0	0	0
66	25	2	6	1	0	0	0
67	28	0	Not preg.	4	0	0	0
68	37	1	Not preg.	1	0	0	0
69	35	3	7	1	0	0	0
70	37	5	8	0	0	0	0
71	21	2	6	1	0	0	0
72	30	2	12	0	0	0	0
73	27	2	Not preg.	1	0	0	0
74	21	1	Not preg.	0	0	1	0
75	34	2	5	1	0	0	0
76	26	3	6	6	0	2	0

Variable or parameter	**Range**	**Mean ± SD**
Age (years)	16-47	28.355 ± 7.445
Parity (n.)	0-9	Mode zero median:1
Miscarriages (n.)	0-6	Mode: 1 median:1
Gestational age (weeks)	6-16	3.46± **SD** 4.419
History of congenital abnormality	0-2	0: 73.7%(56), 1:18.4%(14) 2: 7.9%(6)
History of I.U.D	0-2	0:69(90.8%), 1:4(5.2%), 2:2(2.6%), 4:1(1.3%)
History of neonatal death	1	

Table.2 show serological data and results of PCR of study group.

Type of test	Result	Number	Percentage %	P- value
IgM	+	18	24	<0.0001
IgM	-	58	76	
IgG	+	68	89	<0.0001
IgG	-	8	11	
PCR	+	17	22	<0.0001
PCR	-	59	78	

Table.3 PCR test results for maternal blood according to IgM test results

IgM	Positive PCR	Negative PCR
Positive	10	8
Negative	7	51
Total	17	59

Odratio: 9.1 times positive PCR than IgM

Sensitivity= 58% Specificity =86%

Positive predictive value = 55% Negative predictive value = 87%

Table.4 PCR results for maternal blood according to IgG results

IgG	Positive PCR	Negative PCR
Positive	16	57
Negative	1	2
Total	17	59

Sensitivity = 94% Specificity = 33%

Positive predictive value = 22% Negative predictive value = 66%

Congenital CMV infection is an important cause of hearing impairment, mental retardation and cerebral palsy. Principal sources of infection during pregnancy are young children and intimate contacts. Prevention of maternal and congenital CMV infection depends on counseling women regarding the sources of infection and hygienic measures that might prevent infection.

There is currently insufficient evidence to support use of antiviral treatment or passive immunization for post exposure prophylaxis of pregnant women or as maternal treatment aimed at preventing fetal infection. Vaccines for CMV are under development but it will be a number of years before one is licensed (27). The rate of CMV infection in study group did not increase with age, but instead was consistently high in women of less than 30 years of age (11 out of 17 women 64%). This is in agreement with Australian study in which the rate was 66% (13).

Risk factors for CMV infection have been correlated with the socioeconomic status within a community (28, 29). Most of the women in our study were of middle or low socioeconomic status. This is similar to published data from the United States and Western Europe, in which women of childbearing age, of upper class socioeconomic status, have a lower seroprevalence rate of CMV (30).

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The results demonstrated that the serological tests had a low diagnostic performance in identifying CMV infection in pregnant and non pregnant women. The fact that the serological tests showed a reduced diagnostic performance when compared to the PCR test is important because it means that a pregnant woman who is nonreactive to immunoglobulin M for CMV may still be undergoing viral replication through recurrent infections or viral reactivation.

There are limitations to the interpretation of the test results for immunoglobulin M, and these should be kept in mind. The presence of CMV IgM is not solely indicative of primary infection. CMV IgM is detectable when re-infection or reactivation of CMV infection occurs. Other disadvantages include false negative results and false positive results due to low titers from cross-reaction to rheumatoid factor (32).

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A positive PCR result during pregnancy identifies patients who are undergoing viral replication within the cell but does not clarify the risk for disease development and fetal transmission.

Aitken et al. conducted a study showed that viral loads found in patients with primary infections were higher than those in patients with recurrent infections; since recurrent infections are more common during pregnancy the viral load may be enough to detect the risk of vertical transmission (40).

Therefore, pregnant woman with a risk of CMV fetal transmission could be identified using the PCR test for viral replication and, consequently, adequate follow-up could be established in order to monitor the fetus for infections and sequel. Real-time PCR is a promising method for detection of CMV and helps in discrimination of viral load from viral replication and differentiation between latent from active infection, with the more advantage of avoiding post PCR handling that can be the source of DNA carryover (41). Several studies have reported the utility of this technique for quantification of CMV load in blood or urine (42, 43, 44, 45, and 46).

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