Screening for Cytomegalovirus Infection During Pregnancy in a Teaching Hospital, Western province, Sri Lanka

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Abstract

Primary Cytomegalovirus (CMV) infection during pregnancy is a serious threat to the fetus. Although vertical transmission is likely to occur as a result of maternal infection, the rate of permanent sequelae is higher among infants born to mothers with primary CMV infection. Diagnosis of CMV infection among healthy adults generally relies on serological testing as most of them are asymptomatic. CMV infection in pregnancy is barely studied in Sri Lanka. This study aims in screening for CMV infection of selected pregnant population in a major maternity hospital and to describe associated factors with seropositivity. This study included 385 pregnant women in second trimester presented during a period of 6 months. Mothers with immunocompromised states were not recruited. Questionnaire was used to gather sociodemographic and clinical factors. All serum samples were tested for CMV-IgG and IgG-avidity assay for seropositives. CMV-IgM test was carried out on samples with negative CMV-IgG and low/equivocal-avidity levels. All 3 sero-markers were tested using commercial Enzyme Linked Immunosorbent Assays. Data were analyzed by SPSS. Majority (97%) of the population were positive for CMV-IgG. CMV-avidity was low, equivocal or high in 0.25%, 2.75% and 97% of IgG seropositives respectively. Age, bad obstetric history and multiparity didn't show association with IgG seropositivity. In conclusion, CMV seroprevalence was high in this study population with low rates of recent primary infection. Education regarding preventive measures for CMV infection in antenatal care has a value as some proportion were seronegative for CMV with prone to get new infections.

Key Words: CMV, pregnancy, primary infection, IgG- avidity

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INTRODUCTION

Cytomegalovirus (CMV) is a leading cause for the congenital infections causing severe malformations in the fetus and the newborn with an affected rate of 0.2%-2.5% worldwide[1]. It causes intracerebral calcification, ventriculomegaly, microcephaly, sensory neural hearing loss (SNH), retinitis, hepatosplenomegaly and intrauterine growth retardation[2].

CMV is a ubiquitous virus with varying seroprevalence worldwide according to the country and population. In developed countries with good socioeconomics, CMV seroprevalence in pregnant population ranges 40% - 60% while it is 90%-100% in developing countries[3]. Young children excrete the virus for a longer period and mothers with children or workers of day care centers are at risk for CMV infection[4].

CMV infection in pregnancy can be categorized in to two types, primary or recurrent infection due to reactivation or re-infection from a different strain[5]. Primary infection during first half of the pregnancy transmits the infection to the fetus in about 30%- 50% but only 10-15% of infected babies show signs of congenital CMV at birth with 10% of perinatal mortality. From the 90% of newborns who are asymptomatic at birth develop symptoms like SNH in later life. In contrast, recurrent CMV infection carries the rate of newborn infection of about 1% with the 1%-10% of babies present with symptoms at birth[6, 7]. Children with congenital CMV infection following first trimester maternal infection are more likely to have central nervous system sequelae, whereas infection acquired in the third trimester has a high rate of intrauterine transmission but a favorable outcome[8].

Therefore identification and differentiation of primary CMV infection from recurrent infection in early pregnancy is an important goal in newborn health. As most of maternal infections are asymptomatic, laboratory screening tests for CMV infection play a major role[3]. If mother is diagnosed to have primary infection in early pregnancy serial ultrasound scans (USS) and fetal tests should be done to rule out the congenital infections and malformations. And also there are preventive measures including CMV hyperimmunoglobulin (HIG) and antivirals to reduce congenital malformations which are being evaluating in clinical trials at present. If the mother is found to be seronegative during the screening, mother should be strictly advised regarding hygienic practices like frequent hand washing and avoiding close contact with young children to minimize the acquisition of new infection during pregnancy[9, 10].

Serological diagnosis of recent viral infection mainly depend on detection of virus specific IgM, but it has several limitations with false positives and unable to differentiate primary from recurrent infection[5, 11]. In early phase of CMV infection, avidity of IgG is low and it becomes high during maturation procedure with time. Therefore CMV IgG-avidity can be used to differentiate primary from non primary infection especially in first half of pregnancy[12, 13].

Some European countries offer routine screening to pregnant mothers for CMV infection with well established testing algorithms[14]. In Sri Lanka, still there are no studies and data available on seroprevalence of CMV in pregnancy and burden of congenital CMV infection. The basic data concerning CMV infections during pregnancy is important for health planners and care providers and also the results of this study would add data to the national CMV data bank.

Objective of this study is to screen a selected pregnant population for CMV infection and to describe associated factors with the seropositivity in a major maternity teaching hospital, Sri Lanka.

MATERIALS AND METHODS

This was a descriptive cross sectional study done for a one year period starting from August 2015. This was included randomly selected 385 pregnant women. Sample size was calculated using WinPepi software to get the 95% confidence interval by adding 50% of prevalence data since the local seroprevalence data is not available[15].

Pregnant patients who were in second trimester coming for antenatal care to the clinics in De Soyza Maternity Hospital, Colombo were included in the study. Pregnant mothers with underlying immunocompromised states such as HIV positive mothers, mothers on immunosuppressive agents were excluded from the study.

Venous blood (03 mL) was drawn from each eligible mother into a plain container using a 05mL disposable syringe under aseptic venipuncture technique. Samples were kept for 1 hour at room temperature to separate the serum and transported to the Virology Reference Laboratory-Medical Research Institute within 6 hours.
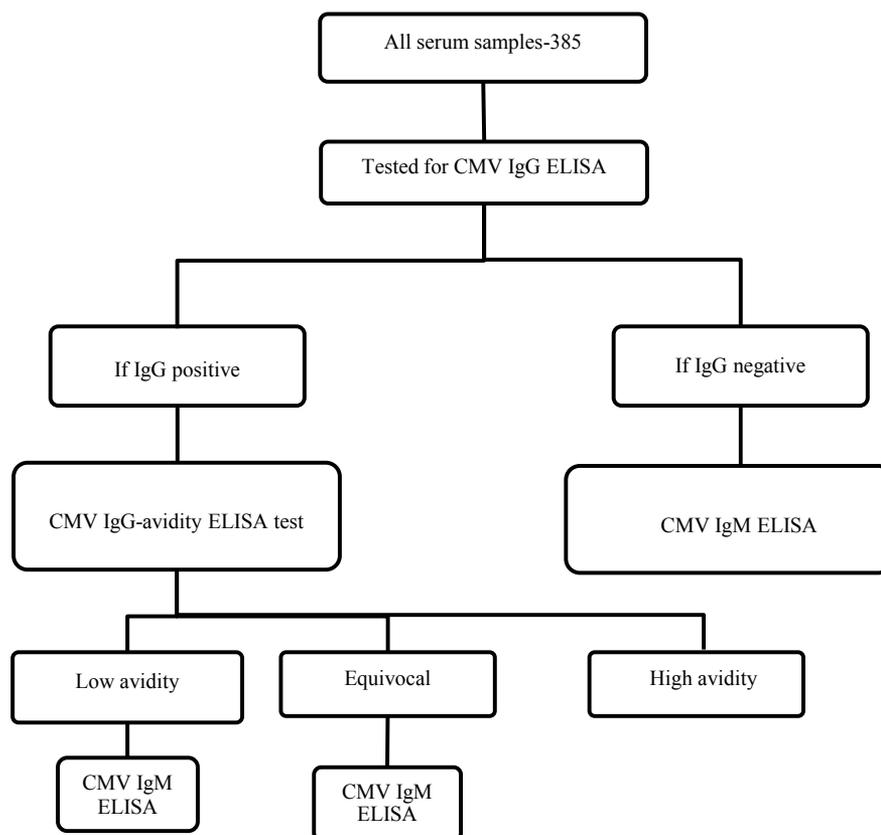
All samples were tested for CMV IgG and positive IgG samples were further analyzed using CMV IgG-avidity assay. CMV IgM test was carried out on the samples negative for CMV IgG and also on samples with low avidity or equivocal results in IgG-avidity according to Illustration 1. All 3 serological markers (IgG, IgG-avidity, and IgM) were tested using commercially validated Enzyme Linked Immunosorbent Assays (ELISA). Euroimmun anti-CMV IgM, IgG and IgG avidity kits were used for testing according to the manufacturer's instructions. Both IgM and IgG kits had a specificity and sensitivity of 99%-100%[32].

The CMV IgG-avidity test was performed with urea as denaturing agent using commercial diagnostic kit (Euroimmun – ELISA for avidity of CMV-IgG). The IgG Relative avidity index (RAI) was calculated and expressed as percentage by dividing the extinction of the sample with urea treatment by the extinction of the sample without urea treatment. The interpretation of RAI results has been determined as follows: RAI < 40% indicated low avidity antibodies; RAI 40-60% indicated equivocal range and RAI > 60% indicated high avidity antibodies[32].

Interviewer based questionnaire was used to gather basic sociodemographic data and other information on parity and past medical history by a relevant medical officer before sample collection.

Ethical approval was taken from Ethical review committee, Medical Research Institute. Each study participant was provided with the information sheet describing the purpose of this study and informed written consent was obtained prior to enroll them.

Statistical analysis was done using descriptive statistics by Statistical Package for Social Sciences (SPPSS) version 21.

Illustration 1-Testing algorithm for CMV serology

RESULTS

SOCIO-DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Total number of 385 pregnant females in their second trimester was enrolled in the study. They were in age range of 16 years – 46 years with the mean age of 27 years and standard deviation (SD) of 6.18.

Out of total number of pregnant females, 60/365 (16.4%) had a previous bad obstetric history in past with miscarriages, intrauterine death or intrauterine growth retardation.

According to the parity, 180/385 (47%) participants were in their first pregnancy- P_1C_0 and 21/385(5%) of total were in second or third pregnancy without any previous live births/children-P_2C_0/P_3C_0. And rest of the participants- 184/385 (48%) was in second or subsequent pregnancies with previous live births/children.

CMV IgG-AVIDITY ASSAY

All IgG positive samples were tested for CMV IgG-avidity. From IgG positive samples, only 1/374 (0.25%) showed low avidity, 10/374 (2.75%) showed equivocal results while majority of 363/374 (97%) showed high avidity IgG.

CMV IgM RESULTS

CMV IgG negative samples (eleven) and low (one) and equivocal (ten) IgG avidity samples were tested for CMV IgM. All (22/22) were negative for CMV IgM.

DISCUSSION

This cross sectional study was carried out in a major maternity hospital in Western province, Sri Lanka among pregnant patients of second trimester.

As discussed by Walker et al. 2013, screening for CMV could potentially reduce the burden of congenital CMV in one of three ways. Primary prevention through advising seronegative mothers

detection is not specific for diagnosing primary infection and not provides information on whether CMV infection occurred before or after the conception.

Assays based on IgG avidity have the capacity to fill the gaps and provide information on timing of infection. Detection of CMV specific IgG antibody in association with low IgG avidity demonstrates primary CMV infection[29].

There are various testing algorithms in literature including first to check IgM and if it is positive only, to proceed with CMV IgG-avidity assay. But there are evidence that CMV IgG positive but IgM negative mothers with low avidity IgG indicating high risk for congenital infection[17,18]. This might be due to the narrow time window with reversion of CMV IgM but still low IgG-avidity. Testing algorithms that direct avidity testing only in IgM positive samples may miss some high risk mothers. For this reason, this study used the algorithm that all sera first tested for CMV IgG and all IgG positive samples to be tested for CMV IgG-avidity with subsequent IgM.

In this study, majority of patients (97%) were positive for CMV IgG which indicated serological evidence of past CMV infection. Since published data on CMV seroprevalence for pregnant population in Sri Lanka is not available, data of this study is compared with regional and global data.

Seropositivity rates lie between 50%-60% in developed countries, and the rates are between 90%- 100% in developing countries[19,20]. CMV seropositivity rates are higher in women of reproductive age especially in low socioeconomic status. In different parts of India, serological surveys have shown 80-90% prevalence of CMV IgG antibodies in women of childbearing age[19,20]. However, in some of European countries, low CMV infection rates have been reported, Australia (56.9%) and France (46.8%)[21].

Among IgG seropositive group, 18% had bad obstetric history and 53% were in second or subsequent pregnancies. Some studies in literature showed significant association of number of parity with IgG seropositivity[22]. Age didn't show significant association with IgG positivity. In literature there are different concerns on maternal age and CMV infection. Most studies were showed elderly females

Table 1   CMV serology results

CMV IgG		CMV IgG-avidity			CMV IgM	
positive	negative	high	low	equivocal	positive	negative
374	11	363	1	10	0	22
(97%)	(3%)	(97%)	(0.25%)	(2.75%)		(100%)
Total 385		**Total 374**			**Total 22**	

CMV IgG RESULTS

Majority of the study population, 374/385 (97%) were positive for CMV IgG. Mean age of IgG positive group and negative group were similar and it was 27 years. There was bad obstetric history in 59/374 (18%) of IgG positive group while only 1/11 (9%) gave a bad obstetric history in IgG negative group. 199/374 (53%) of IgG positive group and 6/11 (54%) of IgG negative group were in second or subsequent pregnancies with children at home. Chi square test was done to analyze the association of age, bad obstetric history and parity with the CMV IgG positivity. There were no significant association found between these variables and IgG positivity.

regarding precautions of hygienic measures is important in minimizing seroconversion. Among women who seroconverted during pregnancy, CMV HIG show significant reduction of perinatal transmission (secondary prevention), and when given to pregnant mother of already infected fetus also have some effect on reducing the risk of malformations (tertiary prevention)[9,10].

Diagnosis of CMV infection usually relies on serological testing. But confirmation of CMV seroconversion during pregnancy carries several practical challenges. Usually the diagnosis of recent viral infection can be identified by detecting the seroconversion of IgM. However, CMV IgM may found in both primary and recurrent infections and in some patients, IgM may persist for many months after the primary infection[16]. Therefore CMV IgM

are at a high risk, while others reporting contrary[23,24, 25].

Low affinity CMV IgG are produced in the first 18-20 weeks after infection[26,27]. A subsequent maturation process generates IgG antibodies with higher avidities. Therefore a high IgG-avidity excludes a recent primary infection with low risk of congenital infection. Conversely, IgG –low avidity antibodies together with reactive CMV IgM strongly support the diagnosis of maternal primary infection in the preceding 3 or 4 months[26, 27].

India has higher seroconversion rate (2%-2.5%) among pregnant population, whereas only one sample (0.25%) showed low affinity antibodies indicating primary infection[19,20]. Equivocal results were found in 2.75% of IgG positive samples. As equivocal results do not exclude recent infection, all those were tested for CMV IgM[28] and all were negative for IgM suggesting no recent infection with CMV.

Majority of total IgG positive samples (97%) showed high avidity results suggesting past CMV infection and less risk for the fetus[29].

IgG negative samples (3% of total study population) were tested for CMV IgM and all were negative. Both CMV IgG and IgM negativity indicate no serological evidence of CMV infection. And this 3% of seronegative study population is prone to get a new primary infection in present pregnancy or in subsequent pregnancies. The rate of susceptibility at the beginning of pregnancy varies by the country and also within the country with highest rates occurring among developing countries. Among women of childbearing age, 40% - 80% are susceptible (seronegative) to CMV at the beginning of pregnancy according to available global data[30].

CMV is transmitted from person to person via close non-sexual contact, sexual activity, breastfeeding, blood transfusions, or organ transplantation [31]. For pregnant women, important sources of infection include sexual activity and contact with the urine or saliva of young children, especially their own children [4].Therefore these mothers should be counseled regarding the risk and preventive measures including frequent hand washing and avoidance of direct contact particularly with body fluids from preschool children [2]. This is more important as 54% of seronegative mothers of this study were having their own children at home.

CONCLUSION

This will be the first published study on CMV infection among pregnant women in Sri Lanka and showed maternal CMV IgG seroprevalence at a high level which revealed as 97%. Since there is a naïve cohort for CMV infection among pregnant population, education programmes regarding preventive methods to minimize new CMV infection are important in antenatal care.

However, further studies with large sample volume are needed to analyze with cost effectiveness and feasibility of CMV screening during pregnancy to minimize congenital CMV disease.

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