ORIGINAL ARTICLE

Seroprevalence of Human Cytomegalovirus (HCMV) infection in pregnant women and outcomes of pregnancies with active infection

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Abstract

Objective: To determine the prevalence of cytomegalovirus in pregnant women and types of overt congenital infection in neonates.

Methods: This cross-sectional study was conducted at the Pakistan Institute of Medical Sciences and Federal Government Services Hospital in Islamabad, Pakistan, from March 2010 to June 2011, and comprised blood samples of pregnant women. Seroprevalence of human cytomegalovirus, immunoglobulin G and immunoglobulin M was determined by enzyme-linked immunosorbent assay while its deoxyribonucleic acid was detected by nested polymerase chain reaction.The congenital human cytomegalovirus infection was also identified in newborn babies from actively infected pregnant women. SPSS 18 was used for data analysis.

Results: Of the 409 pregnant women enrolled, 399(97.55%) were seropositive for cytomegalovirus immunoglobulinG and 52(12.71%) for immunoglobulinM, while cytomegalovirus deoxyribonucleic acid was detected in 82(20%). Of the cytomegalovirus immunoglobulinM-positive women, sera of 40(80%) had immunoglobulinG avidity >50%. The remaining 12(23%) sera had avidity assay value <50%.

Among the 82(20%) infected pregnant women, 70(85.4%) were successfully followed up. Among them, the virus was isolated from 41(58.5%) newborns babies, of which 15(21%) were symptomatic while 26(47.2%) were asymptomatic. Of the former, 4(26.6%) had hepatosplenomegaly.

Conclusion: Human cytomegalovirus infection in pregnant women was the main reason of congenital defects among neonates.

Keywords: Human cytomegalovirus, Congenital HCMV infection, Seroprevalence, Hepatosplenomegaly. (JPMA 66: 1009; 2016)

Introduction

Human cytomegalovirus (HCMV), also known as human herpesvirus-5 (HHV-5), belongs to the beta herpesviridae family. HCMV has emerged as a major cause of congenital infection in humans, with prevalence rate of 0.15 to 3.0% depending on the population, and can cause birth defects and childhood disabilities.[1] HCMV infection during pregnancy can induce abnormal foetal development, leading to abortion, premature delivery, foetal development retardation, birth defects, long-term effects after birth and other serious outcomes. The vertical transmission of HCMV accredited either recurrent maternal viral infection or through primary infection.[2]

HCMV seroprevalence is usually high in both developing as well as developed countries among those of lower socioeconomic status.[3] For instance, Pakistan is considered among the countries where pregnancy-related deaths ratio is high (260 deaths per 100,000 live births).[4] The worldwide ratio of neonates mortality is approximately 40% and nearly 36% of these deaths are due to infections.[5] Pakistan accounts for 7% of global neonatal deaths with an estimated 41 and 70 deaths per 1,000 annually for neonates and infants, respectively.[4]

HCMV seroprevalence status among pregnant and childbearing age women is the main focus of various worldwide studies due to the severe consequences for offspring. Different studies from developed countries demonstrate that severe HCMV congenital infection is due to primary maternal infection or due to reactivation of latent infection or re-infection of different virus strain.[6] In developing countries having the high seroprevalence rate, mostly congenitally infected neonates are born to women with recurrent HCMV infection during pregnancy and majority of the infants are asymptomatic.[7]

A low HCMV seroprevalence has been reported from Australia, Germany[8] and UK[9] compared to countries

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like Brazil, Taiwan, Turkey, Qatar and Saudi Arabia, with around 90% prevalence rate.[10] According to a regional study from Khyber Pakhtunkhwa (KP) province of Pakistan, seroprevalence was reported at 94.5%.[11]

The foetus and infant can either be infected via viral transmission through the placenta, during delivery via cervical secretions and blood or from the mother via breast milk.[12] The prevalence of congenital cytomegalovirus (CMV) infection in developed countries varies from 0.2% to 2%, with higher overall rates in countries with higher maternal seroprevalence.[13] In developing countries, the prevalence of this infection varies considerably, both within and among the countries, ranging from 6-14%.[14] Infection in symptomatic infants ranges from minor to severe problems with life-threatening disease and may lead to 20% perinatal death.[15] In symptomatic newborn babies, up to 80% sequelae may occur like mental obstacle, visual imperfections or sensorineural hearing loss. Moreover, 8-15% of babies who are asymptomatic at birth will later develop complications, mainly neurodevelopmental defects and deafness.[16]

Congenital CMV infection may not only lead directly to increased morbidity at birth and during the early years, but may also impact indirectly on the health of infected children through immunosuppression. However, population-based studies on the seroprevalence of CMV among pregnant women and its association with neonatal outcomes are scarce. The current study was planned to determine the CMV status with reference to seroprevalence and active infection in pregnant women and its relation to neonatal outcomes.

Materials and Methods

This cross-sectional study was conducted at the Pakistan Institute of Medical Sciences and Federal Government Services Hospitalin Islamabad, Pakistan, from March 2010 to June 2011, and comprised blood samples of pregnant women. Ethical approval was obtained from the two institutional review committees and the National Institute of Health (NIH). Informed consent was obtained from all the participants.

Recent maternal HCMV infection was defined as the detection of the HCMV Immunoglobulin M (IgM) antibodies while viral deoxyribonucleic acid (DNA) presents active infection. Congenital CMV infection was defined as detection of the virus in saliva/urine during the first three weeks of life by polymerase chain reaction (PCR). In clinical setting, symptomatic congenital CMV was considered into account when virus isolated within three weeks of life was accompanied by pneumonia, jaundice, hepatitis, microcephaly, petechiae, hearing impairment, thrombocytopenia, hyperbilirubinaemia, hepatosplenomegaly, anaemia, thrombocytopenia, and/or abnormal liver function or abnormal cranial computerised tomography (CT) findings. Pregnant women aged 16 years or above and feeling unwell, and newborns of selected pregnancies were included.

Socio-demographic, obstetrical and clinical data was collected using standard questionnaire. The subjects were inquired for demographic details, including age, residence, occupation and education level. Clinical history included obstetric history (jaundice, gestational period, gravidity and history of miscarriage), presence of underlying disease, prolonged fever of unknown origin, flu, sore throat, hepatitis, lymphadenopathy and extreme fatigue.

Neonates, born from mothers having active CMV infection, were screened for congenital CMV infection during first 3 weeks after birth by detection of viral DNA in urine and saliva swab through PCR. Infants were considered symptomatic if they showed any clinical sign and symptoms suggestive of congenital CMV infection that included intrauterine growth retardation, hepatosplenomegaly, hydrocephaly, microcephaly, skin petechiae/purpura, thrombocytopenia (platelet count<100000/mm^3), jaundice with direct bilirubin (>3mg/dL), alanine aminotransferase (ALT) elevation (>80IU/L), pneumonia and poor sucking. Demographic and clinical data was collected from the medical reports of newborns and from parents or legal guardians of the infants after their permission.

Moreover, 5cc of blood was collected from each pregnant woman in vacutainer tubes, allowed to clot and centrifuged at room temperature. Sera and plasma were stored at -20°C till further processing. Furthermore, neonatal outcome was closely followed up by qualified physicians and through personal communication with mothers carrying active CMV infection. Saliva samples were collected by swabbing the inside of the buccal cavity of newborns with a cotton-tipped applicator and transported in viral transport mediums. Urine specimens were collected without preservatives and transported on melting ice to the laboratory where they were processed immediately.

Enzyme-linked immunosorbent assay (ELISA) was used for detection of CMV immunoglobulin G (IgG) and IgM antibodies by using commercial diagnostic kits (Human Gesellschaftfur Biochemica und

Diagnosticamb H, Germany) according to manufacturer's protocol. The samples were considered positive if their absorbance value was more than 15% of the cut-off value for IgG and IgM and vice versa. The calibrators and controls agreed the validation check recommended by the manufacturer of the kits. Tests were performed at the Department of Virology and Immunology of the NIH.

Women having recent infection were further analysed for anti-HCMV IgG avidity index by using a commercial micro-particle enzyme immunoassay (ARCHITECT CMV IgG Avidity assay) and those with low IgG avidity (<50%) indicated the primary CMV infection as per manufacturer's protocol.

DNA was extracted from plasma, saliva and urine by using Nucleospin® blood kit (MACHEREY-NAGEL GmbH & Co. KG Germany) according to the manufacturer's protocol, eluted in 100µl buffer and stored at -20°C until further testing. Nested PCR was carried out in a 50µl volume of 10X PCR buffer, 2.5mM magnesium chloride (MgCl2), 10µM of each deoxynucleotide triphosphates (dNTPs), 5 units of Taq DNA polymerase and 10pmol of 2 primers set specific to the major immediate-early (MIE), a highly conserved region of the HCMV genome described elsewhere.[17] The cycling conditions for both rounds consisted of denaturation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 30 seconds, 58°C for 40 sec, 72°C for 50 sec, followed by terminal extension at 72°C for 3 min. The amplified product was run on 2% agarose gel and visualised under ultraviolet (UV) transilluminator.

SPSS 18 was used for data analysis. Means, standard deviation (SD) and frequencies (percentages) of the socio-demographical and clinical characteristics were calculated for HCMV infections. The independent sample t-test and Pearson's chi-square were used for comparison of quantitative variables and chi-square test for qualitative variables. Univariate and multivariate analysis were used to find out the association between the characteristics of subjects and HCMV seropositivity or active infection. Using the Enter method, odds ratios (OR) as well as 95% confidence intervals (CI) were calculated by the multivariate analysis. P<0.05 was considered significant.

Results

Of the 409 pregnant women enrolled, 358(87.5%) were aged between 16 and 30 years while 51(12.5%) between 31 and 40 years. The overall mean age was 26.8±3.8 years (range: 16-40 years). Besides, 275(67.2%) participants belonged to rural areas; 396(96.82%) were housewives; 13(3.18%) were doing outdoor jobs; 118(28.8%) had history of abortion; 34(8.31%) of jaundice; 204(49.87%) were in their 3rd trimester; 160(39.1%) in 2nd; 45(11%) in the 1st trimester; 148(36%) were carrying their first pregnancy (primiparous); and 261(64%) were multiparous (Table-1). Increasing age showed significant association (odds ratio [OR]=1.76; 95% confidence interval [CI]=1.02-2.75;p=0.04) with CMV infection when analysed by multivariate analysis (Table-2).

Further analysis showed that 399(97.55%) subjects were seropositive for CMV IgG and 52(12.71%) for IgM. HCMV DNA was found in 82(20%) pregnancies that indicated an active infection. All the women with IgM and DNA positive for CMV had IgG positive too, indicating that the majority of the subjects had recurrent infection. Among the 52(12.7%) pregnant women having history of febrile illness, 32(61.53%) had active infection (p=0.04). Other clinical and obstetric characteristics, including history of hepatitis, lymphadenopathy, sore throat, flu, trimester cycle of pregnancy, and history of miscarriage had no association with CMV infection. There were 10(2.4%) seronegative pregnant women, who were unprotected and fell within the range at which infection was likely to occur.

Of the HCMV IgM-positive women, sera of 40(80%) had IgG avidity >50%, which indicated that most of the pregnant women underwent recurrent HCMV infection. The remaining 12(23%) sera had avidity assay value <50%, considering HCMV as primary infection.

There were 15(3.67%) infants with symptomatic and 26(6.36%) with asymptomatic congenital CMV infection. All of the symptomatic infants belonged to the group where public health clinics were the source of perinatal care compared to 24(92.3%) asymptomatic children. No significant differences were found between asymptomatic and symptomatic infants born with respect to maternal age, number of previous pregnancies and other clinical and demographic parameters (p>0.05).

Of the 82(20%) pregnancies with active HCMV infection, 5(6.1%) resulted into abortion or intrauterine foetal deaths, while 7(8.54%) cases could not be followed up due to non-cooperative behaviour of their family. Of the remainder 70(85.4%) pregnancies that were successfully followed up until deliveries and investigated for any congenital HCMV infection, 15(21%) infants were found with symptomatic infection while 26(47.2%) cases were asymptomatic.

Table-1: Socio-demographic and obstetric features of pregnant women and prevalence of HCMV infection.

Variables	No.Examined	HCMV Immunoglobulin				HCMV DNA	
		IgG Positive (%)	p value	IgM Positive (%)	pvalue	PCR Positive (%)	pvalue
Age (in years)							
16-30	358	348(97.2)	0.45	18(5.0)	0.05	45(12.5)	0.04
31-40	51	51(100)		34(66.6)		37(72.5)	
TOTAL	409	399(97.5)		52(12.71)		82(20.0)	
Educational Level							
Illiterate	74	73(98.6)	0.77	22(29.7)	0.81	24(32.4)	0.62
Primary	99	97(97.9)		12(12.1)		16(16.1)	
Matric	160	154(96.2)		8(5.0)		29(18.1)	
F.A/F.Sc	39	38(97.4)		6(15.3)		7(17.9)	
Graduate	20	20(100)		4(20.0)		5(25.0)	
Masters	17	17(100)		0		1(17.6)	
TOTAL	409	399(97.5)		52(12.7)		82(20.0)	
Residence area							
Rural	275	269(97.8)	0.82	44(16.0)	0.52	52(18.9)	0.52
Urban	134	130(97)		8(5.9)		30(22.3)	
TOTAL	409	399(97.5)		52(12.7)		82(20.0)	
Occupation							
Unemployed	396	386(97.4)	0.5	51(12.8)	0.36	82(20.7)	0.14
Employed	13	13(100)		1(7.6)		0	
TOTAL	409	399(97.5)		52(12.7)		82(20.0)	
Parity							
Primiparous	148	145(97.9)	0.41	8(5.4)	0.05	28(18.9)	0.46
Multiparous	261	254(97.3)		44(16.8)		54(20.6)	
TOTAL	409	399(97.5)		52(12.7)		82(20.0)	
History of jaundice							
Yes	34	33(97)	0.3	14(41.1)	0.21	22(64.7)	0.26
No	375	366(97.6)		38(10.1)		60(16.0)	
TOTAL	409	399(97.5)		52(12.7)		82(20.0)	
History of miscarriage							
None	291	283(97.2)	0.52	19(6.5)	0.13	26(8.9)	0.41
1-4	118	116(98.3)		33(27.9)		56(47.4)	
TOTAL	409	399(97.5)		52(12.7)		82(20.0)	
Gestation Trimester							
1st	45	43(95.5)	0.62	6(13.3)	0.15	9(20.0)	0.43
2nd	160	156(97.5)		22(13.7)		31(19.3)	
3rd	204	200(98)		24(11.7)		42(20.5)	
TOTAL	409	399(97.5)		52(12.7)		82(20.0)	

HCMV: Human cytomegalovirus
DNA: Deoxyribonucleic acid
IgG: Immunoglobulin G
IgM: Immunoglobulin M
FA: Faculty of Arts. FSc: Faculty of Science.

Table-2: Multivariate analysis of the selected features of women and association with active HCMV infection.

Feature	Odds ratio	95% confidence interval	P value
Age (31-40 years)	1.76	1.02-2.75	0.04
Febrile illness fever	1.45	0.89-2.3	0.03
More than 2 children	2.04	0.97-2.8	0.15
Rural residence	1.56	0.82-2.6	0.13
History of jaundice	0.8	0.78-1.49	0.09

HCMV: Human cytomegalovirus.

Table-3: Clinical abnormalities found in newborns with symptomatic congenital CMV Infection.

Findings/Abnormality	Positive/Total Examined (%)
Bronchopneumonia	1/15 (6.6)
Congenital Cataracts	1/15 (6.6)
Developmental Delay	1/15 (6.6)
Hepatosplenomegaly	4/15 (26.6)
Hydrocephaly	1/15 (6.6)
Thrombocytopenia	2/15 (13.3)
Platelet count <100000/mm^3	
Microcephaly	1/15 (6.6)
Neonatal Jaundice	2/15 (13.3)
Respiratory Distress	1/15 (6.6)
Petechiae	1/15 (6.6)
Petechiae with Jaundice	1/15 (6.6)

CMV: Cytomegalovirus.

Of the infants with symptomatic HCMV infection, 4(26.6%) had hepatosplenomegaly, whereas 2(13.3%) each had neonatal jaundice and thrombocytopenia (Table-3).

Discussion

In Pakistan, a large number of people belong to low socio-economic status; therefore, pregnant women are usually exposed to many infections including HCMV. To the best of our knowledge, the current study was the first available one conducted in Islamabad. Based on seropositivity to HCMV IgG and IgM antibodies and its DNA, pregnant women had latent, active and/or acute infection.

Our data regarding seroprevalence of HCMV IgG in pregnant women (97.55%) was comparable to a regional study conducted in KP[11] as well as to some developing countries like Qatar,[9,10] Iraq[18] and Iran[19] while the percentage was higher as compared to developed countries like the United Kingdom[9] and Germany.[8] It is likely that in developed countries, pregnant women are generally more informed on good hygienic practices such as hand washing, thus accounting for a reduced risk of acquiring HCMV infection. For instance, only 4(0.9%) women in our study had information of HCMV infection. The high prevalence rate indicates the endemicity of infection and can be related to socio-economic, clinical and climatic factors. We observed that 61.53% of mothers with CMV infection noted a febrile illness fever during pregnancy, however, given the numerous causes of fever, it represented a non-specific indicator of maternal CMV infection.

Moreover, 10(2.4%) of the women were susceptible to CMV with no antibodies detected in their samples. This group had a high risk of transmission of the virus to the foetus, if infected during the pregnancy. The percentage of active or recent infection or reactivation of HCMV among pregnant women in this study was higher as compared to other studies from Iran,[18,19] Qatar,[10] Sudan[20] and India.[21]

Since the percentage of vertical transmission (19.6%) is higher in women with recurrent infection,[22] Pakistani infants are considered at high risk of perinatal CMV infection. According to a case study in Brazil, frequency of congenital CMV infection is higher in population with higher seroprevalence rate.[23] A concluded recent study has found that maternal CMV seropositive status plays an important role in congenital infection as well as CMV-related hearing loss among offspring.[24] However, data from other studies reveals that vertical transmission is higher in primary CMV infection as compared to recurrent infection.[25] Our study showed that in Pakistan recurrent infection was the leading cause of active infection as most of the cases had high avidity IgG that was determined through molecular studies.

In our study, the incidence of symptomatic congenital CMV infection was 21%, with hepatosplenomegaly and neonatal jaundice being prevailing clinical features. Similar clinical features have been reported in other countries along with other notable symptoms like thrombocytopenia, microcephaly, bronchopneumonia, congenital cataract, etc.[21] The majority of the congenitally infected children seemed to be asymptomatic at birth, but neurological sequelae may develop later after months or even years. Fowler et al.[14,15] reported that after a mean follow-up of 4.7 years, 8% of children from mothers with recurrent CMV infection, exhibited one or more sequelae, especially hearing loss which may not often be present immediately after birth. A longitudinal study by Dahle et al.[26] reported that 7.4% of children with asymptomatic CMV infection developed sensorineural hearing loss (SNHL) as compared to 40.7% born with symptomatic CMV infection.PCR and an antibody (ELISA IgM) assay are common methods used for the detection of CMV infection; however, the PCR may lead to more accurate diagnostic method for maternal as well as congenital CMV infection in newborns. The negative IgM does not essentially rule out CMV infection as samples might be collected early in the course of viral infection and may not have detectable levels of IgM. In order to determine strategies for those at risk and to prevent sequelae, accurate perinatal diagnosis of congenital CMV infection is important.

Conclusion

There was a high seroprevalence of HCMV in Pakistani population. The maternal HCMV infection caused a

considerable disease burden on society in terms of anomalies and birth defects in babies. Good hygienic conditions, proper diagnosis, and introduction of vaccines and antiviral therapies may help in controlling HCMV-related congenital abnormalities in newborns. A comprehensive study would be required with long-term follow-up of offspring born to HCMV-infected mothers in order to provide an accurate status of congenital HCMV infection in Pakistan.

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