Serofrequency of Cytomegalovirus among pregnant ladies attending antenatal care at Omdurman Maternity Hospital, Sudan

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Abstract

Background: Cytomegalovirus (CMV) is a member of the family herpesviridae .This study was conducted to determine the serofrequency of cytomegalovirus (CMV) among pregnant ladies at Omdurman Maternity Hospital between the period April to July 2014. Vertically transmitted Cytomegalovirus is the most common cause of preventable congenital infection. Till date, there is no consensus on routine antenatal screening worldwide.

Materials and Methods: This is a laboratory based descriptive study. It included 90 pregnant ladies attending Maternity hospital. Blood specimens was taken from pregnant women that came for delivery and investigated for cytomegalovirus specific immunoglobin G (IgG) and immunoglobin M (IgM) antibodies using enzyme-linked immunosorbent assay (ELISA). Demographic and clinical data were collected by questionnaire after a written consent. Ethical clearance was obtained from the Ethical Committee of AL-Neelain University.

Results: The serofrequency of cytomegalo virus among Ninety pregnant ladies attending antenatal care at Omdurman Maternity Hospital was 67 (74.4%) for IgG were positive , and 13 (14.4%) for (IgG,IgM) , while 10 (11.1%) were negative for both antibodies .

Conclusion: The serofrequency of CMV showed high rate of IgG and low rate of IgM, however, there is need for voluntary screening of pregnant ladies as the part of antenatal care.

Key words: Serofrequency , ELISA, IgG, IgM. Cytomegalovirus (CMV), pregnant ladies

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Introduction

CMV is DNA virus. Human are only known host like other members of herpes virus family and cause latent infection and infect people on all age and socioeconomic group. It is acquired early in life and can be transmitted vertically and horizontally, also through sexual intercourse, blood transfusion, organ donation and contact with infected saliva and urine . It is the most common cause of congenital infection because it occurs after both primary and recurrent infection in pregnancy(Nigro, 2009) and common cause of deafness and intellectual impairment worldwide. The risk of CMV transmission to the fetus is higher among pregnant women with primary infection compared to those who were IgG positive prior to pregnancy . CMV infection in pregnancy is largely asymptomatic, but association with some obstetric complications has been reported. Its clinical manifestations range from asymptomatic forms (90% of cases) to severe fetal damage, and in rare cases, death due to miscarriage. Furthermore, 10 to 15% of the children who are asymptomatic at birth may develop late sequelae, especially hearing defects, after a period of months or even years (Massimo *et al.*, 2009). CMV infection is considered a significant public health problem because it can cause disease in unborn babies and in people with a weakened immune system. In Sudan High CMV seroprevalence (98.3%) among pregnant women was reported (Nahla *et al.*, 2011). Previous study conducted at Omdurman Maternity Hospital revealed that the seroprevalence of CMV IgG antibodies among pregnant women was 95% (Kafi *et al.*, 2009).

Materials and Methods

This was descriptive-cross sectional study which had been conducted in Omdurman Maternity Hospital, during the period from April to July 2014.Nintey pregnant ladies attending Omdurman Maternity Hospital randomly were included in this study.

Data was collected by using direct interviewing questionnaire; ethical clearance was obtained from research ethic committee of Neelain University and verbal consent will be taken from pregnant women.

ninety blood specimens were collected under strict condition , Venous blood samples (5 ml) was collected after disinfected of skin by 70% alcohol ,and then blood was poured in plain container and centrifuged at 2000rpm for 5 minutes to obtain the serum. Serum was stored at -20°C until used.

Specimens were processed by enzyme immunoassay for the qualitative detection of (IgM, IgG) antibodies to CMV in human serum (ACON Laboratories, USA).

Enzyme immunoassay for the detection of IgM and IgG antibodies to CMV in human serum (the same method of both)

All reagent and specimens were allowed to reach room temperature (18-30°C) before used. working wash buffer was prepared by diluting the concentrated wash buffer 1:25. Microtiter plate was used,A1 well for blank, negative control duplicate in wells (B1,C1), positive control duplicate in wells (F1,G1) ,Cut-off calibrator duplicate in wells (D1,E1), specimen diluents started in well H1 samples or tested in other wells.

One hundred micro liters of specimen diluents, calibrator and controls was dispensed into the appropriate wells. five micro liters of specimen was added started from well H1 mixed gently on flat bench for 30 second and covered the micro well plate with the plate sealer and incubated at 37°C for 30 minutes. At the end of the incubation period, the liquid from all wells was removed. The microtiter wells were rinsed 5 times with 350 micro liters of working wash buffer in each wells and then 100 micro liters of enzyme conjugate(contain recombinant CMV antigens bound to horseradish peroxides with preservatives) was dispensed to each well except for the blank well.

The plate was incubated at room temperature for 30 minutes. Then enzyme conjugate was removed from all wells, as in first washing. Fifty micro liters of substrate A(citrate phosphate buffer contain hydrogen peroxide with preservative) was dispensed to each well .and fifty micro liters of substrate B (buffer contain tetramethylbenzidine(TMB) with preservative) was dispensed to each well and then incubated at room temperature for 10 minutes .Then fifty micro liters of stop solution was added to each well then the yellow color was developed in wells contained positive specimens. The optical density was read at 450 nm within 30 minutes with microtiter plate reader

Calculation of the results: Calculation of the (cut-off) calibrator of the mean of negative control in well (D1,E1) then subtracted the blank absorbance.

Interpretation of results of IgM antibodies: Cytomegalovirus IgM EIA index less than 0.25 was considered seronagative for IgM antibody to CMV and greater than 0.25 was considered seropositive.

Interpretation of results of IgG antibodies: Cytomegalovirus IgG EIA index less than 0.09 was considered seronagative for IgG antibody to CMV and greater than 0.09 was considered sero positive.

Results

A total of ninety serum specimens of pregnant ladies attending antenatal care at Omdurman Maternity Hospital, their age range (15-45 years old), out of them 67 ladies (74.4%) were CMV IgG positive while 13 (14.4%) were positive for both antibodies (IgG, IgM), and 10 ladies (11.1%) were negative (figure 1). The results showed that the highest anti-CMV IgG seropositivity rate was among those in 25-35 years age range (Table1), 3 ladies (4.5%) were reported on having past history of congenital abnormalities, Present of history abortion in 20 ladies (62.5%) (Table 2), Also high positive results were observed among third trimesters group (Table 3) and among multigravidae ladies (Table 4). According to chi-square analysis between age, abortion , trimester, gravidity and presence there was insignificant correlation of CMV antibodies ($P > 0.05$) (Table 5).

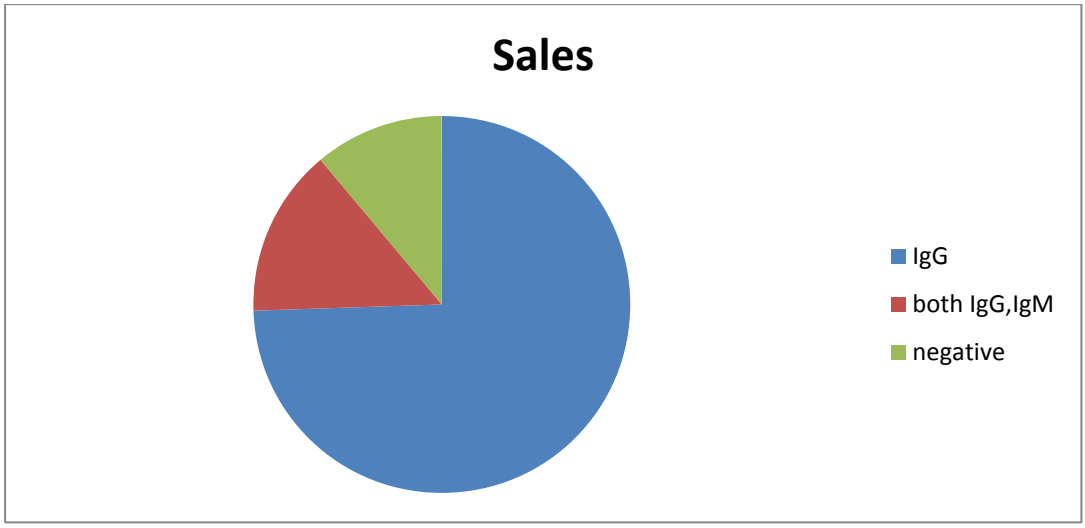

Figure 1 seropositivity of CMV among study group

Table 1. Distribution of CMV seropositive women according to age group

Age groups	IgG seropositive	IgM-IgG seropositive	Negative	Total
15-24	26 (83.9%)	3 (9.7%)	2 (6.5%)	31(34.4%)
25-34	39 (70.9%)	8 (14.5%)	8 (14.5%)	55(61.1%)
35-45	2 (50.0%)	2 (50.0%)	0 (0.0%)	4(4.4%)
Total	67 (74.4%)	13 (14.4%)	10 (11.1%)	90(100%)

Table 2. Distribution of CMV seropositive women according to history of abortion

Abortion groups	IgG seropositive	IgM-IgG seropositive	Negative	Total
Yes	20 (62.5%)	7 (21.9%)	5 (5.6%)	32(35.6%?)
No	47 (81.0%)	6 (10.3%)	5 (8.6%)	58(64.4%)
Total	67 (88.9%)	13 (14.4%)	10 (11.1%)	90(100%)

Table 3. The effect of frequency of parities on CMV IgG and IgM seropositivity among pregnant ladies

Trimesters groups	IgG seropositive	IgM-IgG seropositive	Negative	Total
First	13 (68.4%)	4 (21.1%)	2 (10.5%)	19(21.1%)
Second	17 (77.3%)	4 (18.2%)	1 (4.5%)	22(24.4%)
Third	37 (75.5%)	5 (10.2%)	7 (14.3%)	49(54.4%)
Total	67 (74.4%)	13 (14.4%)	10 (11.1%)	90(100%)

Table 4. Distribution of CMV seropositive ladies according gravidity group

Gravidity groups	IgG seropositive	IgM-IgG Seropositive	Negative	Total
Primagravidae	17 (70.8%)	5 (20.8%)	2 (8.3%)	24(26.7%)
Multigravidae	50 (75.8%)	8 (12.1%)	8 (12.1%)	66(73.3%)
Total	67 (74.4%)	13 (14.4%)	10 (11.1%)	90(100%)

Table 5. Relation between CMV antibodies and certain factor(age, abortion, trimester, gravidity, chi-square test)

Immunoglobulin	Factors	P-value (0.05)
IgM	Age	0.09
IgG	Age	0.39
IgM	Abortion	0.14
IgG	Abortion	0.31
IgM	Trimesters	0.44
IgG	Trimesters	0.48
IgM	Gravidity	0.29
IgG	Gravidity	0.61

Discussion

CMV must be acknowledged as one of the most successful human parasites. It has learned to survive in its human host infecting both vertically and horizontally.

This study revealed that the serofreqency of CMV among pregnant ladies is high, CMV IgG antibodies were found in 74.4% of the cases, while 14.4% were positive for both antibodies (IgG, IgM) and only 11.1% were negative.

The detection of CMV IgG indicated that the pregnant ladies had previously been infected with CMV. After CMV infection, IgG remains in the body for life and protects considerably against the next infections. This indicates that a negative results of CMV IgG test means that the women have not been infected with the virus. The serofrequency of CMV IgG observed in this study was semi similar to the results reported in Sudan by Nahla *et al.*, (2011) which was 98.3% and Kafi et al. (2009) which was (95%). The picture of CMV prevalence in different countries is almost similar to our results; 96% in Egypt (El-Nawawy *et al.*, 1996), 97.2% in Nigeria (Akinbami *et al.*, 2011), 97.3% in Turkey (Uyar *et al.*, 2008), 98.1% in Korea (Seo *et al.*, 2009), and 95.6% in China (Meng *et al.*, 2011). However, the results of this study were higher than those reported by Picone *et al.*, (2009) in France (46.8%), Alanen *et al.* (2005) in Finland (56.3%), and Staras *et al.* (2006) in the United State (60.0%).

Also this study showed the rate of positive of CMV IgM was 14.4% among tested pregnant ladies, which reflected an active recent infection or reactivation of the virus. This finding was higher than that of Hamdan *et al.* (2011) in Western Sudan who reported the rate of positive CMV IgM as 2.5%, however, far higher CMV IgM seroprevalence (94.3%) in neonates was recently reported in Sudan (Nahla *et al.*, 2011). Variable IgM positivity were reported

worldwide, only 1.0% in Turkey (Uyar *et al.*, 2008), 2.5% in Iran (Bagheri *et al.*, 2012) and 1.7% in Korea (Seo *et al.*, 2009).

In this study results showed insignificant correlation between age, abortion, parities and gravidity (P > 0.05) associated with presence of CMV antibodies ,this finding agreed with the results of Bate *et al.* (2010) in the United States . The fact that there were no differences related to the age of the women indicates the same behavior at different ages.

Conclusion

This work has demonstrated that a high proportion (74.4%) IgG and low proportion (14.4%) for both antibodies (IgG, IgM) of pregnant ladies in our environment is exposed to CMV infection. It is recommended in this study that there should be voluntary screening of all pregnant women, as part of antenatal care, so that seropositive women with primary infection could be offered the opportunity for prenatal screening and be informed of intervention options. For seronegative women identified during screening, they should be counseled on appropriate preventive measures such as hand washing and to avoid practices such as kissing and sharing food with children.

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