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High Seroprevalence of Cytomegalovirus Infection among Pregnant Women in Ilorin, Kwara State, Nigeria.

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Abstract

Cytomegalovirus (CMV) is an important public health problem worldwide and has been shown to be an important aetiological agent of intrauterine infection in pregnant women that results into congenital malformations such as cerebral palsy, blindness and sensor neural loss. This study was conducted to determine the seroprevalence of human cytomegalovirus among pregnant women in Kwara State. Two hundred and twenty five (255) blood samples were collected from pregnant women attending antenatal clinics in two selected state Hospitals in Kwara State. Serum obtained from the blood samples were examined for the presence of CMV IgM and IgG antibodies by ELISA method. Out of the 225 pregnant women tested, 221 (98.2%) and 56 (24.9%) were CMV IgG and IgM positive, respectively. A significant statistical relation was observed between CMV IgG seropositivity and gestational age, occupation and marriage type, while the age, parity and gestational age were significantly associated with CMV IgM seropositivity. This extremely high level of CMV infection among these pregnant women as observed in this study indicates that the virus is prevalent in the study area. It is therefore imperative to implement a routine CMV infection screening in pregnant women within the State and also need for vaccination in order to prevent birthing of children with avoidable birth defects.

Keyword: Seroprevalence, Cytomegalovirus, IgG, IgM, Pregnant women.

1. Introduction

Human Cytomegalovirus (HCMV) is a ubiquitous enveloped DNA virus and a member of the family Herpesviridae and belongs to the subfamily beta herpesviridae. CMV has worldwide distribution, infects humans of all ages and all socioeconomic groups, and with no seasonal or epidemic patterns of transmission (Sotoodeh *et al.*, 2010). In developing countries, CMV infection during pregnancy has been reported to be a major cause of congenital infection with

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a prevalence of 0.2-2.4% of live births (Stagno and Britt, 2006). Its clinical manifestations range from asymptomatic forms (90% of cases), a severe fetal damage and in some cases miscarriage. Reports have shown that 10 to 15% of the children who are asymptomatic at birth may develop late sequelae, most commonly hearing defects, after a period of months or even years (Saidu *et al.,* 2015).

HCMV can be transmitted via saliva, sexual contact, placental transfer, breast feeding, blood transfusion and solid-organ transplantation (Bowden, 1991). CMV can be transmitted to the infant in utero, perinatally, or postnatally by infected mothers. In utero infection can result following maternal primary infection during pregnancy but can also occur in women with natural immunity, either due to re-infection with a different strain, or reactivation of latent infection. Perinatal infection can result during delivery following exposure to cervical and vaginal secretions (Emmanuel *et al.,* 2015). Postnatal, CMV is also spread from mother to child through breastfeeding and close contact (Emmanuel *et al.,* 2015). However, during primary maternal infection, the risk of congenital infection is much higher with transmission rate of 30%-40% compared to reactivation of reinfection (Alghalibi *et al.,* 2016).

Studies among pregnant women worldwide have shown different seroprevalence rates for previous infection. In Australia, Belgium, France and Poland, a relatively low seroprevalence rate of 30%-60% was reported (Basha *et al.,* 2014), while a high CMV seroprevalence of greater than 90% was reported in Iran, Turkey, Nigeria and India (Josheghani *et al.,* 2015). In Nigeria, past studies have revealed CMV prevalence of 84.2%, 91.1%, 97.2%, 98.7% in Bida, Kano, Lagos and Sokoto respectively among pregnant women, indicating increase in prevalence.

Detection of virus-specific IgG antibody in the blood of a pregnant woman who was previously

seronegative, or detection of specific IgM antibody associated with low IgG avidity is diagnostic of primary maternal CMV infection (Sonoyama *et al.*, 2012). IgM antibody is present for 4 to 8 weeks after primary infection and can persist for years. Nevertheless, false positive results can occur due to cross-reactivity with other diseases, such as autoimmune disorders or other viral infections (Johnson and Anderson, 2013). CMV infection among pregnant women is of great concern because of their immunocompromised state and risk of infection to the foetus whose immune system is under developed.

Despite the precarious consequence that CMV infection poses during pregnancy, no national screening test for CMV infection is available during pregnancy in Nigeria. There are no published data on CMV in pregnant women in General and Civil Service Hospitals in Ilorin, Nigeria. The basic data on CMV infection during pregnancy is vital for health planners and health care providers. So seroprevalence studies of CMV in Nigeria are crucial to raise awareness of CMV infection and inform on appropriate and rational interventions which will help in management of the infection and prevent secondary spread in the country.

2. Materials and Methods

Study Area

This research was a hospital based cross sectional study of Pregnant Women attending the Ante-natal Clinics of General Hospital Ilorin and Civil service clinic both of which are reference hospitals in Ilorin. Ilorin is the state capital of Kwara state in Nigeria and located on 8030‘N 4033‘E /8.5000N 4.5500E.

Study population/sampling technique

The sample size was calculated using the Fishers formula and the study population consisted of 225 pregnant women who met the inclusion criteria at the antenatal clinic of the Department of Obstetrics and Gynecology of the Hospitals.

Socio-demographic data

A well-structured questionnaire containing open and close ended questions was designed to obtain information from the patients. This was administered to patients who met the inclusion criteria and the response to the questionnaire gave information about the age, religion, marital status, parity, gestational age, educational level and occupation.

Laboratory methods

Blood sample collection

From each of the 225 pregnant women woman, five ml (5ml) of venous blood was collected aseptically in plain blood sample bottles marked with a unique number that tallied with the number on their questionnaire, centrifuged on same day and the serum stored at -20^{o}C until assay.

Assay

The preserved sera were screened for CMV IgG and IgM antibodies using Enzyme Linked Immunosorbent Assay method (ELISA). Analysis and interpretation of results was done according to the manufacturer's instruction (Diagnostic Automation/Cortez Diagnostics, Inc.)

Statistical analysis

Results were analyzed using the Statistical Package for the Social Science (SPSS) version 21.0 software package. The significance of the values obtained was determined at $P<0.05$

Ethical approval

Ethical approval was granted by the Ethical Review Committee of the Kwara State Ministry of Health, Ilorin. Informed consent was obtained from each subject after a careful interpretation of the study.

3. Result and Discussion

The study on seroprevalence of cytomegalovirus IgM and IgG antibodies was carried out on two hundred and twenty five pregnant women attending ante-natal clinics in two hospitals in Ilorin, Kwara State. From the 225 blood samples collected, 24.9% (56/225) and 98.2% (221/225) respectively for IgM and IgG respectively. A total of 225 pregnant women between the ages of, and a mean gestation period of 5.2 months, were enrolled for this study.

The prevalence of Cytomegalovirus was found to be 24.9% (56/225) and 98.2% (221/225) respectively for IgM and IgG respectively. The age groups of the women enrolled in the study were between the ages of 16 and 40 with a mean age of 28 years. The results on Table 1 showed that the highest CMV IgM antibodies (22; 39.2%) was recorded in the age group of 21-25 while the highest CMV IgG antibodies (82;37.1%) was recorded in the age group 25-30. The result shows that there was a significant association between age group and CMV IgM antibody (P = 0.000) while no statistical association was observed between age group and presence of CMV IgG antibody (P = 0.581) (Table 1).

Analysis with respect to the subject's gestational age, majority of the subjects 124(55.1%), were in their second trimester, out of which 5(4.0%) were IgM positive and 52(42.0%) were IgG positive. A statistical association was recorded with P values= 0.000 and 0.020 for IgM ad IgG respectively. Distribution based on parity, higher seropositivity was observed among women who are parous as compared to those who are nulliparous for both CMV IgM and IgG with statistical association observed for CMV IgG antibody (P=0.040) (Table 2). More than half of the test subjects were Muslims and 32(24.6%) and 126(97.0%) were IgM and IgG positive respectively, with no statistical significance.

Further analysis of the data based on educational level of the respondent showed that none out of 3(1.3%) subjects without any form of education, 2(3.6%) of 2(0.9%) that attended only primary school, 21(37.5%) of 103(45.8%) with secondary education and 33(58.9%) of 117(52.0%) subjects that had tertiary education were positive to CMV-IgM (P=0.032). None out of the subjects with no formal education, 2(0.9%) subjects in the primary education group, 103(46.6%) subjects in secondary education group and 113(51.1%) tertiary group were positive for CMV-IgG antibodies (P=0.289).

There was an observed relationship between level of education and IgM seropositivity as increase in educational level showed a corresponding increase in seropositivity. Subjects with tertiary education had the highest seropositivity for CMV IgM and IgG antibodies with 33(28.2%) and 113(96.6%) respectively (Table 3). With respect to marital status, 7(3.1%) were single while 218 (96.9%) were married. Out of which 2(3.6%) subjects that were single and 54 (94.4%) that were married tested positive for CMV-IgM antibody (P=0.819) while 7 (3.2%) single and 214 (96.8%) married pregnant women tested positive for anti-CMV IgG (P=0.718) (Table 3). With respect to the marriage type, 208(92.4%) of the test subjects were from monogamous families of which 52(25.0%) were seropositive for IgM and 208(95.0%) for IgG (Table 1). A statistical association was observed between the marriage type and positivity to CMV-IgG (P= 0.000).

Considering the occupation of the respondents, out of the 102(45.3%) subjects who were business women, 23(21.9%) and 102(97.1%) were IgM and IgG (P= 0.000) positive respectively. Fifty four (24%) subjects were positive for both CMV IgM and IgG in the study (re-infection). 2(0.9%) were negative for both CMV IgM and IgG depicting at risk /susceptible status. 167 (74.2%) were IgM negative and IgG positive (indicating a past infection), and 2(0.9%) were IgM positive and IgG negative (primary infection) (Table 4).

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

Age (Years)	Number Tested	Anti-CMV IgM Positive	Anti-CMV IgG Positive	χ2 (IgM/IgG)	P values (IgM/IgG)
>20	10	2(3.6)	10(4.5)	74.268/20.040	0.000/0.581
21-25	63	22(39.2)	63(28.5)		
26-30	86	14(25.0)	82(37.1)		
31-35	47	10(17.9)	47(21.3)		
36-40	19	8(14.3)	19(8.6)		
Total	**225**	**56(24.9)**	**221(98.2)**		

Table 2: Seroprevalence of CMV among Pregnant Women in Kwara State by Gestational Age and Parity

Gestational age (Months)	Number Tested	Anti-CMV IgM Positive (%)	Anti-CMV IgG Positive	χ2 (IgM/IgG)	P values (IgM/IgG)
First trimester	49	6(10.7)	47(21.2)	27.88/16.608	0.000/0.020
Second trimester	124	45(80.7)	122(55.2)		
Third trimester	15	5(9.0)	52(23.5)		
Parity					
Nulliparous	115	33(58.9)	110(49.8)	1.823/3.895	0.177/0.040
Multiparous	110	23(41.1)	111(50.2)		
Total	**225**	**56**	**221**		

Table 3. Demographic characteristics of the respondents in relation to CMV IgM and IgG status.

Demographic characteristics	Number Tested	Anti-CMV IgM Positive (%)	Anti-CMV IgG Positive	χ2 (IgM/IgG)	P values (IgM/IgG)
Religion					
Christianity	95	24(42.9)	95(43.0)	0.912/2.976	0.912/0.085
Islam	130	32(57.9)	126(57.0)		
Educational Level					
No formal Education	3	0(0)	3(1.4)	0.032/3.759	8.834/0.289
Primary	2	2(0.9)	2(0.9)		
Secondary	103	21(45.8)	103(46.6)		
Tertiary	117	33(58.9)	113(51.1)		
Total					
Marital Status					
Single	7	2(3.6)	7(3.2)	0.052/0.137	0.819/0.718
Married	218	54(94.4)	214(96.8)		
Marriage Type					
Monogamous	208	52(96.3)	208(95.0)	1.038/56.48	0.308/0.000
Polygamous	15	2(3.7)	11(5.0)		
Occupation					
Unemployed	15	6(10.7)	15(16.8)	2.811/72.29	0.590/0.000
Student	12	4(7.1)	102(46.2)		
Business	102	102(45.3)	8(3.6)		
Artisan	62	14(25.0)	62(28.1)		
Civil servant	34	9(16.1)	34(15.4)		
Total	**225**	**56**	**219**		

Table 4: Serological status of CMV among the pregnant women

CMV serology	No(%) of patients
IgM (-) IgG (+)	167 (74.2)
IgM (-) IgG (-)	2 (0.9)
IgM (+) IgG (+)	54 (24)
IgM (+) IgG (-)	2 (0.9)

This study revealed that the prevalence of CMV in pregnant women is very high, anti-CMV IgG antibodies was found in 98.2% of the cases, while 24.9% of the subjects tested positive for anti-CMV IgM. The detection of CMV IgG shows that the pregnant women had previously been infected with CMV. After CMV infection, IgG persists in the body for life and confers a considerable immunity the subsequent infections. This indicates that a negative results of CMV IgG test means that the women have not been infected with the virus and are at a high risk of CMV infection.

The high percentage level of maternal CMV IgG antibodies observed in this study is similar to previous studies in Nigeria (Olatunji and Oluwajana, 2017; Glory *et al.,* 2016; Hamid *et* al., 2014; Akinbami *et al.,* 2011). The picture of CMV prevalence in different developing countries is almost similar to our result; 97.5% in Sudan, 96% in Egypt (Khairi *et al.,* 2013), 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). This however, differs from those reported from developed countries where seroprevalence rate is lower (Glory *et al.,* 2016). It can be seen that the prevalence of CMV infection recorded in this study was similar to that reported in other developing communities but however higher than in the developed countries. This could be as a result of the low socioeconomic status and poor hygienic practices which might play important roles in increasing the rate of transmission of CMV infection. It was previously reported that seroprevalence of CMV among women varies with geographical location, socioeconomic status and occupation (Khairi *et al.,* 2013).

In the present study, the rate of positive CMV IgM was 24.9% among tested pregnant women, which reflected an active recent infection or reactivation of the virus. This prevalence is almost similar to that reported in Iran with a prevalence of 33% (Arabzadeh, 2007). However a lower prevalence rate has earlier been the rate of 3.1% positive CMV IgM was reported in Ilorin by Olatunji and Oluwajana (2017), this higher rate of current infection in this study indicates an increase in the CMV transmission and acquisition in the state. Variable IgM positivity have been reported worldwide, only 1.0% in Turkey (Uyar *et al.,* 2008), 2.5% in Iran (Bagheri *et al.,* 2012), 6.0% in Sudan (Khairi *et al.,* 2013) and 1.7% in Korea (Seo *et al.,* 2009).

In this study, a significant association ($P < 0.05$) was found between the age of pregnant women and CMV IgM seropositivity. Most of the respondents had acquired immunity at age above 20 years. This finding disagrees with the results of Ndako *et al.* (2016) in Northern Nigeria where it was reported that most of the subjects had acquired immunity before 20 years of age. However, our finding was in consonance with the results obtained by Hamdan *et al.* (2011). As observed in this study, pregnant woman below 20 years of age were at higher risk of CMV infection. The high prevalence rates seen among subjects aged, 21-30 could also be attributed to the fact that subjects within this age group are among the sexually active age hence the likelihood of being infected through the sexually transmitted route (Glory *et al.,* 2016).

Our findings showed that subjects in their second trimester of pregnancy recorded the highest prevalence followed by those within the third trimester and lastly those in first trimester. A statistically significant association was recorded for both anti-CMV IgM and IgG ($P<0.05$) in the three trimesters. The result obtained in this study with regards to gestational age agrees with the work of Ndako *et al.* (2016) and Okwori *et al.* (2008) where the highest seroprevalence of CMV antibodies was recorded among pregnant women in their second trimester followed by those in their third trimester with prevalence. The result from our study is however is in contrast with the result obtained by Edward *et al.* (2015) where women at their third trimester

had the highest prevalence followed by the first and second trimester with prevalence of respectively. Women at all stages of pregnancy could be at high risk of intrauterine transmission but those at higher risk are those who were infected within the first 20 weeks of pregnancy (Ndako *et al.,* 2016). Babies delivered to these women in their second trimester are at risk of getting congenital CMV infection (Sheevani and Aggarwal, 2005).

With respect to parity, it was observed that a larger percentage of study respondents were nulliparous (51.1%) as compared to those that were parous. The highest prevalence rate was found in the nulliparous group, however, there was no significant association between parous and nulliparous and the risk of acquiring CMV infection in the study. This agrees with the findings of Olatunji and Oluwajana, 2017 which reported that there was no significant relatedness between parity status and the risk of acquiring CMV infection. In contrast however, a significant statistical association was observed between parity and presence of CMV IgM antibody. This result agrees with the findings of Hamdan *et al*. (2011) that reported parity to be a significant risk factor for CMV infection. This could be so as increase in parity also implies increase in age, which according to previous reports is a significant predictor of CMV infection (Stadler *et al.,* 2013).

In this study, the seroprevalence rate increased insignificantly with increase in education with higher degree of seropositivity being observed in the group with tertiary education. The increase in seroprevalence disagrees with a previous report that showed that illiterate women are at higher risk of CMV infection as a result of contact with contagious secretions from their own children and poor hygienic practice (Yeroh *et al.,* 2015). The marital status shows that most of the respondents are married with only 3.1% of the population being single. Cytomegalovirus infection is higher in married women than in singles which suggest continuous circulation of the virus within the area, although no significant statistical association

was observed. This is in consonance to a study that also recorded increased seropositivity among married women as compared to single ladies (Douglas *et al.,* 2012).

The distribution of seroprevalence rates of CMV among pregnant women by type of marriage shows that most of the respondents were involved in the monogamous marriage type, study showed a significant association between CMV IgM detection and type of marriage (P=0.000). Of the tested subjects, 0.9% have never been exposed to CMV, this shows that these women are susceptible to CMV infection, and are at risk of developing a primary CMV infection on coming in contact with the virus for the first time during pregnancy, thus increasing their chances of giving birth to congenitally infected children.

The findings of our study indicated high prevalence of CMV seropositivity among pregnant women in Ilorin, Kwara State. Furthermore, the results showed that maternal and gestational age were main risk factors for CMV reinfection or new infection. Although most of the considered risk factors were not of statistical significance, this study however shows that CMV is highly associated with pregnant women in Ilorin indicating that there is possibility of corresponding increase in the incidence of congenital CMV infection among infant born in the state. Thus, increased public awareness about CMV infection is required across Nigeria, and this can be done through primary health care channels such as antenatal clinics where the transmission, consequences of infection on foetus and its control and preventive measures can be discussed. Efficient blood screening for CMV before transfusion to pregnant women, routine screening of pregnant women for CMV should be adopted in all health care settings and babies born to seropositive mothers should be screened and examined immediately after delivery for possible signs of hearing and vision defect for early management.

4. Conclusion

A significant number of pregnant women in Ilorin metropolis are exposed to CMV infection. We hereby recommend that health policies should incorporate routine screening for both anti CMV IgM and IgG antibodies among pregnant women. Also compulsory CMV antibody screening of blood to be transfused to premature babies, pregnant women and immunocompromised individuals should be adopted.

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