Serological Evidence and Sociodemographic Risk Factors of Recent Cytomegalovirus Infection in Pregnant Women Attending a Tertiary Hospital in Maiduguri, Nigeria

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Received Nov 01, 2014; accepted Dec 16, 2014

INTRODUCTION

Human cytomegalovirus (HCMV) is a very common viral infection and one of the most common causes of congenital infections in humans. Its clinical manifestations range from asymptomatic forms to severe fetal damage, and in rare cases, fetal death due to abortion. This prospective cross-sectional study was designed to determine the seroprevalence of HCMV in pregnant women attending antenatal clinics of the University of Maiduguri Teaching Hospital, Nigeria, and to identify its possible risk factors. Methods: Blood samples were collected from 182 pregnant women aged 16 to 40 years. Samples were tested for anti-CMV specific IgG and IgM antibodies using the commercial ELISA Kits. A brief structured questionnaire was used to obtain some of their sociodemographic characteristics. Results: Seroprevalence of CMV-specific IgG and IgM were 79.1% and 2.2%, respectively. Of 182 women, 141 had previous exposure to CMV [IgG (+) IgM (-)], 3 had CMV reactivated infection [IgG (+) IgM (+)], 37 were susceptible to CMV [IgG (-) IgM (-)], and only one woman had recent infection [IgG (-) IgM (+)]. There was no significant association between seroprevalence and any of the studied sociodemographic data (p>0.05). Conclusion: The findings of this study indicated that a large number of the studied pregnant women were non-immune (susceptible) to HCMV infection, while four of them had active HCMV infection, which places their unborn children at risk of acquiring congenital HCMV infections. Therefore, it is necessary to screen pregnant women for CMV infection as part of their antenatal care and follow-up them to assess the effect that CMV might have on their fetuses. J Med Microbiol Infect Dis, 2014, 2 (2): 49-55.

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Serological surveys have shown HCMV infections almost exist in every population that have been tested with seropositivity ranging from 40-100% in various parts of the world [15,17]. For example, a serological survey of over 20,000 women in London found that 54.4% of these women were seropositive for HCMV [18].

Maternal infection poses the risk of congenital CMV infection, which occurs in 0.5%-2.5% of all life births [19]. Risk of congenital infection is much higher during primary infection of the mother with transmission rate of 30%-40% compared to 0.15%-2.2% during reactivation and reinfection [20].

Seroprevalence studies among pregnant women worldwide have indicated seropositivity rates for previous infection, which ranges from 50% in highly developed countries to 100% in developing countries [21].

A serological survey conducted by Okwori et al. on HCMV among pregnant women in Bida, Nigeria gave a seroprevalence of 84.2%. Another study carried out on pregnant women in Lagos, Nigeria gave a seroprevalence of 97.2% [22]. The high prevalence of CMV observed in these studies conducted in Nigeria, shows that the prevalence of CMV infection in Nigeria is on the rise.

The prevalence of HCMV among pregnant women has been studied in relation to other causes of congenital infections, such as Toxoplasma gondii and rubella (TORCH agents). In one of these comparative studies conducted on 1972 pregnant women in western region of Turkey, HCMV was found to be the highest in prevalence with a seroprevalence of 96.4% for IgG, 0.7% IgM, and 1.9% IgG+IgM and the least for T. gondii with a seroprevalence of 48.3% for IgG, 0.4% IgM, and 1.6% IgG+IgM [23].

The present study aimed to provide serological data of recent primary and reactivated HCMV infection among pregnant women attending antenatal clinics of the University of Maiduguri Teaching Hospital, Nigeria and to justify the need for including voluntary CMV screening in antenatal visits.

MATERIAL AND METHODS

Study area. This is a descriptive cross-sectional study, which was carried out in the WHO National Polio Reference Laboratory, University of Maiduguri Teaching Hospital, Borno State, Nigeria. The study was approved by the Ethical Research Committee, University of Maiduguri Teaching Hospital, Nigeria. Maiduguri, the capital city of Borno state, Nigeria, located in northeastern Nigeria shares borders with neighboring countries, such as Niger Republic, Chad, and Cameroon. Within Nigeria, Maiduguri shares borders with other states, such as Adamawa, Yobe, and Gombe and has Sahel savannah vegetation. The annual average temperature of Maiduguri ranges from 19.1°C to 34.7°C and average annual precipitation is 562 mm. In Nigeria, there has been no consensus of opinion regarding conducting CMV screening or other TORCH panel tests for pregnant women during their antenatal visits.

Study population. The study population consisted of pregnant women attending antenatal clinic of the University of Maiduguri Teaching Hospital, Borno State, Nigeria. The median age of the women was 28 years ranging 16-40 years.

Inclusion criteria. All pregnant women attending antenatal clinic within the study period who consented to participate in the study.

Exclusion criteria. All pregnant women who declined to participate in the study or refused to consent, were excluded.

Sample size. The sample size was determined using prevalence rate demonstrated by Okwori et al. [16]. This was 0.842.

Data collection. Questionnaires were used to collect sociodemographic data, such as age, city of residence, gravida, gestational age, educational status, occupation, marital status, number of marriages, history of blood transfusion, and history of congenital deformity.

Sample collection and preparation. Samples were collected between December 2013 and March 2014. Five ml of blood was collected aseptically into plain vacutainer tubes. The tubes were then appropriately labelled with patients’ laboratory number. Sera from these blood samples were separated by allowing the blood to clot at room temperature and centrifuged at 2500 rpm for 10 min. The sera were then separated using clean Pasteur pipettes, transferred into serum containers, and stored at -70°C until laboratory analysis.

Laboratory diagnosis. Serum samples were analyzed by enzyme-linked immunosorbent assay (ELISA) using ELISA CMV IgM and IgG kits (NovaLisa™ Immunodiagnostica, Germany) with product numbers CMVM0110 and CMVG0110, respectively.

Test Procedure. All samples and reagents were brought to room temperature. The test was performed according to manufacturer’s instructions. The optical density (OD) was read using a GF-M3000 microplate reader at 450 nm wavelength. Samples were considered positive if the absorbance value was higher than 10% over the cut-off and negative if the absorbance value was lower than 10% below the cut-off. The cut-off is the mean absorbance value of the cut-off control determinations.

Informed consent. The purpose of this work was explained to the participants before obtaining their written consent. The consent form was filled in by the investigators, after which each participants signed her corresponding forms.

Ethical approval. Ethical approval for this study was obtained from the Ethical Committee of the University of Maiduguri Teaching Hospital before embarking on the research.

Statistical analysis. The data obtained from the questionnaire and the results of the laboratory analysis were entered into Microsoft Excel and analyzed using SPSS (statistical package for social sciences, version 20). The results obtained were reduced to percentages and figures. The Pearson Chi square test at a 95% confidence interval and a significance level of 0.05 was used to determine the relationships between demographic data and prevalence rates.
RESULTS

One hundred and eighty-two samples were tested and the study population were attendees of antenatal clinics of the University of Maiduguri Teaching Hospital for their usual periodic check-up. The mean age of the studied women was 25.1±6.66 ranging 16 to 40 years. Thirty-two (17.6%) women were between 15-20 years of age, while fifty-two (28.6%) were between 31-40 years (Table 2a).

Seroprevalence. At the first screening, the results of the serological assays were categorized into 4 types of responses. The first group was immune to CMV [IgG (+) and IgM (-)], which consisted of 141 women. In the second group, three women had reactivated infection [IgG (+) and IgM (+)]. In the third group, there was only one woman who had recent infection [IgG (-) and IgM (+)]. In the fourth group there were 37 women who were susceptible to CMV infection [IgG (-) but had IgM (-)]. Thus, anti-CMV IgG seropositivity was 79.1% and anti-CMV IgM seropositivity was 2.2% (Table 1).

Table 2a shows the distribution of CMV-specific IgG and IgM seropositivity across ages of pregnant women. CMV IgG seropositivity was mostly observed among those ≥31 years, 49 (26.4%) and least among those 15-20 years, 23 (12.6%).

Table 2b shows the distribution of CMV-specific IgG and IgM seropositivity across educational level of pregnant women. 9 (4.9%) women had no form of formal education, 45 (24.7%) had primary education, 80 (43.9%) had secondary education, while 48 (26.4%) had tertiary education. CMV IgG seropositivity was highest among those with secondary education, 64 (35.1%) and least among those with no formal education, 7 (3.8%).

Table 2c shows the distribution of CMV-specific IgG and IgM seropositivity across gestational age of pregnant women. Ninety-three (51.1%) women were in their third trimester, 79 (43.4%) were in their second trimester, while only 10 (5.5%) were in the first trimester.

Table 2d shows the distribution of CMV-specific IgG and IgM seropositivity across parity distribution of pregnant women. Fifty-one (28.0%) women were carrying their first pregnancy (primigravida), 25 (13.7%) had only one child, and 32 (17.6%) had more than 4 children.

There was no significant association between any of the sociodemographic data studied and seroprevalence of HCMV (p<0.05).

Figure 1 shows that majority of pregnant women with CMV IgG seropositivity were at their third trimester, 73 (51%), and least among those at their first trimester, 7 (5%).
Table 2c. Human CMV-specific IgG and IgM seropositivity across gestational age of pregnant women

<table>
<thead>
<tr>
<th>Item</th>
<th>No. of Subjects Tested (%)</th>
<th>No. of Subjects positive for CMV antibodies (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG+ and IgM- (%)</td>
</tr>
<tr>
<td>First trimester</td>
<td>10 (5.5)</td>
<td>7 (3.8)</td>
</tr>
<tr>
<td>Second trimester</td>
<td>79 (43.4)</td>
<td>61 (33.5)</td>
</tr>
<tr>
<td>Third trimester</td>
<td>93 (51.1)</td>
<td>73 (40.1)</td>
</tr>
<tr>
<td>Total</td>
<td>182 (100)</td>
<td>141 (77.4)</td>
</tr>
</tbody>
</table>

Note: p-value= 0.085

Table 2d. Human CMV-specific IgG and IgM seropositivity across parity distribution of pregnant women

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<tr>
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<th>No. of Subjects Tested (%)</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>IgG+ and IgM- (%)</td>
</tr>
<tr>
<td>0</td>
<td>51 (28.0)</td>
<td>41 (22.5)</td>
</tr>
<tr>
<td>1</td>
<td>25 (13.7)</td>
<td>16 (8.8)</td>
</tr>
<tr>
<td>2</td>
<td>33 (18.1)</td>
<td>23 (12.6)</td>
</tr>
<tr>
<td>3</td>
<td>19 (10.4)</td>
<td>11 (6.0)</td>
</tr>
<tr>
<td>4</td>
<td>22 (12.1)</td>
<td>18 (9.9)</td>
</tr>
<tr>
<td>≥ 5</td>
<td>32 (17.6)</td>
<td>32 (17.6)</td>
</tr>
<tr>
<td>Total</td>
<td>182 (100)</td>
<td>141 (77.4)</td>
</tr>
</tbody>
</table>

Note: p-value=0.06667

IGG = Immunoglobulin G
IgM = Immunoglobulin M
+ = Seropositivity
- = Seronegativity

![Number of IgG seropositivity](image)

Fig. 1. Pie chart representation of anti-CMV IgG seropositivity across gestational age

**DISCUSSION**

The findings of this study revealed that 77.5% of the pregnant women had anti-CMV IgG with no corresponding anti-CMV IgM antibodies. Development of IgG antibodies to CMV indicates that these women had previous infection with CMV in their life time. The level of maternal immunity observed in this study compares favorably with the findings of previous studies in Nigeria and other developing countries [24-27]; however, it differs from those reported in developed countries, where seroprevalence rates were lower (58% and 49%) [28, 29].

The differences in the prevalence of maternal CMV infection between developed and developing countries may reflect the ignorance on the disease and low hygienic standard and cultural practices, which can increase infection transmission in developing countries. It is likely that in developed countries, pregnant women are generally more informed on the disease, good hygienic practices, and...
other preventive measures, which account for a reduced risk of acquiring CMV infection. In addition, the high socioeconomic status of women in developed countries could account for this difference, due to the higher risk of primary maternal infection in women with low socioeconomic status than in those with high socioeconomic status [30]. Ludwig and Hengel observed that low socioeconomic status is a risk factor for congenital CMV infection.

There was an exponential increase in the prevalence of CMV IgG during the first (7%), second (61%), and third trimesters (73%) of the pregnancies. Three (3.8%) women had reactivated CMV infection (positive for both anti-CMV IgG and IgM antibodies) during the second trimester. These are not considered primary HCMV infection; this speculation is supported by a report showing that a significant increase in anti-CMV specific IgG antibody titre with or without the presence of specific IgM antibodies is an indication of non-primary CMV, which must have been acquired before pregnancy [31].

Seo et al. demonstrated that pregnant women who were positive for both CMV IgG and IgM antibodies, also had high IgG avidity index, and were at low risk of transmitting CMV infection [32]. In their report, none of the neonates of the women showed evidence of congenital CMV infection on routine neonatal examination. In our study, IgG avidity test was not performed on the 3 women who had both anti-CMV IgG and IgM. This was due to subsequent closure of Maiduguri neighbouring borders, where IgG avidity ELISA kit can be delivered to us if purchased, as a result of security tension.

The study by Ludwig and Hengel showed that the overall CMV seroprevalence in women of childbearing age depends on age, ethnicity, parity, and social status, and is different between countries and regions. In contrast to that report, seroprevalence in our study was not significantly associated with age, parity, gestational age, and educational status of the women studied. Further studies with larger sample size are required to determine the actual association between CMV antibody prevalence and these sociodemographic data.

In this study, 3 (1.6%), 14 (7.7%), and 20 (10.9%) of the pregnant women were non-immune (IgG- and IgM-) during their first, second, and third trimesters, respectively. They are at risk of acquiring primary infection, which increases the chances of intrauterine transmission to fetus [33]. Women infected with CMV during late gestation are most likely to transmit the virus to their unborn child than women infected early in gestation. Failure to detect seroconversions in late gestation may lead to failure to detect infected asymptomatic neonates [33].

It is therefore imperative for pregnant women to be informed of hygienic practices and behavioral measures, such as avoiding direct contact with organic materials and frequent thorough hand-washing, which can reduce the chance of infection [34].

Although, anti-CMV IgM antibodies are good indicator of recent infection, it does not always correlate with primary infection. This is because pregnant women can produce IgM during reactivations or reinfections. Virus-specific IgM may persist for months after natural infection and can be detected in pregnant women, 6-12 months after the end of the acute phase of primary infection [2, 35]. In addition, false-positive results are common and may arise in patients with other viral infections (e.g. parvovirus B19 and Epstein-Barr virus) or autoimmune diseases or due to interference with rheumatoid factor of the IgM class, or laboratory methods used [32]. Usually, all positive CMV IgM should be tested for low avidity IgG antibodies, which can persist for up to 20 weeks after primary infection, and then they are replaced by high avidity IgG antibodies. Therefore, our inability to perform IgG avidity test on the CMV IgM positive women, due to limited resources renders this result inconclusive in terms of the primary CMV infection status. Nevertheless, there is a need to follow-up these cases through neonatal examination.

The low prevalence (0.5%) of recent infection (anti-CMV IgM seropositivity) in this study is in agreement with other studies [35, 36]. However, higher seroprevalences have been documented by other researchers [37, 38]. These discrepancies may be attributed to differences in sociodemographic setting and this can be inferred from the work of Stagno and Whitley, which demonstrated that the risk of primary maternal infection was approximately three times higher among the high-income susceptible women (45%) compared to the low-income groups (15%). This may be viewed from the point that there are likely more seronegative women among those with higher educational background (especially tertiary education), due to better hygiene compared to those with lower social class and educational background, making the former more susceptible to primary infection. However, it would be reasonable to study a larger sample size of this group to obtain a meaningful conclusion on risk factors.

The low prevalence of IgM antibodies observed in this study may also be due to the fact that majority of the women would have recovered from primary infection, with loss of IgM, by the time they reach child bearing age [39]. Although, the prevalence of recent infection among pregnant women is low, they are a critical group because the risk of congenital CMV infection is much higher during recent infection in the mother [40, 41].

Therefore, it would be beneficial to properly inform this category of women on the need for further investigations, such as ultrasonography, magnetic resonance imaging and amniocentesis to detect prenatal infection and planning of proper interventions, such as use of hyperimmune globulin or termination of pregnancy.

In Nigeria and most other countries, routine screening is not widely used for maternal CMV infection, while prenatal CMV screening remains controversial and has not been approved by any professional organization or public health authority worldwide [42, 43]; however, in some European countries and in Israel, prenatal CMV testing is more widely performed, even if there are no recommendations for routine screening [43]. Our study indicates that CMV screening in pregnant women may gain more importance in the future antenatal care, and diagnostic value of readily
Serological Evidence of Recent CMV in pregnancy

ACKNOWLEDGEMENT
We will like to acknowledge Mr. Bamidele Oderinde and Mr. Ajayi Babajide of the departments of Immunology and Medical Laboratory Sciences, University of Maiduguri Teaching Hospital, Nigeria for their technical support.

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest associated with this manuscript.

REFERENCES