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**ORIGINAL ARTICLE**

# Seroprevalence and risk factors for *cytomegalovirus* infection among pregnant women in southern Nigeria

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**ABSTRACT**

**Objective:** Vertically transmitted *cytomegalovirus* (CMV) is the most common cause of preventable congenital infection, including deafness and intellectual impairment worldwide. Till date, there is no consensus on routine antenatal screening worldwide. The objective of this study was to determine the prevalence of previous and primary CMV infection among antenatal women at the University of Benin Teaching Hospital (UBTH) and the effect of age, socioeconomic class, parity and gestational age on prevalence.

**Methods:** In this descriptive cross-sectional study, blood samples were collected from each of 200 consecutive pregnant women attending the antenatal clinic of the university. Samples were analyzed for CMV specific IgG and IgM using ELISA test kits. A semi-structured researcher administered questionnaire was used to obtain socio-demographic information which included; age, socioeconomic class, parity and gestational age.

**Results:** Seroprevalence of CMV- specific IgG and IgM were 92% and 4% respectively, while 4% were seronegative. There was no significant association between seroprevalence and any of the risk factors ($p>0.05$).

**Conclusion:** There is need for voluntary screening of pregnant women as part of antenatal care to detect primary and seronegative susceptible women. *J Microbiol Infect Dis 2013; 3(3): 123-127*

**Key words:** *Cytomegalovirus*, seroprevalence, pregnancy, Nigeria

## Güney Nijerya'da hamile kadınlar arasında cytomegalovirus seroprevalansı ve risk faktörleri

**ÖZET**

**Amaç:** Sağırlık ve zekâ geriliğine de yol açan vertikal *cytomegalovirus* (CMV) enfeksiyonu dünya'da önlenebilir konjenital enfeksiyonlarının en sık sebebidir. Halen dünya'da rutin antenatal tarama konusunda bir fikir birliği yoktur. Bu çalışmanın amacı Benin Üniversitesi Hastanesi'nde hamile kadınlar arasında doğum öncesi dönemde geçirilen primer CMV enfeksiyon prevalansını ve yaş, sosyoekonomik sınıf, doğum sayısı ve doğum yaşının prevalans üzerine olan etkisinin araştırmaktır.

**Yöntemler:** Bu tanımlayıcı kesitsel çalışmada üniversite hastanesinin doğum öncesi kliniğinde takip edilen ardışık 200 hamile kadının her birinden kan örneği alındı. Örnekler özgül CMV IgG ve IgM varlığı açısından ELISA test kitleriyle analiz edildi. Araştırmada ayrıca hamile kadınlara yaş, sosyoekonomik sınıf, doğum sayısı ve doğum yaşı hakkında bilgi elde edilmesi amacıyla yarı-yapılandırılmış bir anket uygulandı.

**Bulgular:** Özgül CMV IgG ve IgM seroprevalansı sırasıyla % 92 ve % 4 olarak bulundu. Kadınların %4'ü seronegatif idi. Seroprevalans ve CMV enfeksiyonu riskleri arasında istatistiksel olarak önemli bir bağlantı bulunamadı ($p >0,05$).

**Sonuç:** Antenatal dönemde primer CMV enfeksiyonu ve seronegatif olup enfeksiyona duyarlı hamilelerin taranması bu bölgede isteğe bağlıdır.

**Anahtar kelimeler:** *Cytomegalovirus*, seroprevalans, hamilelik, Nijerya

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## INTRODUCTION

Human *cytomegalovirus* (CMV) is a member of the family Herpesviridae and belongs to the subfamily betaherpesviridae. CMV has worldwide distribution and infects humans of all ages and socioeconomic group, with no seasonal or epidemic patterns of transmission.[1] It is the most common cause of congenital infection and a common cause of deafness and intellectual impairment worldwide.[2] CMV is acquired early in life and can be transmitted vertically and horizontally. Infection can be classified as congenital if acquired before birth, perinatal at the time of delivery or as postnatal if acquired later in life. Horizontal transmission is more common than vertical transmission and most infections are acquired by direct close contact with individuals who are shedding the virus in body fluids such as saliva or urine in young children.[2] It is also acquired through sexual intercourse, blood transfusion as well as bone marrow and solid organ transplant.[1] Infections are asymptomatic most times, but once a person becomes infected, the virus remains latent with the possibility of reactivation later in life when the body immunity is suppressed. CMV infection in pregnancy is largely asymptomatic, but association with some obstetric complications has been reported.[3]

Maternal infection poses the risk of congenital CMV infection which occurs in 0.5%-22% of all life births.[4] Risk of congenital infection is much higher during primary infection of the mother with transmission rate of 30%-40% compared with 0.15%-2.2% during reactivations and reinfection.[5] Seroprevalence studies among pregnant women worldwide have shown seropositivity rates for previous infection ranging from 50% in highly developed countries to 100% in developing countries.[6,7] There is dearth of data on CMV prevalence among pregnant women in our environment and whereas studies from other regions have implicated age, parity and social class as associated factors.[2,6] the few available studies did not explore this relationship. There is also no available data to justify the need for routine antenatal screening. The objective of this study was to determine the prevalence of previous and primary CMV infection among antenatal women and the effect of age, socioeconomic class, parity and gestational age on prevalence, with a view to determining the desirability or otherwise of antenatal screening.

## METHODS

This was a descriptive cross-sectional study. The study procedure was approved by the Ethics Committee of UBTH. The antenatal women selected for the study were well informed and their consent taken. Blood samples were collected from 200 pregnant women attending antenatal clinic in the Obstetrics and Gynaecology Department of the hospital between November and December 2010.

The samples were analyzed in the medical microbiology laboratory for CMV-specific immunoglobulin (Ig) IgG and IgM by using the ELISA test kits (Clinotech®, Richmond BC, Canada) as follows: The test samples were diluted 1:51 by adding 5µl of the test sample to 250 µl of sample diluents in separate tubes. 100 µl of each diluted sample from the tubes were added to the wells (coated with CMV antigen). Also, 100 µl each of the negative control, positive control, and calibrators (provided in the kit as prediluted) were also added into separate wells. The wells were covered and incubated at room temperature (for IgM assay at 37°C) for 30 minutes after which each well was washed 5 times with diluted wash buffer. 100 µl of Horse Radish Peroxidase (HRP) conjugate solution was added to each well and incubated for 30 minutes at room temperature (for IgM assay at 37°C). The wells were again washed 5 times with diluted wash buffer. 100 µl of TMB substrate solution was then added to each well and incubated for 10 minutes at room temperature, followed by the addition of 100 µl of stop solution to each well. Finally, the Optical Density (OD) of each well was read using the Microplate Reader at 450 nm wavelength. Specimen OD ratio was calculated (Specimen OD/Calibrator OD) and interpreted as Negative when OD ratio was ≤ 0.90, and Positive when OD ratio was ≥0.90.

Risk factors were identified using a structured questionnaire administered by the researchers. Information sought in the questionnaire included; age, occupation, husband's occupation, marital status, gestational age, number of children, history of blood transfusion and HIV-status of respondents. Socioeconomic status was determined using the protocol for Social Classification by Olusanya et al [8] as follows;

### A. Husband's occupation

**Score:** 1. Professionals, top civil servants, politicians and businessman, 2. Middle-level bureaucrats, technicians, skilled artisans and well-to-do traders, 3. Unskilled workers and those in general whose income would be at or below the national minimum wage.

### B. Wife level of education attainment

**Score:** 0. Education up to university level, 1. Secondary or tertiary level below the university (e.g. college of education, school of nursing etc), 2. No school or up to primary level only.

Social class = A+B

### Statistical analysis

All data obtained from questionnaire forms and laboratory analysis of specimens was entered into a Microsoft Windows version 16.5 data-based statistics program, Statistical Package for Social Sciences (SPSS Inc. Chicago, IL). Descriptive statistics which included; frequency tables were used to compute percentages and averages. Cross-tabulations were used to examine the relationship between variables (respondents' demographics and results of specimen analysis). Categorical variables were compared using the Chi-square test, although Fisher's exact test was used when the data was sparse. Tests were two-tailed and a difference of $P<0.05$ was accepted as statistically significant.

## RESULTS

Two hundred pregnant women were investigated for seroprevalence of CMV infection. Table 1 shows their sociodemographic characteristics. The mean age was 29.3 ± (SD) 3.7, with a range of 22 to 45 years. One hundred and thirty two (66%) were between 20-30 years of age, while only 2 (1%) fell above 40 years. Seventy-eight (39%) fell into social class 2 category, 54 (27%) were in social class 3, while social class 5 were 12 (6%).

**Table 1.** Sociodemographic characteristics of pregnant women (n=200)

| Characteristic | Number | % |
|---|---|---|
| Age groups (years) | | |
| 20 – 30 | 132 | 66 |
| 31 – 40 | 66 | 33 |
| 41 – 50 | 2 | 1 |
| Social classes | | |
| Class 1 | 38 | 19 |
| Class2 | 78 | 39 |
| Class 3 | 54 | 27 |
| Class 4 | 18 | 9 |
| Class 5 | 12 | 6 |
| Gestational age (Trimesters) | | |
| 1st Trimester | 24 | 12 |
| 2nd Trimester | 55 | 27.5 |
| 3rd Trimester | 121 | 60.5 |
| Parity | | |
| 0 | 110 | 55 |
| 1 | 48 | 24 |
| 2 | 24 | 12 |
| 3 | 8 | 4 |
| ≥4 | 10 | 5 |

One hundred and twenty one (61%) women were in their third trimester. Fifty-five (27.5%) were in their second trimester, while only 24 (12%) were in first trimester. Also more than half, 110 (55%) of the women were carrying their first pregnancy (primigravida). Forty-eight (24.0) had only one child, while only 10 (5%) had more than 3 children.

Table 2 shows the results of the serologic assays. These were categorized into 4 types of responses. The first category were immune to CMV [IgG (+) plus IgM (-)]. This constituted 92% of the women. The second group was those with primary infection [IgG (+) plus IgM (+)] and this consisted of eight respondents. The third group also had eight women who were seronegative [IgG (-) plus IgM (-)]. The last category of women was those with [IgG (-) plus IgM (+)]. None of them was in this category. There was no significant association between any of the risk factors examined and seroprevalence of CMV (Table 3).

**Table 2.** Seroprevalence of CMV-specific IgG and IgM antibodies among pregnant women (n=200).

| Immune responses | Number | % | Interpretation |
|---|---|---|---|
| 1gG(+) 1gM(-) | 184 | 92 | Previous exposure |
| 1gG(+) 1gM(+) | 8 | 4 | Primary infection |
| 1gG(-) 1gM(-) | 8 | 4 | Susceptible |
| 1gG(-) 1gM(+) | 0 | 0 | Recent primary infection |
| Total | 200 | 100 | |

**Table 3.** Comparison of the CMV-specific IgG by age, gestational age social classes and parity of pregnant women (n=200)

| | CMV-specific IgG | | | |
|---|---|---|---|---|
| Characteristic | Positive | Negative | Total | p-value |
| Age groups (yr), *n* (%) | | | | |
| 20 – 30 | 121 (91.7) | 11 (8.3) | 132 (66.0) | 1.0 |
| 31 – 40 | 61 (92.4) | 5 (7.6) | 66 (33) | |
| 41 – 50 | 2 (100) | 0 (0) | 2 (1) | |
| Social classes, *n* (%) | | | | |
| Class 1 | 34 (89.5) | 4 (10.5) | 38 (10.9) | 0.886 |
| Class2 | 72 (93) | 6 (7.7) | 78 (39) | |
| Class 3 | 51 (94.4) | 3 (5.6) | 54 (27) | |
| Class 4 | 17 (94.4) | 1 (5.6) | 18 (9) | |
| Class 5 | 10 (83.3) | 2 (16.7) | 12 (6) | |
| Gestational age (Trimesters), *n* (%) | | | | |
| 1st Trimester | 23 (95.8) | 1 (4.2) | 24 (12) | 0.636 |
| 2nd Trimester | 51 (92.7) | 4 (7.4) | 55 (27.5) | |
| 3rd Trimester | 110 (90.8) | 11 (9.2) | 121 (60.5) | |
| Parity, *n* (%) | | | | |
| 0 | 103 (93.6) | 7 (6.4) | 110 (55) | 0.508 |
| 1 | 42 (87.5) | 6 (12.5) | 48 (24) | |
| 2 | 21 (87.5) | 3 (12.5) | 24 (12) | |
| 3 | 8 (100) | 0 (0) | 8 (4) | |
| ≥4 | 10 (100) | 0 (0) | 10 (5) | |

## DISCUSSION

The overall seroprevalence of CMV infection among pregnant women in this study was 96%. While 92% of subjects were immune, 4% had primary infection and 4% were vulnerable to infection. The level of maternal immunity observed in this study substantiates previous studies in Nigeria and other developing countries.[9-11] This however differs from those reported for developed countries where seroprevalence rates were lower.[12,13] The differences in the prevalence of maternal CMV infection between the developed and developing countries may reflect the low hygienic standard and cultural practices that propagate disease transmission in developing countries. 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 CMV infection. For instance, of the 200 women interviewed in this study, only six (3%) had an information of CMV infection.

The low prevalence of primary infection in this study is in agreement with several other studies.[14-16] However, a higher seroprevalence, has been documented by other researchers.[17,18] These discrepancies may be attributed to differences in socioeconomic setting and this can be inferred from the work of Stagno and Whitley[19], which demonstrated that the risk of primary maternal infection was about three times higher among the high income susceptible women (45%) than the lower income group (15%). This may be viewed from the point that there are likely more seronegative women among the high social class, on account of better hygiene than the low social class, making the former more susceptible to primary infection. Only eight women had primary infection in this study, it would be reasonable to study a larger sample size of this group to make for a meaningful conclusion on risk factors. The low prevalence of IgM antibodies observed in this study is possibly due to the fact that majority of the women would have recovered from primary infection, with the loss of IgM, by the time they reach child bearing age.[2]

Although the prevalence of primary infection among pregnant women is low, they are a critical group because the risk of congenital CMV infection is much higher during primary infection in the mother.[20,21] It would be beneficial therefore 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 appropriate intervention such as use of hyperimmune globulin or termination of pregnancy as an option. In the past, lack of evidence for treatment efficacy to prevent congenital CMV infection in women with primary infection has made routine maternal screening for primary infection ineffective and uneconomical. However, recently published data have demonstrated that universal screening for maternal primary infection by using IgG avidity testing which can help to distinguish primary CMV infection from reactivation, and treatment with hyperimmune globulin was efficacious and cost-effective.[22] It is known that reactivation is usually associated with a very low rate of vertical transmission.[5,23,24]

Also observed in this study, was that, eight (4%) of the women were susceptible to CMV. This group has a high risk of transmission of the virus to the fetus, if infected during the pregnancy.[25] Routine screening of pregnant women would provide opportunity to identify those susceptible women who can be counseled on appropriate preventive measures, especially in relation to their behavior with children, who are the major source of infection. Despite the benefit universal screening may offer, there is still no consensus in the scientific community concerning the implementation of screening, and it is not recommended by any public health system because of its cost/benefit ratio. Presently, most obstetricians in Israel, Italy, Belgium and France do test all pregnant women for CMV. In Austria, Switzerland, Germany and Japan, it is performed on specific request, whereas in the United Kingdom, the United State and the Netherland, when there are symptoms in the mother.[26] In Nigeria, there is no screening for CMV infection in pregnancy and there is no policy or protocol in place.

There was no association between maternal immunity and social class. While this finding agree with a previous study, it differs from several other reports which demonstrated that CMV infection was higher in the lower socioeconomic class.[27,28] There was also no difference in maternal immunity between age groups. This is in accord with some previous reports in an urban area in Italy and India but differs from other investigations in Nigeria, Singapore and several others,[2,9,17,29] where seroprevalence increased with age. The reasons adduced for the increase in seroprevalence with age, in other studies, is that majority of the women have already been exposed and recovered from primary infection by the time they reach childbearing age.

The use of CMV-specific IgM as an indicator for primary infection in this study had its limitation. A negative IgM result does not necessarily rule out a primary infection with CMV as samples collected

too early in the course of a primary infection may not have detectable levels of IgM. Furthermore, CMV specific IgM may reappear during reactivation of CMV infection. It was not possible to distinguish between primary infection and reactivation in this study. IgG avidity assay if available would be preferable as the presence of low IgG avidity has been shown to be a more unique and reliable serologic indicator of primary CMV infection.[30]

In conclusion, this work has demonstrated that a high proportion (92%) of pregnant women in our environment is exposed to CMV infection. Maternal exposure was not affected by age, social class, gestational age or parity. 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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