

# Seroprevalence of cytomegalovirus antibodies amongst normal pregnant women in Nigeria

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**Objective:** Cytomegalovirus (CMV), a ubiquitous virus belonging to the herpes family, is known to be transmitted frequently to developing fetuses in pregnancy. In an immunocompromised state like pregnancy, primary infection through blood transfusion or reactivation of a latent CMV infection can cause severe illness. The study was carried out to determine the seroprevalence of the immunoglobulin G (IgG) antibody to cytomegalovirus amongst pregnant women in correlation with previous exposure to blood transfusion.

**Methods:** A cross sectional study was carried out amongst 179 HIV negative pregnant women attending the antenatal clinic of Lagos State University Teaching Hospital (LASUTH), Ikeja, Nigeria. Five mL of blood was collected and stored in a plain bottle, centrifuged on the same day and the serum stored at  $-20^{\circ}\text{C}$ . All samples were screened for anti-CMV IgG antibodies using the enzyme linked immunosorbent assay (ELISA). Consenting participants were instructed to fill a semi-structured questionnaire to obtain demographic and other related information. Statistical analysis of the results was done using Pearson's chi squared test for analytical assessment.

**Results:** A total of 97.2% of the pregnant women recruited for this study were anti-CMV IgG positive. Out of the 179 recruited for the study 174 responded to the question on previous history of blood transfusion, 14.9% of the respondents (26 of 174) had a previous history of blood transfusion and all tested positive to the anti-CMV IgG antibody. However, past history of blood transfusion and educational level were found to be insignificant to the risk of acquiring CMV infection.

**Conclusion:** The seroprevalence of the CMV antibody amongst pregnant women in this environment is high in relation to findings in other developing countries. There is the need to assess anti-CMV immunoglobulin M antibodies in pregnant women, which is a determinant of active infection.

**Keywords:** CMV, IgG, pregnant women

## Introduction

Cytomegalovirus belongs to the subfamily of herpes known as  $\beta$  herpes and it is found universally in various geographic locations. CMV infection is also known to be frequently transmitted to a developing fetus. This virus remains the leading cause of congenital viral infection and a significant cause of transfusion-acquired infections in patient populations.<sup>1</sup> Its clinical manifestations include asymptomatic forms, severe fetal damage, and death in rare cases due to spontaneous abortion.

For most healthy people who acquire CMV infection after birth or through blood transfusion, there are few symptoms and no long term sequelae. Therefore, for the vast majority of individuals, CMV infection is innocuous.<sup>2</sup> However, CMV

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infection is important in pregnant women because of their immunocompromised state and risk of infection to the fetus whose immune system is not fully developed. 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 years.<sup>3</sup>

Once a person becomes infected, the virus remains alive but usually dormant within the individual's body for life. Recurrent disease rarely occurs unless the immune system is compromised. It was reported that the risk of fetal damage is greater if the primary infection occurs during the first trimester of pregnancy.<sup>4-6</sup> The prevalence of congenital infection ranges from 0.2% to 2.5% in different populations,<sup>7-13</sup> with groups at greater risk including Black or Asian women, those from a low socioeconomic background, and those that are born prematurely.<sup>10</sup>

The prevalence of congenital infection varies with the prevalence of the infection in the population.<sup>14</sup> The seroprevalence of CMV among women of childbearing age ranges from 35% to 95% in different countries.<sup>5,14-17</sup> It is more widespread in developing countries and in areas of lower socioeconomic conditions.<sup>18</sup>

The rate of seropositivity of anti-CMV immunoglobulin G (IgG) enzyme linked immunosorbent assay (ELISA) antibodies of pregnant women in Turkey was reported to be 98.5%<sup>19</sup> and 84% in Spain.<sup>20</sup> These rates are much higher than the typical European rate but similar to the rate obtained amongst Black pregnant women.

An overall rate of 87% of anti-CMV IgG ELISA antibodies in pregnant women was reported in Singapore,<sup>21</sup> 100% in Thailand,<sup>21</sup> and 93% in Iranian women of childbearing age.<sup>22</sup>

Since CMV is transmitted through blood transfusion, the strict use of blood during pregnancy and labor cannot be overemphasized as some of the pregnant women could be CMV negative.

Several studies and data support the transmission of CMV from seropositive blood donors to susceptible recipients in a variety of settings.<sup>23,24</sup> Topin et al<sup>25</sup> provided the first biochemical evidence for transfusion associated CMV infection. The study reported that monocyte latently infected with CMV represent the primary vector for Transfusion Associated-CMV (TA-CMV) which can be largely abrogated by transfusing at risk patients with either seronegative units or blood filtered to remove white blood cells. Lamberson et al<sup>26</sup> also determined that a decreased incidence of transfusion associated with CMV (TA-CMV) infection occurred when only blood products that tested negative for CMV immunoglobulin M (IgM) were used.

Two hypotheses have been investigated as possible mechanisms for CMV transmission by blood. First, since CMV resides in a latent state in the infected host, it has been proposed that all CMV-seropositive blood donors can transmit the latent virus,<sup>27,28</sup> following transfusion. In this case latent virus is reactivated and may cause active CMV infection in the recipient. Latency following a primary infection may be punctuated by periodic reactivation that gives rise to recurrent infections, and in utero transmission may occur during either primary or recurrent infections.<sup>29</sup> Yeager and colleagues published an excellent study of TA-CMV infections in newborns to prove this fact.<sup>30</sup> CMV-specific antibody of the IgM class has been recognized as a marker of active or recent primary infection with the virus. Recent reports have shown a positive correlation between post transfusion CMV infection and the receipt of blood from CMV IgM-positive donors.<sup>31</sup>

Such units of blood may be used for CMV negative pregnant women thus increasing the incidence of CMV in the population with its consequent embryopathy such as sensorineural hearing loss, chorioretinitis, mental retardation and fetal death. CMV embryopathy should be a major concern for public health irrespective of the percentage of babies affected. Therefore, the need to determine the seroprevalence of the CMV antibody in pregnant women cannot be overemphasized. This will underscore the significance of CMV screening in blood units for use in pregnancy and labor. This is necessary to avoid the transmission of CMV infected blood to women who are CMV seronegative.

## Subjects and method

### Study population

A cross sectional study was carried out using the antenatal clinic of Lagos State University Teaching Hospital (LASUTH), Ikeja, Nigeria. The clinic registers about 150 patients daily and all consenting newly registered pregnant women were recruited for the study between July 1 and August 15, 2008. However, approval was obtained from the institution's research and ethics committee (LASUTH Health Research and Ethics Committee). Participants were instructed to fill out a structured questionnaire including demographic information and previous exposure to blood transfusion. Immunocompromised participants with a history of chronic illness, such as hepatitis, sickle cell disease, renal disorders, gestational diabetes and HIV infection were excluded.

## Collection of samples

A blood sample of 5 mL was collected into a sterile, nonanticoagulated bottle from each consenting participant. The sample was centrifuged and the serum separated into a sterile bottle and stored at  $-20^{\circ}\text{C}$ .

## Cytomegalovirus serology

Sera were tested for IgG CMV using the ELISA test. The CMV-specific IgG antibodies were studied by the commercial Dia.Pro Diagnostic Bioprobe CMV IgG (Milan, Italy) according to the manufacturer's instructions. All specimens were analyzed using the enzyme immunoassay test. The cutoff of IgG was set at 0.5 World Health Organization (WHO) IU/m (calibrator 2) by the kit's manufacturer.

Samples with a concentration  $\geq 0.5$  WHO IU/mL were considered positive for CMV IgG, while samples with a concentration below the cut off were considered as negative results. The controls and the calibrators passed the validation check recommended by the manufacturer of the kit.

## Statistical analysis

Statistical analysis of results was done using the statistical package for social science (SPSS 16.0, IBM, USA). The Pearson chi squared test and multiple comparisons involving post-hoc analysis such as the Bonferonni correction were used for the analytic assessment and the differences were considered to be statistically significant when the  $P$ -value obtained was  $< 0.05$ .

## Results

A total of 179 pregnant women were enrolled into the study. About 50.8% of them were between the ages of 25–30 years and 0.60% were under 18 years (Table 1). Almost all of them (97.20%) had anti-CMV IgG antibodies. The majority of those (89 of 179) who had anti-CMV IgG antibodies were between parity 1 and 4 (Table 2). It was observed that most (70.40%) of the patients had tertiary education, followed

**Table 1** Age in years with CMV serostatus

Age in years	anti-CMV IgG +ve	anti-CMV IgG –ve
<18	2	0
19–24	18	1
25–30	89	2
31–35	45	1
36–40	17	0
41 and above	3	1

**Note:**  $P = 0.14$ .

**Abbreviations:** CMV, cytomegalovirus; IgG, immunoglobulin G.

**Table 2** Parity with CMV seropositivity

Parity	anti-CMV IgG +ve	anti-CMV IgG –ve
0	60	3
1–4	89	2
5 and above	25	0

**Note:**  $P = 0.42$ .

**Abbreviations:** CMV, cytomegalovirus; IgG, immunoglobulin G.

by 24.60% with secondary education. Less than 2% had no formal education (Table 3). However, levels of education did not correlate with CMV status ( $P = 0.14$ ).

The majority (86.03%) of the subjects were married and most of them had anti-CMV IgG antibodies, 11.17% were single and 2.7% were separated. ( $P = 0.11$ ) (Table 4).

One hundred and seventy four of the participants responded to the question on past history of transfusion while 14.94% of the 174 respondents (26 of 174) had a previous history of blood transfusion and all tested positive to the anti-CMV IgG antibody. Equally, 148 of the 174 (85.05%) subjects had no past history of blood transfusion and 143 of the 148 (96.62%) tested positive to anti-CMV IgG antibodies, but only 5 of 148 (3.37%) tested negative to the IgG CMV  $P = 0.77$  (Table 5). Hence an association cannot be established between transfusion history and anti-CMV IgG positivity.

Multiple comparisons involving post hoc analysis such as the Bonferonni correction were done on level of education/CMV serostatus, marital status/CMV serostatus, parity/CMV serostatus, and age/CMV serostatus. The tests of homogeneity of variances were 0.73, 0.11, 0.78, and 0.74 respectively which were not significant, hence the use of Bonferonni rather than the Tamhane's T2 post-hoc test. None of the mean differences between the groups compared by the test was significant at 0.05 levels. Post-hoc analysis could not be performed on previous history of blood transfusion and anti-CMV seropositivity because there were fewer than three groups.

## Discussion

The 97.20% seropositivity rate of IgG anti-CMV antibodies observed in this study amongst pregnant women is in keep-

**Table 3** Education with CMV seropositivity

Education	anti-CMV IgG +ve	anti-CMV IgG –ve
None	3	0
Primary	5	1
Secondary	42	2
Tertiary	124	2

**Note:**  $P = 0.14$ .

**Abbreviations:** CMV, cytomegalovirus; IgG, immunoglobulin G.

**Table 4** Marital status with CMV seropositivity

Marital status	anti-CMV IgG +ve	anti-CMV IgG -ve
Single	18	2
Married	151	3
Separated	5	0

Note:  $P = 0.11$ .

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G.

ing with several studies. As early as 1973, Krech reported that CMV antibodies are more prevalent in developing countries and areas of lower socioeconomic conditions in comparison to developed countries.<sup>18</sup> Tookey et al,<sup>32</sup> in a prospective study amongst pregnant women in London, found a seroprevalence as high as 88.2% in the first pregnancy of women. Their study was, however, ethnically diverse, with the highest prevalence recorded in Asian women at 88.2%, 77.2% in Black women, and the lowest in White women at 45.9%.

The lowest percentage of 45.9% derived amongst the White pregnant women population reported by Tookey et al, is similar to the overall rate of anti-CMV IgG ELISA of 55.1% reported amongst French women,<sup>33</sup> with 68.3%,<sup>34</sup> 30.4%,<sup>35</sup> 39%–94%<sup>36</sup> and 56.8%<sup>37</sup> in Italy, Ireland, the USA, and Australia respectively.

It is noteworthy in this study that age, parity, marital status, and educational level were not associated with the risk of CMV positivity, because they did not reach significant levels.

Blood transfusion-associated CMV infection has been proven extensively.<sup>9–24</sup> Akinbami (2009) et al<sup>38</sup> reported a 96% IgG anti-CMV and 19.5% IgM anti-CMV antibodies seroprevalence amongst healthy blood donors in Lagos, Nigeria. This is quite significant because one out of every four units of blood donated is acutely infected by CMV because IgM anti-CMV antibody is a marker of active or recent primary infection with the virus. A pregnancy-related hemorrhage is still a major cause of peripartur morbidity and mortality in developing countries and blood transfusion rates may be high in these countries. In a Nigerian audit of 463 consecutive cesarean sections and blood transfusions within a period of 3 years,<sup>39</sup> 25.2% cesarean section cases were transfused.

**Table 5** Relationship between blood transfusion and anti-CMV IgG seropositivity

Past transfusion history	anti-CMV IgG +ve	anti-CMV IgG -ve
Yes	26	0
No	143	5

Note:  $P = 0.77$ .

Abbreviations: CMV, cytomegalovirus; IgG, immunoglobulin G.

A total of 67.2% of caesarean sections of these cases were emergency.<sup>39</sup> The rate of blood transfusion for the various indications were as follows: malpresentation (excluding breech) 66.7%; placenta previa 59.1%; uterine rupture 55.6%; breech delivery 32%; obstructed labor 28.2%; previous baby 25%; previous cesarean section 17.0%; severe preeclampsia 11.1%; fetal distress 10.7%; and others, 10.3%. Akinola et al<sup>40</sup> also reported in a similar study a transfusion rate of 13.6% amongst cesarean section patients in southwestern Nigeria.

Despite the reported rate of lower seroprevalence of anti-CMV IgG ELISA antibodies amongst higher socioeconomic classes and in developed nations,<sup>18</sup> lower rates of blood transfusion of 4.9% and 5.4% were reported by Dathie et al<sup>41</sup> and Rouse et al<sup>42</sup> respectively. In Canada<sup>43</sup> the overall rate of blood transfusion for postpartum hemorrhage was 0.31%, and 0.49%, 0.28%, 0.23% for emergency caesarean section, vaginal delivery, and elective caesarean section respectively.

In developing countries, the higher rate of anti-CMV IgG seroprevalence associated with higher requirements for blood in pregnant women poses a grave risk of CMV embryopathy. There is the need to assess anti-CMV IgM antibodies in pregnant women in these countries which is a marker of acute infection and more directly related to CMV embryopathy.

Previous history of blood transfusion and socioeconomic status were, however, found in this study not to be significant factors to CMV antibody positivity. This might be related to the high seroprevalence of anti-CMV IgG in the Nigerian population. Future studies in populations with similarly high seroprevalence may need higher recruitment numbers.

## Conclusion

The seroprevalence of anti-CMV IgG is very high among pregnant women in Nigeria. Based on previous studies that showed a decrease in the incidence of CMV disease when blood was screened for CMV (IgM), the incidence of the disease can be decreased in Lagos if blood is screened for CMV. Previous blood transfusion exposure was insignificant in the risk of transmission.

## Limitations of this study

1. Reliability of information on blood transfusion provided by patients and the possible reasons for finding small numbers of those who have been previously transfused.



- The use of IgG ELISA assay rather than IgG and IgM in determining seroprevalence of CMV in pregnant women.

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## Disclosure

The authors report no conflicts of interest in this work.

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