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The Prevalence of Cytomegalovirus among Eligible Blood Donors in Keffi, Nigeria

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A B S T R A C T

Cytomegalovirus also called human herpesvirus type 5 (HHV 5) is known to be a significant cause of morbidity and mortality following blood transfusion. In immunocompetent individuals primary CMV infection is usually asymptomatic, the immunosuppressed population for which CMV seronegative blood products are required is increasing due to advances in medical care especially to premature infants, AIDS patients, increasing use of transplantation procedures and immunosuppressed cancer therapies. The aim of this study was to determine the prevalence of cytomegalovirus infection among eligible blood donors in Keffi, Nigeria. Blood samples were collected from 208 consenting participants in the Heamatology Unit of Federal Medical Centre, Keffi. The sera were evaluated for CMV IgG antibody using an ELISA kit (Cortez Diagnostic, Inc. USA). The overall prevalence for CMV IgG was 74%. The prevalence of CMV IgG antibody based on sex was 73.9% in males and 74.2% in the females. There was no statistically significant association between gender and CMV IgG prevalence ($p > 0.05$). Seroprevalence was found to increase with age from 52.5% among those aged < 20 years to 81.8% among those aged 30-39 years. Neither occupation nor history of blood transfusion was significantly associated with CMV IgG prevalence among the participants. Of those that have had a blood transfusion 70.6% were positive to the virus while among those that have never had a transfusion the prevalence of the virus was 74.3%. This study reported a high CMV infection prevalence in this study area. This therefore underscores the need to include CMV screening before blood transfusion and the importance of using strategies such as leukoreduction before any transfusion in this area. Also the medical records of seronegative eligible donors should be kept for emergency contact when patients who are at high risk of developing severe CMV infection are in dire need of transfusion. Proactive strategies of educating people on prevention and control measures should be put in place.

Introduction

Cytomegalovirus (CMV), a member of the human herpes family of viruses, transmissible through blood transfusions, is an important cause of concern worldwide (Shaiegan *et al.*, 2015). It is a ubiquitous organism found universally in all geographic

locations. However, CMV is more common in developing countries and in people belonging to lower socio-economic status (Ojide *et al.,* 2012; Arun *et al.,* 2012). The seroprevalence of CMV is 80 -100% in Africa and Latin America (Souza *et al.,* 2010). Like most other herpes viruses, CMV remains latent in the host after primary infection and persists for life in the organism. Latent CMV is associated with white blood cells which aids its transmissionby transfusion of cellular blood components(Matos *et al.,* 2010). Nevertheless, these viruses can be reactivated in immunosuppressed individuals and can be an important cause of morbidity and mortality. CMV can be transmitted by blood transfusion, transplacental route or by transplantation of hematopoietic stem cells and solid organs from infected donors (Zeimann and Hennig, 2014). Most studies suggest that 13-38% of immunocompromised patients will contract CMV from transfusion of unscreened and unfiltered cellular blood components (Souza *et al.,* 2010; Arun *et al.,* 2012; Oladipo *et al.,* 2014). Therefore, the most effective way to minimize the risk of CMV transmission in high risk recipients would be to administer CMV free blood products. The immunosuppressed population for whom CMV free blood products are requested is increasing due to advances in medical care (Lopo *et al.,* 2011).

The diagnosis of CMV infection can be relied on different techniques including: electron microscopic detection of typical CMV virion, histologic or cytologic detection of typical CMV cytopathology, isolation of virus, detection of CMV antigen in blood and tissues, detection of CMV genome in tissues, DNA amplification, and serology techniques based on CMV antibodies detection. The diagnosis of CMV infection in immunocompromised patients can be difficult as it requires virus detection and determination of CMV as the cause of the disease (Shaiegan *et al.,* 2015). Several other tests are available including: DNA probe techniques, polymerase chain reaction (PCR), and immunofluorescence technique for detection of CMV early antigen in circulating leucocytes (Furui *et al.,* 2013). One of the most common available serologic tests to detect CMV IgG and CMV IgM antibodies is based on enzyme-linked immunosorbent assay (ELISA). IgG positive result is indicative of a person infected by CMV during his or her life (Furui *et al.,* 2013). CMV IgM presence could be interpreted as new infection, acute infection or re-activation of CMV. It has been reported that CMV infection rate increases with blood donor age. Transfusion transmissible infections (TTIs) are a very serious complication of blood transfusion. These infections continue to pose a great challenge to transfusion medicine, especially in Africa, due to a high transfusion demand (Zeimann and Hennig, 2014). This study was therefore carried out to determine the seroprevalence of Cytomegalovirus among eligible blood donors in Keffi, Nigeria.

Materials and Methods

Study Area and Population

The area of study for this research was Keffi. It is approximately 68Km from Abuja, the Federal Capital Territory and 128Km from Lafia, the capital of Nasarawa State. Keffi is located between latitude 8 5'N of the equator and longitude 7 8'E and situated on an altitude of 850m above sea level (Akwa *et al.,* 2007).

The study population was made up of 208 consented eligible blood donors randomly selected from both sexes. Socio-demographic information of the participants

was obtained through oral interview. Such information includes; age, sex, occupation, history of blood transfusion.

Eligible Blood Donors

Blood was collected only from those that fulfilled the criteria of being eligible donors. For the purpose of this study, such a participant must have been;

≥ 50kg in weight
Physically healthy
Aged ≥ 18 years

Sample Collection

After obtaining a verbal informed consent from each participant, 5ml blood sample was collected by vein puncture into a sterile vacuum plain bottle, allowed to clot for 30 minutes and centrifuged at 3000rpm for 5 minutes. Each resultant serum was harvested into a sterile serum bottle, labeled and stored at -200C until ready for ELISA test.

Test Procedure

The sera were analysed for CMV specific IgG using ELISA kits (Cortez Diagnostic, Inc. USA) according to the manufacturer's instruction.

The CMV antigen coated strips was placed into the holder. A 1: 40 dilution was prepared for each test sample; negative control, positive control and calibrators by adding 5µl of each of these to 200µl of sample diluents. One hundred µl of diluted calibrator, controls (positive and negative) and serum was dispensed into appropriate wells. For the reagent blank, 100µl sample diluentswas dispensed in 1A well position of the micro titer plate. The holder was tapped to remove air bubbles from the liquid contact of the wells and mixed by slight agitation. The plate was incubated for 30 minutes at room temperature. The liquid was then removed from all the wells and the plate washed three times with washing buffer. One hundred µl f enzyme conjugate was dispensed into each well and further incubated for 30 minutes at room temperature. The enzyme conjugate was removed from wells, the plate was washed three times with washing buffer and 100µl TMB chromogenic substrate was dispensed to each well and the plate incubated for 30 minutes at room temperature. After the incubation period, 100µl of stop solution was added to stop further reaction. Precaution was taken to ensure that there was no air bubble in each well before taking the readings. Optical density (O.D) was read at 450nm with a microwell ELISA reader. (BIO-RAD PR2100). Results were interpreted according to the manufacturers' recommendation

Ethical Approval

Approval for this study was obtained from the Ethical Review Commmittee on Human Research, Federal Medical Centre, Keffi, Nigeria.

Statistical Analysis

The data obtained were subjected to descriptive statistical analysis using SPSS version 17.0. C hi-square was used to determine associations and values obtained were considered statistically significant at $p \leq 0.05$.

Results and Discussion

Two hundred and eight voluntary blood donors were recruited into this study. Of these, males were 55.3% while females were 44.7%. CMV IgGseropositivity among male donors was 73.9% and 74.2% among female

donors. There was no statistically significant difference in the prevalence of the viral infection between the sexes.

Similarly, there was no statistically significant difference in the viral infection prevalence in the different age groups. It ranged from 52.2% among those aged less than 20 years to 100% among those aged above 50 years.

There was also no correlation observed between the viral prevalence of infection and either history of blood transfusion or occupation. The 74.0% CMV-IgG prevalence among eligible blood donors in this study is relatively high. This suggests that many people in the study area have been exposed to the virus, thus the virus can be said to be endemic in Keffi, Nigeria. It also means that there are just about 26% of eligible blood donors that can comfortably donate blood (with respect to safety of CMV infection) to at risk patients in this environment. The seropositive rate reported in this study is lower than prevalence rates observed in previous studies carried out in other parts of Nigeria. It was 92% in Jos (Alao *et al.,* 2008), 95.8% in Benin (Ojide *et al.,* 2012), and 96% in Lagos (Akinbami *et al.,* 2009) prevalence.

Lower rates were observed in Ogbomoso 25.8% (Oladipo *et al.,* 2014). Several studies on the prevalence of CMV IgG antibody in different parts of the world have also shown different rates. For example, Ghana 93.2% (Adjei *et al.,* 2008), Iran 94.82% and 92% (Arun *et al.,* 2012; Shaiegan *et al.,* 2015), Turkey 97.2% (Mutlu *et al.,* 2008) and Brazil 96.4% (Souza *et al.,* 2010) These differences might be as a result of different screening methods, environmental and climatic factors and socioeconomic status of the study populations. The prevalence of CMV-IgG antibody in this study was shown to vary with age. It ranged from 52.2% among those aged <20 years to 100% among those aged ≥ 50 years. This result is in consonance with the reports from Benin (Ojide *et al.,* 2012) which recorded 100% seropositivity among those aged ≥ 50 years but in contrast to reports from Ogbomoso (Oladipo *et al.,* 2014) and from Chennai (Arun *et al.,* 2012) which recorded a higher prevalence in lower age groups. The 100% prevalence recorded in this group might be as a result of the paucity of samples screened in that age bracket. In the study area, it is very uncommon to find people of this age group donating blood so it was not easy to get many samples from this age group. There was no statistical association between the seropositivity of CMV-IgG antibody and the age group of participants ($p > 0.05$) but infection was more common among the youth aged 20 – 39 years. A similar observation was reported from Lahore, Pakistan (Rizvi *et al.,* 2015). In their contribution, Hecker *et al.,* (2012) had posited that seroconversion is a lifelong event and is associated with age.

A history of blood transfusion was also not found to be associated with seropositivity for the viral infection among eligible blood donors ($p > 0.05$) in the study. This may imply that previous blood transfusion is not the only risk factor for CMV infection. This outcome is in consonance with the outcome of other researchers (Akinbami *et al.,* 2009; Oladipo *et al.,* 2014). The possible explanation is that there are various routes through which CMV is transmitted including intrauterine, breastfeeding, sexual contact and spread from children (Shaiegan *et al.,* 2015).

In this study there was no statistically significant association between the viral IgG antibody prevalence and occupation ($p > 0.05$), implying that occupation has no relationship with CMV infection. This report

is in agreement with the work of Oladipo *et al.,* (2014) which also recorded that occupation is not related with the viral infection.

Table.1 Seroprevalence of Cytomegalovirus with respect to Risk Factors among Eligible Blood Donors in Keffi, Nigeria

Risk Factors	No. Tested	No. Positive (%)	p value
Gender			
Male	115	85 (73.9)	> 0.05
Female	93	69 (74.2)	
Age (years)			
<20	23	12 (52.2)	
20-29	141	107 (75.9)	
30-39	33	27 (81.8)	> 0.05
40-49	8	5 962.5)	
≥ 50	3	3 (100)	
History of Blood Transfusion			
Yes	17	12 (70.6)	> 0.05
No	191	142 (74.3)	
Occupation			
Civil servants	30	23 (76.7)	
Students	154	114 (74.0)	> 0.05
Artisans	24	17 (70.8)	

There was no association between gender and CMV infection in this study (p> 0.05). The seroprevalence was higher in females (74.2%) than their male (73.9%) counterparts. This is similar with the report of Oladipo *et al.,* (2014) who recorded higher prevalence in females. However it is in contrast with the work of Arun *et al.,* (2012) where the seroprevalence rate was higher in males (76.03%) than females (23.97%). It has been reported that infected infants and children especially those under 30 months old actively excrete the virus in their saliva and urine and so serve as the common source of infection for adults (Matos *et al.,* 2010). In the study area, children are known to be carried by both male and female adults. So this might have been the reason for the similarity in CMV infection prevalence among males and females. Some researchers have also reported that personal close contact rather than poor hygiene was more important for the transmission of the virus (Fowotade and Nwadike, 2012; Sukruili *et al.,* 2012; Furui *et al.,* 2013; Shaiegan *et al.,* 2015).

In conclusion, a high seroprevalence of CMV IgG antibody (74%) was reported in this study among eligible blood donors although none of the studied risk factor was significantly associated with the viral infection prevalence. Despite the high prevalence, the routine screening of blood donors for CMV infection in such a resource limited environment might not be feasible. However, the 26% seronegative donors could be a source of the needed blood for immunosuppressed patients and this can be achieved by keeping an inventory of CMV seronegative blood in the blood bank. Alternatively, leukoreduction filtration could be adapted to minimize transmission.

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