Arch. Bas. App. Med. 8 (2020): 63 – 67
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Research Article

Prevalence of Cytomegalovirus among Children Aged 0-6 Months in Ibadan, Nigeria

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Received: March, 2020; Accepted: June, 2020

Abstract
Cytomegalovirus infection is the most common cause of congenital infections in humans and it produces considerable morbidity in newborns. There is the paucity of information on the detection of CMV DNA which is an indication of active infection among infants and their mothers in Nigeria. This study was designed to determine the prevalence of CMV DNA among infants and their mothers in Ibadan, Nigeria. Clinical, demographic information, and blood samples were collected from 80 consenting mothers attending three selected health facilities in Ibadan, Nigeria. Saliva was also obtained from their 82 babies once within the first six months of life. The blood and saliva samples were tested for the presence of CMV DNA using PCR while blood samples from mothers whose babies tested positive for CMV DNA were analyzed for CMV specific IgM antibodies by ELISA technique. Eleven (13.4%) of the 82 infants tested positive for CMV DNA most of whom were aged 4-6 months, while CMV DNA was not detected in any of the maternal samples. None of the mothers of the CMV DNA positive infants was positive for CMV IgM antibodies. Also, none of the babies had any visible sign of congenital abnormalities. This study shows a high prevalence of cytomegalovirus infection among children aged 0 – 6 months in Ibadan, Nigeria.

Key Words: Cytomegalovirus, DNA, infants, Ibadan, Nigeria

INTRODUCTION

Cytomegalovirus (CMV), otherwise known as Human herpesvirus type 5 is a double-stranded DNA enveloped virus approximately 230 nm in diameter and a member of the Herpesviridae family (Drew LW, 2004). The virus shares certain similar characteristics with other herpesviruses, including genome, virion structure, and the ability to cause latent and persistent infections (Tandon and Mocarski, 2012). CMV has the largest genome among the herpesviruses. Based on the time of synthesis of viral progeny after infection, viral replication may be categorized into immediate-early, delayed early and late gene expression (Ross et al., 2011).

Cytomegalovirus infection is the most common cause of congenital infections in humans and it produces considerable morbidity in the newborn (Ross et al., 2011). About 10% of CMV infected infants who acquired infection from their mothers during pregnancy can be symptomatic at birth (CDC, 2018). According to the CDC (2010), the majority of adults would have been infected with CMV by the age of 40 years while 30-50% of women of childbearing age remain susceptible to CMV infection. The incidence of primary CMV infection in pregnant women varies from 1% to 4% in the United States and 2.5-12% in Africa (Hamdan *et al.,* 2011; Maingi and Nyamache, 2014; CDC, 2018).

CMV is highly endemic in developing countries and in particular in sub-Saharan Africa with a seropositivity rate that often approaches 100% (Chakraborty et al., 2003; Schlesinger et al., 2005; van der Sande et al., 2007; Miles et al., 2008). The presence of HIV has also contributed to increased circulation and prevalence of CMV especially in sub-Saharan Africa (Pennap and Ahmed 2016 ; Gumbo et al., 2014). CMV can cross the placenta and cause both fetal and placental infections, a serious risk factor for congenital CMV infection in the unborn child (Bakare et al., 2009). Infection in infants could also be acquired during vaginal delivery, through breast milk and contact with other children as well as through blood transfusion, but later in life, infection is acquired through sexual contact. Infected infants and young children usually shed the virus especially in their saliva and this aids transmission of the virus to their mothers and daycare staff and other day care attendees (Tandon and Mocarski, 2012).

The prevalence of congenital abnormalities continues to rise worldwide especially in developing countries (Bates et al. 2014). However, the causes of some of the birth defects are not known due to inadequate or missed diagnosis among other reasons (Bakare et al., 2009). Congenital CMV infection may be one of the causes of congenital abnormalities in children in Nigeria. Previous studies in Nigeria have provided data on the seroprevalence of CMV especially among women (Hamid

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et al., 2014; Yeroh, Aminu and Musa, 2014). However, there is the paucity of data on the burden of CMV DNA which is an indication of active infection among infants and their mothers in Nigeria. It is important to know the burden of CMV DNA as this denotes active replication and circulation of the virus in positive individuals. Persons with CMV DNA especially in their saliva are also most infectious and serve as a major source of transmission of the virus. Most infants with congenital CMV infection do not manifest clinical abnormalities at birth; rather, the infection is asymptomatic. However, sensorineural hearing loss eventually develops in approximately 10 to 15% of CMV-positive children. High titers of CMV are shed in the saliva of infected newborns. Also, because of the ease of collection, saliva specimens appear to be a better and less invasive type of sample for CMV testing among newborns than blood or urine. This study was undertaken to determine the prevalence of CMV DNA in infants aged six months and below and their mothers.

MATERIALS AND METHODS

Study design: This is a cross-sectional descriptive study involving infants within the first six months of life and their mothers. After ethical approval from UI/UCH ethical review board, consenting participants were enrolled at the pediatric vaccination unit and pediatrics wards within the health facilities of Naomi Medical center in Ibadan Northwest LGA (urban), St Mary's Catholic Hospital, Eleta in Ibadan south-east LGA (urban) and Ido LGA primary health center, Omi-Adio, Ibadan (rural) in Oyo State, Nigeria. A total of 80 mothers and 82 babies' (37 were males and 45 females) were enrolled for the study. The sample size was determined using the formular (Z^2pq/d^2) and a prevalence of 3% reported by Olusanya *et al*., (2016), where P=prevalence, q=1-p and d=0.05 at 95% confidence interval.

Specimen Collection: Informed consent was obtained from each participating mother and mother's assent for the children. Saliva swab samples were collected from babies by placing a sterile swab stick underneath the baby's tongue and holding the swab in the baby's mouth for two minutes. The saliva samples were collected at least 20 minutes after the last breastfeeding of the baby. The cotton head or the swab was placed into cryovial containing virus transport medium (2% MEM containing antibiotics and antifungal treatment) and then transported to the laboratory in a cold box to maintain the cold chain. In the laboratory, the swab was vortexed to further elute the saliva into transport medium and stored at -20°C until analyzed.

A blood specimen was collected from each participating mother by finger prick and blotted on a filter paper card. The dry blood spot (DBS) cards were allowed to air dry and stored at room temperature in a sealed bag containing desiccants until tested for the presence of CMV DNA and IgM antibodies.

DNA extraction from saliva swab samples: DNA was extracted and purified from the saliva swab by adapting the technique previously described by Wang *et al*., [2011] using lysis buffer containing 24 grams of guanidine thiocyanate 20 mL of Tris-HCl, (pH 6.4), 4.4 mL of 0.2 M EDTA (pH 8.0) and 0.5mL Triton X-100. Three hundred microliter of lysis buffer was pipetted into a 1.5mL microcentrifuge tube containing 200µl of transport medium and saliva swab sample and properly mixed by pipetting up and down. The resulting mixture was incubated at room temperature for 20 minutes after which 400µl of isopropanol was added. After thorough mixing, the tube was centrifuged at 14,000rpm for 15 minutes at room temperature. The supernatant was then decanted and 1mL of 70% ethanol added. The tube was then covered and vortexed for 3-5sec, centrifuged at 14,000g at room temperature after which the supernatant was decanted, pellets were allowed to dry properly and 50ul of nuclease-free water added to each tube, which was then vortexed vigorously to re-suspend the DNA pellets.

Extraction of DNA from dried blood spot (DBS): The protocol used for DNA extraction from DBS was adopted from Barbie *et al.,* (2000). Briefly, blood was eluted from cards by soaking three disks punched from the card in 25µl cell culture medium (2% MEM) in a 1.5mL microcentrifuge tube. Tubes containing specimen were placed on the heating block at 55°C for 60 minutes and then transferred to another heating block at 100°C for 7minutes. After which the tubes were rapidly cooled and centrifuged at 10,000rpm for 3minutes. The resulting supernatant containing the DNA was collected and kept frozen at -80°C for at least 1hour.

Polymerase Chain Reaction (PCR) for Cytomegalovirus glycoprotein B gene: The genomic DNA extracted was tested for the presence of CMV DNA by PCR using primers previously published (Lashini *et al*., 2011) targeting the glycoprotein B gene of the virus. The PCR was performed in a total volume of 25ul that contains 13.25µl of nuclease-free water, 2.5µl of PCR buffer, 2.5µl of 50mM Magnesium Chloride ($MgCL_2$), 0.5µl of10mM dNTP mix, 0.5µl of 10ρM of each of both the forward and reverse primer (Table 1), 0.25µl of Taq polymerase and 5ul of template DNA. Positive and negative control samples were included in each PCR run. Amplification was carried out using conventional PCR with the following cycling condition: DNA denaturation at 94°C for 3minutes, followed by 30 cycles of (30 seconds of denaturation at 94°C, annealing at 55°C and elongation at 72°C) and then 72°C for 5 minutes for additional elongation in the last cycle (Lashini *et al*., (2011). The amplified product with the expected band size of 275bp was detected using 2% agarose gel electrophoresis (Fig 1). Previously characterized CMV DNA was used as the positive control.

Cytomegalovirus IgM ELISA

Blood samples from mothers whose children were positive for CMV DNA were tested for the presence of CMV specific IgM using a commercially available ELISA kit (WANTIA CMV IgM ELISA China) according to the manufacturer's instruction.

RESULTS

A total of 80 mother and 82 children were recruited for this study. The children were aged 0-6 (Mean age= 2.9 months) while the mothers were aged 22-41 years (mean age= 26.7 years). Eleven of the 82 saliva samples from the infants tested were positive for CMV DNA, giving a prevalence of 13.4%. Figure 1 shows the image of expected DNA band size after agarose gel electrophoresis. Although the rate of infection was slightly higher among males (16.2%) than the female (11.1%) children, the difference was not significant (Table 2).

Table 1:
Nucleotide sequence of primers used for CMV glycoprotein B gene amplification

	Nucleotide sequence	Genome annealing site
P1 (Forward)	5'-CGGTGGAGATACTGCTGAGGTC-3'	82494-82515
P2 (Reverse)	5'-CAAGGTGCTGCGTGATATGAAC-3'	82729-82750

Source: Lashini *et. al*., (2011).

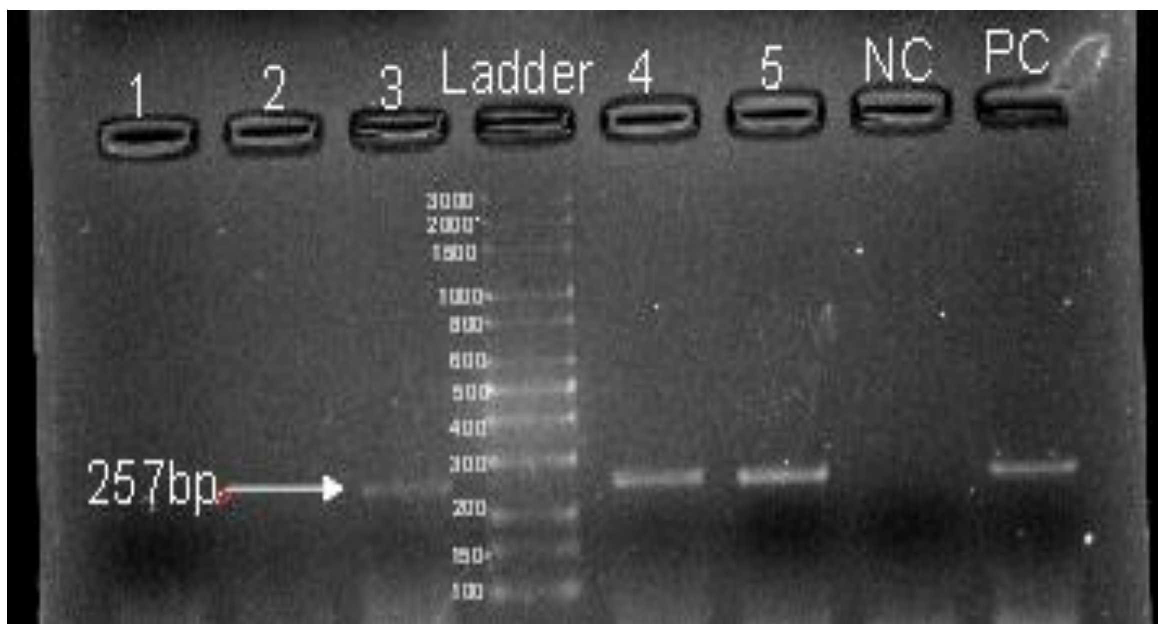

Figure 1
Gel picture showing amplified CMV DNA (glycoprotein B): Lane 3-5contained positive samples, while lane 1 and 2 contain negative samples. Lane NC and PC contained negative and positive control respectively.

Table 2:
Gender distribution of CMV infection among infants in Ibadan

Gender	No. Positive	No. Negative	No. Tested
Male	6	31	37
Female	5	40	45
Total	11	71	82

Table 3:
Distribution of CMV DNA across the different age groups of infants in Ibadan

Age group	Number tested	Number positive (%)	Number negative
0-1 months	29	2 (6.9%)	27
2-3 months	44	2 (4.5%)	42
4-6 months	9	7 (77.8%) *	2
Total	82	11(13.4%)	71

Test of Significance using Pearson Chi-Square <0.00001

The prevalence of CMV DNA increased significantly with the age of the children (Table 3). CMV DNA was not detected in any of the maternal samples. Also, none of the eleven mothers whose babies had detectable CMV DNA were positive for CMV specific IgM antibodies.

DISCUSSION

The aim of this study was to determine the prevalence of active CMV infection among infants aged zero to six months and their mothers in Ibadan, Nigeria by testing CMV DNA in the saliva of the infants and blood of the mothers. We observed a prevalence of 13.4% among the infants while none of the mother tested positive both by DNA PCR and CMV IgM antibodies.

The 13.4% prevalence of CMV DNA found among infants in this study is higher than previously reported 0.57% in Israel using real-time polymerase chain reaction (Galia *et al.,* 2014; Barkai *et al.*, 2014) to identify the presence of cytomegalovirus DNA in the saliva of neonates. In addition, Santos and co-workers reported a prevalence of 6.8% of CMV DNA when urine samples obtained from children at the first week of life in Brazil were examined (SANTOS *et al.*, 2000). The higher prevalence of CMV DNA found in our study could be because our study population included older children, up to six months of life when compared to previous studies that screened neonates. Older children are more likely to be exposed to CMV from sources of infection other than their mothers as previous studies have shown that the rate of CMV infection increases with age (Bate *et al.,* 2010).

There was no significant difference between the rate of CMV detection among male and female children tested in this study (p=0.4997). This is similar to reports by Santos *et al.,* [2000] who looked at CMV infection among children in the first week of life. Some studies, however, described a slightly higher prevalence among males, although this difference was not significant (Bate *et al*, 2010).

A significant association between CMV DNA positivity with infant age was found (P=0.001). The rate of infection was highest among infants within four to six months. This finding is similar to what was reported by Bello & Whittle (Bello and

Whittle, 1991) who conducted a 15 months longitudinal study among children in The Gambia. They found an increase in the detection of CMV in the saliva of children as the age increase. It is also similar to what was described by Stowell and co-workers (Stowell *et al.*, 2014) in the United States. This indicates that as infants grow older, with more environmental exposures and interaction with other people (for example, in the daycare center) there is a higher chance of becoming infected with CMV through other routes than through transplacental infection. During the first 2 to 3 months of life, infants are usually on breast milk, and carried, and fed by their mother, these age group of children are also protected by maternal antibodies if their mother had previously been infected, but by age of 4 months and above when the children are introduced to other food, the child contact with other household and non-household members increases and hence their exposure to infection. Children aged four to six months also have a higher risk of CMV infection in Nigeria because this is usually the age of enrollment in daycare centers for children of working mothers and it has been reported that there is a high rate of CMV transmission in daycare centers (Pass *et al.*, 1984).

In this study saliva samples of the children investigated were used. The ease of collection, handling, and storage of saliva samples, are key factors for it use in this study. Previous studies revealed that saliva is a preferred sample for cytomegalovirus diagnosis in neonates and infants because of their ease of collection using swabs and it is also less invasive and less painful than blood collection. (Simel *et al*., 1991, Johansson *et al*., 1997, Scanga *et al*., 2006, Johansson *et al.*, 1997; Scanga *et al.*, 2006. Also, high titers of CMV are shed in the saliva of infected newborns. Saliva has also been found to be as good as urine as a diagnostic sample of both congenital and postnatal CMV infection (Ross *et al.*, 2011). The outcome of this study confirmed the usefulness of saliva samples in the diagnosis of CMV infection in neonates and infants.

The CMV DNA was not detected in any of the mother's samples, including those mothers whose babies were positive for CMV DNA. Studies have shown that CMV DNA is only detectable during the viremic phase of primary infection, in immunocompetent subjects during which the virus can be isolated from peripheral blood polymorphonuclear leukocytes and monocytes (Rinaldo, Black, and Hirsch, 1977; Revello *et al.*, 1998). CMV DNA is also undetectable in immunocompetent subjects undergoing CMV reactivation while, in the immunocompromised patient, the virus can be detected in both primary and recurrent infections (Gerna *et al.*, 1991). A report of a decrease in DNA detection in maternal blood months after the onset of primary infection. These are probable reasons why CMV DNA could not be detected in all the mothers' blood (Revello and Gerna, 2002). Thus, it could not be ascertained if the babies who had CMV DNA acquired the infection from their mothers. CMV IgM detection or positivity is an indication of primary infection. None of the mothers also tested positive to CMV IgM by ELISA, thus indicating that none of the mothers had a primary infection at the time of sample collection. Patients with both positive IgM antibodies and IgG antibodies indicate a primary infection in the past and reinfection with a different strain of CMV (Gandhoke *et al.*, 2006).

The findings from this study showed a high prevalence of cytomegalovirus infection among children in their first six months of life and that the rate of the infection increases with age. This information is important in the estimation of the burden of cytomegalovirus infection in Nigeria and suggests the need for the development of a vaccine and its deployment early in life.

Disclosure Statement
The authors of this study hereby declare no conflicts of interest.

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