SERO-PREVALENCE OF HUMAN CYTOMEGALOVIRUS INFECTION AMONG PREGNANT WOMEN ATTENDING AMINU KANO TEACHING HOSPITAL

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ABSTRACT
Background: Cytomegalovirus (CMV) is the most common viral cause of congenital infection, Congenital Cytomegalovirus (cCMV) infection is one of the most common intrauterine infections in pregnancy. It is a leading congenital infection that causes mental disorders and sensorineural deafness. Recent CMV infection presents the greatest risk of transmission and severity. cCMV infection is an important concern in pregnant women. Routine serologic testing is performed during pregnancy in most countries for rubella, syphilis, HBV, and HIV. Thus, adding CMV to ongoing serologic testing is most likely to occur.
Methods: This study was conducted to determine the seroprevalence of human cytomegalovirus (HCMV) among pregnant women at AKTH. This study is a descriptive cross-sectional study which was conducted in AKTH Antenatal hospital were blood was taken from pregnant women that came for antenatal care and investigated for cytomegalovirus specific immunoglobin M(IgM) antibodies using enzyme-linked immunosorbent assay (ELISA). Demographic and clinical data were collected by questionnaire after participants consented. A total of 90 pregnant women were included in this study.
Results: Multi-gravid had the highest seroprevalence of 83.3% (5) based on their gravidity (p=0.000), while pri-gravid had 16.7%. (1). Subjects with two children at home had the highest seropositive rate of 50.6%, whereas subjects with 0 child and 3+ had 16.7% and 33.3%, respectively, but subjects with one child had no seropositive, positivity rate (p=0.000).Gravidity and parity were found to have a statistically significant relationship with CMV infection ($p \leq 0.05$).
Conclusion: This study provides data on HCMV epidemiology in northern Nigeria, and also provides an indicator on active CMV infection and the potential for transmission in utero.
Keywords: Congenital Cytomegalovirus, IgM-ELISA, Sensorineural deafness

INTRODUCTION

Human cytomegalovirus (HCMV) belong to the genus Cytomegalovirus, family Herpesviridae and subfamily Beta herpesvirinae, which also includes other mammalian cytomegaloviruses (Petrov *et al*., 2019). CMV is a cosmopolitan virus that affects people of all ages and socioeconomic classes and has no seasonal or outbreak patterns (Mamuye *et al*., 2015).It is more prevalent in developing countries and areas of lower socioeconomic status (Khalil *et al.*, 2017). Factors such as maternal age of more than 30 years and in unmarried women, nonwhite race, lower level of education, and close interaction with young children and their toys are all correlated with seropositivity of CMV (Khalil *et al.*, 2017).Sexual activity and interaction with infected babies or young children are the two primary causes of maternal CMV infection, according to research (Fowler and Pass, 2006). The latter, however, is considered the most significant, since the high prevalence of CMV in infants and preschool-aged children puts seronegative pregnant women caring for these infants at high risk of CMV infection (Grazia *et al.*, 2015).The virus, on the other hand, is shed in bodily secretions such as saliva, sperm, cervical secretion, urine, and feces, it also can be transmitted through sexual interaction (Petrov *et al.*, 2019). CMV has been identified as the most common cause of viral infection during pregnancy, labor, and delivery, as well as in newborns who are breastfed. However, Congenital CMV infection (cCMV) may be caused by primary maternal infection, as well as reinfection

and/or reactivation during pregnancy in HIV-positive mothers (secondary maternal infection) (Deborah *et al.*, 2015).

It is a common congenital infection that causes mental illness and sensorineural deafness in children. CMV infection that has occurred recently poses the greatest risk of transmission and severity (Deborah *et al.*, 2015). Infection with the cCMV virus is a major concern for pregnant women. In most countries, routine serologic testing for rubella, syphilis, HBV, and HIV is done during pregnancy. As a result of the danger posed by CMV in pregnant women, it's likely that testing for this virus will be added to existing serologic testing (Tung, *et al.*, 2012). The objectives of this research was to establish the seroprevalence of CMV infection among pregnant women who visited the Aminu Kano Teaching Hospital (AKTH) Ante-natal Clinic in Nigeria.

MATERIALS AND METHODS

Sampling Method

This is a descriptive cross-sectional study that was carried out at AKTH, Nigeria. This research included 90 consenting women (Cochran *et al.,* 1977), with an average age of 23 years (range 18-48 years). Patients visiting the AKTH antenatal clinic were included in the study. Before sample collection, each participant's permission was obtained, as the investigator completed a well-designed questionnaire.

Ethical Considerations / Approval

Before commencement of the research, the ethical committee of AKTH granted permission to perform the study with the reference number "AKTH/MAC/SUB/12A/P-3/VI/2703" dated September 5, 2019. Only consented participants were included in the study.

Sample Collection And Handling

Blood samples (approximately 2mls) were collected from consented participants using a sterile syringe and needle, the samples were transferred into correctly labeled plain tubes with the patient's identification number. The blood was allowed to clot before being centrifuged for 5 minutes at 3000 rpm. The sera were collected and preserved in clean, sterile bottles at -20°C until they were analyzed.

Sample Analysis

Cytomegalovirus IgM-EIA was performed at EIA bench, hematology laboratory of AKTH. All of the sera were assayed for the presence of Cytomegalovirus IgM antibodies using XEMA CMV-IgM ELISA kit (XEMA Co., Ltd, 105264 Moscow, Russia), with Catalogue Number: K103M

Test Procedure

The assay was performed by strictly following manufacturer instruction.

Reagent Preparation

- All reagents (including unsealed microstrips) were allowed to reach room temperature before use.
- All reagents were mixed by gentle inversion prior to use and formation of form was avoided.
- Washing solution was prepared from the concentrate BUF WASH 26X by 26 dilutions in distilled water.

Assay Procedure

The desired number of microstrips were put into the frame; 4 wells were allocated for control samples CONTROL - CONTROL + (3 and 1 well resp.), which 90µl of EIA buffer was pipetted into each well, followed by 10µl of control samples CONTROL-CONTROL + and unknown samples were put into each wells, the wells were covered by plate adhesive tape (included into kit). They were incubated at 37^{o}C and were continuously shaking at 500-600rpm. After which 250µl of washing buffer was used per well and the strips were washed 3 times and 100µl of CONJ HRP was dispensed into the wells and was covered by plate adhesive tape. They were incubated for 30min at +37oc and were continuously shaking at 500-600rpm. The strips were washed 5 times. And, 100µl of SUB TMB was dispensed into each wells. They were incubated for 20 minutess at room temperature. After which, 100µl of STOP was dispensed into the wells.The OD (optical density).The photometer blank was set on air (Pratiksha. 2015).

Statistical analysis

To assess the relationships between the associated risk factor and seroprevalence rate, the results and data from questionnaires were analyzed using SPSS version 20 and the Pearson Chi square test with a 95 % confidence interval and a significance level of >0.05

RESULTS

Seroprevalence of CMV Infection

The result of this study showed that the prevalence of CMV IGM (infection) among the study subject (pregnant women) were 6.7% (6) as depicted in Table 1.

Table 1 Seroprevalence of CMV among studied subjects

Variables	Frequency (n)	Percentage (%)
Positive	6	6.7
Negative	84	93.3
Total	90	100.

Socio-Demographic Characteristics Associated with CMV Infection

The Socio-demographic characteristics of subjects in relation to CMV sero-status were reported as depicted in Table 2. Subjects aged group 18-28yrs had highest rate of CMV infection 66.7% (4), 29-38yrs had 33.3% (2) while 39-48yrs had no seropositive positivity rate (p=0.894). Positivity rate among ethnic group, all seropositive subjects were Hausa 100% (6) while Fulani, Yoruba, Igbo and others were found seronegative after tests (p=0.471). Subjects with primary education had highest seropositive individuals 66.7% (4) followed by subjects with secondary education 33.3% (2) while those with tertiary or other form of education had no seropositive individuals (p=455). No significant association was observed between seropositivity and Socio-demographic characteristics with CMV Infection which include Age(p=0.894), Ethnicity(p=0.471), and Level of education(p=0.455).

Table 2 Socio-demographic variables of CMV Infection among participants distributed according to CMV seroprevalance

Characteristics	CMV positive (%)	CMV Negative (%)	P value
Age			
18-28	4 (66.7)	59 (70.2)	**0.894**
29-38	2 (33.3)	23 (27.4)	
39-48	0 (0.0)	2 (2.4)	
			0.471
Ethnic Group			
Hausa	6 (100)	52 (61.0)	
Fulani	0 (0.0)	17 (20.2)	**0.455**
Yoruba	0 (0.0)	6 (7.1)	
Igbo	0 (0.0)	3 (3.6)	
Others	0 (0.0)	6 (7.1)	
Level of Education			
Primary Education	4 (66.7)	38 (45.2)	
Secondary Education	2 (3.3)	20 (23.8)	
Tertiary Education	0 (0.0)	23 (27.4)	
Others	0 (0.0)	3 (36.0)	

p>0.05 – statistically no significant difference.

Risk Factors Associated With CMV Infection Among The participants Distributed According To CMV Seroprevalance

According to their gravidity (p=0.000), multi-gravid had the highest seroprevalance of 83.3% (5) while pri-gravid had 16.7% (1). For number of children at home, subjects with 2 children had highest seropositive of 50.6% while subjects with 0 child and 3+ were 16.7% and 33.3% but subjects with 1 child had no seropositive, positivity rate (p=0.000). Gravidity and parity showed statistically significant difference association with CMV infection (p > 0.05) (Table 3). According to their transfusion history, subjects with no transfusion history had the highest seroprevalence 66.7% (4) while subjects with transfusion history was 33.3% (2) seropositivity rate (p=0.952). However, Transfusion history had no statistically significant association with CMV infection (Table 4). Furthermore, presence of child(ren) ≥ 3yrs old at home showed statistically significant associated with CMV infection (p=0.000), subjects with presence of chid(ren) ≥ 3yrs old at home had 50.0% (3) and subjects without presence of child(ren) ≥ 3yrs old at home had 50.0% (3). The financial status had no statistically significant associated with CMV infection (p=0.489), participants with average and good financial status were 50.0% and 50.0% and no low financial status were seropositive (Table 5).

Table 3 The seroprevalance of CMV infection among the subjects according to their gravidity and parity

Factors	CMV positive (%)	CMV Negative (%)	P value
Gravidity			
Pri-gravid	1 (16.7)	70 (83.3)	**0.00**
Multi-gravid	5 (83.3)	23 (27.4)	
Parity			**0.00**
0	1 (16.7)	71 (84.5)	
1	0 (0.0)	7 (8.3)	
2	3 (50.0)	5 (6.0)	
3+	2 (33.3)	1 (1.2)	

p>0.05 – statistically no significant difference

Table 4 The seroprevalance of CMV infection among the subjects according to their transfusion history

Factors	CMV positive (%)	CMV Negative (%)	P value
Transfusion History			
Yes	2 (33.3)	27 (32.1)	**0.952**
No	4 (66.7)	57 (67.9)	

p>0.05 – statistically no significant difference

Table 5 The seroprevalance of CMV infection among the subjects according to their presence of child (ren) ≥ 3yrs old at home and financial status

Factors	CMV positive (%)	CMV Negative (%)	P value
Presence of Child ≥ 3yrs old at home			
Yes	3 (50.0)	3 (3.6)	**0.00**
No	3 (50.0)	81 (96.4)	
Financial Status			**0.489**
Low	0 (0.0)	1 (1.2)	
Average	3 (50.0)	7 (71.4)	
Good	3 (50.0)	23 (27.4)	

p>0.05 – statistically no significant difference

DISCUSSIONS

Our findings showed 6.7% CMV IgM seroprevalence in the study region, indicating that the virus was active and reactivated recently. This findings was slightly lower than a work reported in Kenya 8.1% (Maingi *et al.,* 2014) and slightly similar to the work reported in Sudan 6% (Khairi et al., 2013), in Finland 4.0 %, Australia 5.5 %, and France 5.7 % (Mustakangas *et al.,*2000; Picone *et al.,* 2009; Munro *et al.,* 2005). The differences in prevalence in these areas could be attributed to variation in geographical location, social-economic status, cultural factors and child bearing practices (Ho *et al.,* 1999; Henge *et al.,* 2000). The low prevalence of recent CMV infection in this study is in agreement with several other studies (Hamdan *et al.,* 2011; Satilmis *et al.,* 2007; Rubina *et al.,* 2004). However, a higher seroprevalence, has been documented by other researchers (Deborah *et al.,* 2013; Yeroh *et al.,* 2014). These discrepancies may be attributed to differences in socioeconomic setting and this can be inferred from the work of Stagno and Whitley (Rubina *et al.,* 2004).Geographical location, socioeconomic status, cultural influences, and child-bearing behaviors could all play a role in the disparities in prevalence of CMV in this region. High prevalence of CMV was observed in the 18-28 year old age group. The age group 18-28 years represents sexually mature and active adults who have a proclivity for sexual promiscuity and are therefore more likely to become infected. Prevalence rates of 66.6 % and 33.3 %were recorded for primary and secondary education, respectively, while tertiary and other forms of education had no prevalence. This study agrees that the prevalence rate of CMV infection decreased significantly as education level decreased. This decrease is consistent with a previous study by Hamdan *et al*. (2011) where they reported OR of 14.7 and $P = 0.01$, thereby concluding that education is a significant risk factors for CMV infection which found that illiterate women are more likely to contract CMV due to interaction with infectious secretions from their own children and bad hygienic practices; nonetheless, they are immune to CMV except in the rare case of reactivation or reinfection. Surprisingly, seronegative women with a higher level of education are more vulnerable to primary CMV infection due to non-functional CMV immunity (Diaye *et al.,* 2014). Multigravid women were found to be the most infected in this study, with an infection rate of 83.3%. This is consistent with the findings of another study, which found a high prevalence of multigravid pregnancy in multigravid pregnant women (Okwori *et al.,* 2008; Deborah *et al.,* 2015).There was no statistical association between women who had history of blood transfusion and CMV seropositivity.

This was higher among those that were never transfused 66.7% than those that had been transfused 33.3%.The limited number of transfused women in this study may have contributed to the lack of significance in the relationship. According to the findings of this research, subjects with average and good financial status had a higher prevalence of CMV infection.

Other risk factors, such as education level(p=0.455) and financial status(p=0.489) were not find to be associated with CMV's IgM epidemiology.

The incidence of congenital CMV infection has been reported to be dependent on population epidemiological characteristics, especially maternal CMV seroprevalence, also in populations with elevated CMV seroprevalence, high rates of congenital CMV infection have been consistently demonstrated. Hence, the lower prevalence of CMV infection among pregnant women who visited the AKTH antenatal clinic suggests a low incidence of congenital CMV infection among Kano state infants.

CONCLUSION

Since congenital CMV infection may be both asymptomatic and symptomatic, asymptomatic children can infect other children and those who care for them. Infants born to infected mothers who are asymptomatic should be monitored to see whether they develop clinical sequels and defects as they grow, such as subtle growth retardation and sensor neural hearing loss, so that those organs are not damaged. The findings of this study showed that pregnant women in the population screened had CMV IgM antibodies, suggesting a current infection and the possibility of transmission in utero.

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