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Research Article

SERO-PREVALENCE OF CYTOMEGALOVIRUS IgG ANTIBODIES IN PREGNANT WOMEN ATTENDING ANTE-NATAL CLINICS IN BIRNIN-KEBBI, KEBBI STATE, NIGERIA

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ABSTRACT

Cytomegalovirus has been described as an important etiological agent of intrauterine infection in pregnant women that causes congenital malformations like intrauterine growth restriction, cerebral palsy. The study on seroprevalence of cytomegalovirus IgG antibodies was conducted on one hundred and twenty pregnant women attending, ante-natal clinics in two hospitals in Birnin-Kebbi. The serum samples were examined for the presence of IgG antibodies against cytomegalovirus by Elisa technique. From the 120 blood samples collected, 112 (93.3%) were seropositive for cytomegalovirus IgG antibodies and 8 (6.7%) did not have the CMV IgG antibodies. There was no significant relation of CMV IgG seropositivity with increasing age, occupation, residence, stage of pregnancy and parity, however there was association between level of education and CMV IgG seropositivity. Cytomegalovirus IgG antibodies seroprevalence rate was higher in women from urban rural as compared to those of urban rural areas. All women (100%) who had secondary and tertiary education had CMV IgG antibodies and it was high (100%) in civil servants and urban women (96.2%). CMV IgG antibodies was absent in women who were in 1st trimester (3, 11.5%) and 2nd trimester (5, 8.6%). Women (100%) in 3rdtrimester and who had one and three children had 100% CMV IgG antibodies. There should be voluntary screening of all pregnant women for CMV infection and its antibodies as part of the antenatal care. The identified susceptible/seronegative women should be educated on appropriate preventive measures.

KEYWORDS: Seroprevalence, Cytomegalovirus, IgG antibodies, pregnant women, Birnin-Kebbi

INTRODUCTION

Cytomegalovirus (CMV) is a ubiquitous human beta herpesvirus type 5 belonging to the herpesvirus family [1], [2]. It is the largest, compared to other human herpesviruses, with a genome of ~235kb, encoding ~165 genes [3]. The virion (200-300nm), consists of a double-stranded linear DNA core in an icosahedral nucleocapsid, enveloped by a proteinaceous matrix[4] which are enclosed in a lipid bilayer envelope that is derived from the nuclear

membrane of infected cell and contains viral glycoproteins [4]. Initial infection with CMV commonly occurs during childhood and depending on geographic location and socioeconomic group, 35-90% of population have antibody against the virus by adulthood [5] The CMV is highly species-specific and human disease are produce only by human strains and is transmitted vertically from mother to the fetus through placental transfer, breastfeeding and horizontally through other bodily fluids like saliva, sexual contact, as well as blood transfusion, solid-organ and hematopoietic stem cell transplantation [7] Human cytomegalovirus (hCMV) seroprevalence varies in different populations and age groups. Congenital malformation is the most resulting clinical symptom of CMV intrauterine infection [8],[9], [10], [11]. Up to 15% of intrauterine CMV infections led to congenital symptomatic diseases. Asymptomatic congenital CMV infection develops in 10-15% of infants [12], [13], [14]. CMV persists in a latent form after primary infections and reactivation may occur years later, particularly under such conditions including immunosuppression such as pregnancy [15]. The objective of this study was to determine the prevalence of previous CMV infection among antenatal women and their effect to age, demographic characteristics, parity and gestational age, with a view to determining the desirability of antenatal screening of the infection.

MATERIALS AND METHODS

Sample Collection

A total of 120 pregnant women attending antenatal clinics of the two hospitals: Hajiya Turai Yar' Dua General Hospital and Maya Foundation Clinic and Maternity in Birnin-Kebbi were randomly selected for the study. The study was approved by the ethical committee of the two hospital and informed consent was taken from each subject before sample collection. Demographic information and clinical details were recorded on a standardized questionnaire.

Blood samples (5ml) were collected by venepuncture. Each of the collected blood sample was transferred to clean-dry bottles and allowed to clot. It was then centrifuged at 3000 rpm for five minutes. The sera were then separated and transferred into cryovials (BD Inc, USA) and stored at −20^{o}C until required for use.

Sample Analysis

The samples were analyzed for CMV Immunoglobulin G (IgG) using the ELISA test kits (Dialab, Austria) as follows: All the samples, reagents and calibrators were brought to room temperature an hour before the test. The samples were diluted 1:50 with sample diluents and were carefully mixed before dispensing. The anti IgG antigen coated micro-well plate was labeled calibrators, samples and blank appropriately. 100 µl of the calibrators and samples were dispensed in the wells, leaving the blank well empty. The strip was covered with adhesive films and was incubated at 37^{o}C for 60 minutes. The adhesive film was peeled-out and the reaction solution was aspirated from all the wells. The wells were washed five times with 300 µl of diluted wash buffer. 100 µl of enzyme conjugate was then introduced into calibrators and samples well. The strip was further covered and incubated at 37^{o}C for another 60 minutes. The adhesive was peeled-out and the reaction solution aspirated from all the wells. The wells were then washed five times with 300 µl of diluted wash buffer, the remaining liquid were aspirated off carefully. 100 µl of the substrate A and B were dispensed in all the wells including the blank respectively. The strip was covered with new adhesive film and incubated at room temperature for 20 minutes, protected from light and 100 µl of stop solution was finally introduced into all the wells. With the aid of a multiscan (BDSL, UK), the absorbance of each well was read against the blank well at optical density of 450 nm. According to the manufacturer, the cut-off has been set at 1IU/ml, value of CMV IgG concentration at which an individual is considered protected [16]

DATA ANALYSIS

Data were entered in Microsoft Office Excel Work sheet. Pearson Chi-square test was employed for the statistical analysis of the data obtained in the study. The value of $p < 0.05$ was considered statistically significant.

RESULTS

The study on seroprevalence of cytomegalovirus IgG antibodies was conducted on one hundred and twenty pregnant women attending, ante-natal clinics in two hospitals in Birnin-Kebbi. From the 120 blood samples collected, 8 (6.7%) did not have the CMV IgG antibodies while 112 (93.3%) had the antibody. The age groups of the women involved in the study were between the ages of 16 and 45 years. The results Table **1** showed that the highest CMV IgG antibodies (42; 93.3%) was recorded in the age group of 26-30. All women (100%) in age group of 36-40 years and 41-45 years had CMV IgG antibodies. There was no significant relation of CMV IgG seropositivity with increasing age, occupation, residence, stage of pregnancy and parity, however there was association between level of education and CMV IgG seropositivity.

All women (100%) who had secondary and tertiary education had CMV IgG antibodies and it was high (100%) in civil servants and urban women (96.2%) (Table 2). CMV IgG antibodies was absent in women who were in 1st trimester (3, 11.5%) and 2nd trimester (5, 8.6%). Women (100%) in 3rd trimester and who had one and three children had 100% CMV IgG antibodies Table 3

Table 1: CMV IgG antibodies of the pregnant women involved in the study according to their age groups

	Presence of IgG Antibody			
Age Group(years)	**No.**	**Present (%)**	**Absent(%)**	**p-value**
16-20	20	19(95.0)	1(5.0)	0.898
21-25	34	32(94.1)	2(5.9)	
26-30	45	42(93.30	3(6.7)	
31-35	15	13(86.7)	2(13.3)	
36-40	4	4(100.0)	0(0.0)	
41-45	2	2(100.0)	0(0.0)	
Total	120	112	8	

Key: Figures in parenthesis are the percentages; No. = number

Table 2: Demographic characteristics of the pregnant women recruited in the study showing CMV IgG antibodies

	Presence of CMV IgG Antibody			
Variables	**No.**	**Present(%)**	**Absent(%)**	**p-value**
Education				0.00
Primary	16	13(81.3)	3 (18.7)	
Secondary	48	48(100.0)	0 (0.0)	
Tertiary	24	24(100.0)	0 (0.0)	
Arabic	28	25(89.3)	3 (10.7)	
Non	4	2(50.0)	2 (50.0)	
Total	120	112	8	
Occupation				0.390
Civil servant	16	16 (100.0)	0 (0.0)	
Self employed	28	25 (89.3)	3 (10.7)	
Unemployed	76	71 (93.4)	5 (6.6)	
Total	120	112	8	
Residence				0.091
Urban	78	75 (96.2)	3 (3.8)	
Rural	42	37 (88.1)	5 (11.9)	
Total	120	112	8	

Key: Figures in parenthesis are the percentages; No. = number

Table 3: CMV IgG antibodies according to their stage of pregnancies and previous

Presence of IgG Antibody				
Variables	**No.**	**Present**	**Absent**	**p-value**
Stage of pregnancy				0.141
1st Trimester	26	23 (88.5)	3 (11.5)	
2ndTrimester	58	53 (91.4)	5 (8.6)	
3rdTrimester	36	36 (100.0)	0 (0.0)	
Total	120	112	8	
Parity				0.465
Nil	28	26 (92.9)	2 (7.1)	
1	12	12 (100.0)	0 (0.0)	
2	22	19 (86.4)	3 (13.6)	
3	14	14 (100.0)	0 (0.0)	
4	44	41 (93.2)	3 (6.8)	
Total	120	112	8	

Key: Figures in parenthesis are the percentages; No. = number

DISCUSSION

The seroprevalence of CMV IgG antibodies among the pregnant women involved in this study was 93.3%, as they had been previously exposed to CMV infection thereby having the CMV IgG antibodies and 6.7% were susceptible to the infection because they had not contracted CMV infection before pregnancy. This high percentage level of maternal CMV IgG antibodies observed in this study is similar to previous studies [16],[17].[18] This, however, differs from those reported form developed countries [18], [19] where seroprevalence rate is low. The differences in the prevalence of maternal CMV infection between the developed and developing countries may reflect the low hygienic practices in developing countries. In developed countries, there is reduced risk of acquiring CMV infection because pregnant women are generally more informed on good hygienic practices. Statistical analysis of this study, showed that there was no significant relation of CMV IgG seropositivity with increasing age, occupation, residence, stage of pregnancy and parity, however there was association between level of education and CMV IgG seropositivity. The 8 (6.7%) susceptible women were observed in women less than 35 years of age. They are at risk of contracting CMV infection as they are in their child-bearing age. In this study, it was also observed that the prevalence of CMV IgG antibodies was 100% in age group 36-45 years because with age majority of the women have already been exposed and recovered from primary infection, thus CMV IgG antibodies increase with age[21].

There is high prevalence of CMV IgG among the educated and those residing in urban areas. Like the previous study [23] showed that CMV IgG seroprevalence had no significant correlation with geographical location. Route of infection can be a hypothesis for the probable role of geographical influence upon CMV seroprevalence. In rural areas, saliva is probably the main route through which the virus is transmitted postnatally, in life amongst infants and young children due to poor personal and environmental sanitation [21] On the other hand, in urban areas, sexual transmission seems to be the major route of infection later in life during childbearing age [24]. It has been reported that the risk of fetal damage is greater if the primary infection occurs during the first trimester of pregnancy [8]. So, the fetus of the 3 susceptible women who were in first trimester were more susceptible to acute infection. From the study, it was also observed that parity plays no role in determining the prevalence of cytomegalovirus infection. Transmission of CMV infection to women from children at the home either their own or family member who had acquired infection from elsewhere was associated with the CMV IgG antibodies seropositivity.

Congenital CMV infection manifest as a primary infection, non-primary infection with a new strain of CMV, or reactivation of a latent infection [25]. Generally, the average transmission of CMV infection from mother to fetus is greater during primary maternal infection (40%) with reported range of 24-75%, and congenital infection of the fetus is higher [26] The transmission is 0.15%–2.2% during reactivations or reinfections [27] when, furthermore,

most of the newborns are asymptomatic [28]. The transmission in reactivation or reinfection of CMV is less because IgG antibodies is the only type of antibody that can cross the placenta, provides passive immunization in the fetus throughout pregnancy [29]

Therefore, these women having no CMV IgG antibodies are likely to have first contact with the virus (primary infection). Women with CMV IgG antibodies can have recurrent infection but since primary infection with CMV during pregnancy is much more likely to produce symptoms and sequelea in infants than recurrent maternal [26], [8] thus, their fetus are at high risk. Further investigations are required to determine the proportion of congenital CMV infections in the children giving birth by the 8 (6.7%) susceptible women discovered in this study, especially if there is fetal malformations and adverse neonatal outcome.

CONCLUSION

A high proportion of the pregnant women tested in this study were previously exposed to CMV infection thereby having the CMV IgG antibodies that will somehow protect their fetus, while 8(6.7%) are susceptible to the CMV infection. There should be voluntary screening of all pregnant women for CMV infection and its antibodies as part of the antenatal care. As no effective treatment and vaccine against the CMV is available, the identified seronegative women should be educated on appropriate preventive measures such as good hand washing with soap and clean water as well as wearing gloves when changing baby diapers soaked with urine and avoid responsible sexual practices, limited contact with infected children and sharing of eating and utensils.

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