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Human Cytomegalovirus IgM antibodies seropositivity among pregnant women in ante-natal clinics in Birnin-Kebbi, Kebbi state, Nigeria

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ABSTRACT

Infection with Cytomegalovirus (CMV), especially in pregnancy, may cause pregnancy complications such as congenital infection, non-hereditary deafness, intrauterine growth restriction and other health defects. The objective of this study was to evaluate the prevalence of CMV IgM antibodies in pregnant women attending antenatal clinics at General Hospital, Zauro/Ambursa and Mayo Foundation Clinic and Maternity, Birnin-Kebbi. Serum samples were collected from 92 pregnant women. CMV IgM antibodies were detected using an enzyme-linked immunosorbent assay method for IgM. Of the 92 pregnant women tested, CMV IgM antibody was found in 1(1.1%) of the women while 91(98.9%) did not have the CMV IgM antibodies. The diagnosed CMV IgM antibody seropositive pregnant women was educated, civil servants, residing in the urban areas and in her third trimester in first pregnancy. Although, a low proportion of pregnant women is exposed to CMV current infection, it is recommended that there should be voluntary screening of all pregnant women, as part of their antenatal care, so that seropositive women with primary infection could be offered the opportunity for prenatal screening and be informed of intervention options.

INTRODUCTION

Cytomegalovirus (CMV) is a member of the subfamily of Herpes known as Beta(β) herpes and it is found universally in various geographical locations. [1] Infection by CMV can be classified as congenital, if acquired before birth, perinatal, at the time of delivery or as postnatal, if acquired later in life. [2] The infection is usually asymptomatic in adults but its significance is many times increased when it occurs during pregnancy. CMV infection during pregnancy is a major cause of congenital infection in developing countries with an incidence of 0.3-0.7% of live births. [3] The seroprevalence of CMV among women of childbearing age ranges from 35% to 95% in different countries. [4]

Transmission of CMV from person to person requires intimate contact with secretions of body fluids (e.g. saliva, urine, breast milk cervico-vaginal secretions and semen) of an infected person. This frequently happens by sexual contact, blood transfusion and transfer from mother to fetus by transplacental (intrauterine), intra-uterine and breast milk transmission. [5, 6]

Congenital CMV infection to fetus can occur during either primary or recurrent maternal CMV infection. Recurrent maternal CMV infection, can be resulted by reactivation of latent virus acquired prior to pregnancy or reinfection of seropositive mothers with a new CMV strain during pregnancy. Primary CMV infections are transmitted more frequently to the fetus and are more likely to cause fetal damage than recurrent infections. [7, 8, 9, 10, 11] Latency following a primary infection (first contact with the virus) may be disrupted by periodic reactivations that give rise to recurrent infections. [9] Primary infections occur between 0.5- 4% in seronegative women during pregnancy. [12] The Risk of congenital CMV infection is much higher during primary infection of the mother with transmission rate of 30%-40% compared with 0.15%-2.2% during reactivations and

reinfection [13] and up to 13% of these infections will result in symptomatic congenital disease in the newborn. [11]

Among the most clinically important forms of CMV disease is congenital infection, which can occur at any stage of pregnancy. The majority of congenitally-infected infants develop normally, but about 10% are likely to have permanent CMV-related damage, [2] which subsequently leads to congenital defect which can include permanent hearing loss, vision loss and neurological impairment cerebral palsy, mental retardation, visual impairment and psychomotor delay over time. [3] In a few cases, there are symptoms at birth which include premature delivery, being small for gestational age, jaundice, enlarged liver and spleen, microcephaly, seizure, rashes and feeding difficulties. *[14]*

MATERIALS AND METHODS

Study area

This research was carried out in Usmanu Danfodiyo University Teaching Hospital (UDUTH), Sokoto but blood samples were collected from Hajiya Turai Yar-dua General Hospital, Zauro/Ambursa and Mayo Foundation Clinic and Maternity, Birnin-kebbi, Kebbi State, North-western Nigeria. 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.

Sample collection

With the consent of the women after a brief teaching on cytomegalovirus and its negative effects to the fetus, blood samples of 5ml were collected from the pregnant women by venepuncture. Each of the collected blood sample was transferred into a sterile, non-anticoagulated clean-dry bottles and allowed to clot. It was then centrifuged at 3000 rpm for five minutes using the centrifuge model 800D. The sera were separated and transferred into cryovials and stored at 20°C until required for use. [1]

Sample analysis

Cytomegalovirus IgM antibodies were detected using ELISA micro-well method as follows: All the samples and reagents were brought to room temperature 15 minutes before the test. Following the manufacturer's instructions, 1:40 dilution of wash buffer concentrate was prepared with distilled water by adding 1ml of buffer solution to 39ml of distilled water, 100µl of the sample diluent was added into the appropriate wells except the blank and the two negative wells, then 50µl of the blood specimen was added to the wells beating by pipettor repeatedly until the mixture turned blue. Then, 50µl of negative and positive controls were dispensed into the negative and positive wells separately. The microtiter wells were flicked for 30seconds to mix well, the sealing template was fixed on the wells and incubated at 37°C for 20 minutes.

The wells were inserted into the microplate washer machine to add wash buffer to each well and absorb it after 20 seconds. This was repeated five times until each well was dried. Then, 50µl of Horse Radish Peroxidase (HRP) conjugate was dispensed to each well except the blank well. It was then mixed by gentle vibration, sealing template was affixed and the well was incubated at 37°C for 20 minutes. It was then washed five times with the diluted wash buffer using the microplate washer machine for 20 seconds. Then, 50µl of substrate A and 30µl of substrate B were dispensed into all the wells except the blank well, and it was incubated at 37°C for 10 minutes followed by gentle vibration mixture to mix well and incubated at 37°C for another 10 minutes. After this, 50µl of stop solution was added to the wells except the blank well; mixed and the result was read using the multiscan microwell reader. With the aid of a multiscan (BDSL, UK), the absorbance of each well for IgM antibodies was read against the blank well at optical density of 450 nm. According to the kit manufacturer, the cut-off optical density was set to be the product of 2.1 and the negative control optical density, if the sample optical density is greater than or equal to the cut-off optical density the result is considered positive meaning the person has the CMV IgM antibody. But if the sample optical density is less than the cut-off optical density, the result is negative that is to say the person does not have the CMV IgM antibody.

Statistical 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 IgM

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

Age Group (in years)	Presence of IgM Antibody		Absent (%)	X^2
	No. Tested	Present (%)		
16-20	13	0 (0)	13 (100)	0.886
21-25	21	0 (0)	21 (100)	
26-30	34	1 (3)	33 (97)	
31-35	18	0 (0)	18 (100)	
36-40	4	0 (0)	4 (100)	
41-45	2	0 (0)	2 (100)	
Total	92	1	91	

Key: Figures in parenthesis are the percentages
X^2 = Pearson chi square result

Table 2. : Demographic characteristic of the pregnant women recruited in the study showing CMV IgM antibodies.

Variables	Presence of IgM Antibody			
	No. Tested	Present(%)	Absent (%)	X^2
Education				0.228
Primary	16	0 (0)	16 (100)	
Secondary	38	0 (0)	38 (100)	
Tertiary	14	1 (7)	13 (93)	
Arabic	20	0 (0)	20 (100)	
Non	4	0 (0)	4 (100)	
Total	92	1	91	
Occupation				0.091
Civil servant	16	1 (6)	15 (94)	
Self employed	10	0 (0)	10 (100)	
Unemployed	66	0 (0)	66 (100)	
Total	92	1	91	
Residence				0.441
Urban	58	1 (1.7)	57 (98.3)	
Rural	34	0 (0)	34 (100)	
Total	92	1	91	

Key: Figures in parenthesis are the percentages
X^2 = Pearson chi square result

antibodies was conducted on ninety two pregnant women attending, ante-natal clinics in the two hospitals in Birnin-Kebbi. From the ninety two blood samples collected, ninety one (98.9%) did not have the CMV IgM antibodies while one (1.1%) had the antibody. The age groups of the women involved in the study were between the ages of 16 and 45 years. The CMV IgM antibodies was recorded only in age group of 26-30 years (table 1).

The diagnosed CMV IgM antibody seropositive pregnant women was educated, civil servants, residing in the urban areas, in her third trimester, and in first pregnancy. There was no significant relation of CMV IgM seropositivity with increasing age, level of education, occupation, residence, stage of pregnancy and parity (table 2 &3).

DISCUSSION

The seroprevalence of CMV specific IgM antibody in pregnant women attending antenatal clinics was low (1.1%) in the two hospitals in Birnin-Kebbi. The low prevalence of CMV specific IgM antibody in this study, is in agreement with Hamdan *et al*., 2.5%; [15] Saidu *et al*., 1%; [16] Alghalibi *et al*., 1.8%; [13] and *Usta et al.,* 1.1%. [17] However, a higher seroprevalence, has been documented by other researchers like Lone *et al.*, 16%; [18] Saraswathy *et al*., 7.2%; [19] Hama and Abdurahman, 9.18 %; [20] and Neirukh *et al*., 11.5%. [21]

The presence of CMV IgM antibodies is revealing current infection of CMV and increasing the possibility of transmission of infection *in utero* to the fetus. The use of ELISA CMV-specific IgM in this study had its limitations. CMV specific IgM may reappear during reactivation of CMV infection, thus, it was not possible to distinguish between primary infection and reactivation in this study. The low prevalence CMV IgM antibodies in study area, might be as a result of enlightenment as the women are more educated and have better hygienic environments and condition of living. It is also possible that majority of the women would have already been exposed and recovered from primary infection, with the loss of IgM, by the time of the current pregnancy. [22]

The diagnosed CMV IgM antibody seropositive pregnant women was educated, civil servants and residing in the urban areas. This might be because the educated women in urban areas may have been practicing safe hygienic practices as a way of life without knowing the existence of CMV infection. This practice might have prevented them from previous exposure to CMV

Table 2. : CMV IgM antibodies according to their stage of pregnancies and parity

Variables	Presence of IgM Antibody			
	No. Tested	Present	Absent	X^2
Stage of pregnancy				0.125
1st Trimester	26	0 (0)	26(100)	
2ndTrimester	48	0 (0)	48 (100)	
3rdTrimester	18	1 (6)	17 (94)	
Total	92	1	91	
Parity				0.385
Nil	18	1 (6)	17 (94)	
1	12	0 (0)	12 (100)	
2	20	0 (0)	20 (100)	
3	14	0 (0)	14 (100)	
4+	28	0 (0)	28 (100)	
Total	92	1	91	

Key: Figures in parenthesis are the percentages
X^2 = Pearson chi square result

infection thereby having first contact with CMV infection during pregnancy, which led to the presence of CMV IgM antibodies in their serum than the uneducated ones living in rural areas that did not practice safe hygiene practices. On the other hand, in urban areas, sexual transmission is the major route of infection later in life during childbearing age. [23]

Looking at their stage of pregnancy, the diagnosed pregnant women was in her third trimester in first pregnancy and she is at risk of transmitting the infection to the fetus, since she might be having the recent primary infection. The women infected with CMV during late gestation are more likely to transmit the virus to their unborn child than women who are infected at early gestation [24, 25].

Observed also in this study was that 91(98.9%) negative cases for CMV IgM antibodies, that was considered susceptible for CMV infection. Although the prevalence of CMV IgM antibody among pregnant women is low, the risk of congenital CMV infection is much higher. CMV infection can lead to significant damage to the fetus and as the damage done *in utero* cannot be reverted, control of intrauterine CMV infection is important, through prevention of CMV infection in the pregnant women. As no effective treatment and vaccine against the CMV is available, more emphasis should be laid upon educating women (to maintain good hygiene, limited contact with infected children and responsible sexual practices) and routine screening of pregnant women to reduce the fatal outcome of the pregnancy occurring due to the CMV infection.

CONCLUSION

Although, a low proportion of pregnant women is exposed to CMV current infection, it is recommended that there should be voluntary screening of all pregnant women, as part of their antenatal care, so that seropositive women with primary infection could be offered the opportunity for prenatal screening and be informed of intervention options. Finally, follow-up tests with offspring to CMV IgM-positive mothers are essential, in order to detect the consequences of congenital infection and to allow treatment to occur as early as possible. Education of women about CMV, virus transmission and hygiene strategies to prevent CMV infection should be the most important strategy to reduce the risk of congenital CMV infection.

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Conflict of interest:

The authors report no conflict of interest.

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