Bayero Journal of Pure and Applied Sciences, 4(1): 63 - 66
Received: November, 2010
Accepted: May, 2011
ISSN 2006 - 6996

SERO-PREVALENCE OF CYTOMEGALO VIRUS ANTIBODIES IN PREGNANT WOMEN ATTENDING TWO SELECTED HOSPITALS IN SOKOTO STATE, NORTH-WESTERN NIGERIA

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ABSTRACT
Infection with cytomegalo virus (CMV), especially in pregnancy may cause pregnancy complications such as congenital infection, non-hereditary deafness, intrauterine growth restriction and other high defects. This study was to evaluate the prevalence of CMV in pregnant women attending Antenatal Clinics at Maryam Abacha Women and Children Hospital (MAWCH) and Sokoto Specialist Hospital (SSH). The study enrolled 90 pregnant women (age range: 16-45 years old) and data on demographic and life styles obtained using structured questionnaire. Serum samples were assayed using CMV Ig-G EIA kit. Out of the 90 pregnant women examined, 88(97.8%) had IgG protective antibodies to CMV while 2(2.2%) did not have protective antibodies. The prevalence rate of 2.20% was obtained in pregnant women that did not have the protective CMV IgG antibodies and were in the age range of 16-30 years old. They were also found to be in their second trimester, which could have increased the risk of intrauterine transmission. The risk factors for CMV were observed to have been significantly (P > 0.05) correlated with the socio-economic status, the levels of CMV Ig-G antibodies, stage of pregnancy as well as the symptoms of pregnancy complications observed in the study population while they were insignificant (P < 0.05) in the case of number of previous pregnancies. The outcome of effects on the fetus was usually fatal and irreversible. Therefore, all pregnant women should scrupulously consistently adhere to routine infection control precautions.

Keywords: Cytomegalo virus, Pregnant women, Sero-prevalence, Sokoto.

INTRODUCTION

Cytomegalovirus (CMV) is a ubiquitous member of the Herpes family, and is responsible for a wide range of infection in humans of all ages. It infects between 50% and 85% of adult in the United States over 40 years of age. CMV infection is spread in developing countries and in areas of low socio-economic conditions (Gold and Nankervis, 1976). Once a person is infected, the virus latently persists in the body throughout the person's life with viral excretion (occasionally from several sites) in various body fluids like; blood, urine, saliva, cervical secretions, semen, breast milk and respiratory secretions (Morag, 1986). Transmission of CMV from person to person requires intimate contact with secretions of body fluids of an infected person. This frequently happens by sexual contact, blood transfusion, and transplacental (intrauterine) transfer from mother to fetus (Stagno *et al*., 1986). Among the most clinically important form of CMV disease is congenital infection, which can occur at any stage of pregnancy, which subsequently lead to congenital defect. CMV infection in pregnant women can either be primary or recurrent. Congenital infection can occur in the fetus if the mother has a primary infection or reactivation during pregnancy. Infection is transmitted to the fetus at a higher rate with primary infection during pregnancy with estimated incidence of 25-75% than with reactivation infection with an estimation of 0.2-2% (Stagno *et al*., 1982a, b; Grantgoel-Keros *et al*., 1997). Only 1% of all live births born annually in the United States with congenital CMV infection are overtly symptomatic at birth. The symptoms include any or combination of microcephaly, intracranial calcification, chorioretinitis, jaundice, low birth weight hepatosplenomegaly and purpura. The mortality rate among the symptomatic infant can be as high as 30%; another 5% have milder or a typical involvement, and 90% are born with sub clinical but chronic infection and are likely to develop long-term neurological sequelea, including hearing loss, visual impairment, psychomotor delay, mental retardation (Istas *et al*., 1995; Conboy *et al*., 1987). In Africa and particularly in Nigeria, incidence of CMV infection is not documented. Hence, this research was aimed at determining cases of CMV infection among pregnant women.

MATERIALS AND METHODS

Sample collection

Blood samples (5ml) were collected from 90 pregnant women by venepuncture. Each was transferred to clean-dry bottles and allowed to clot. It was then centrifuged at 3000rpm 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

CMV Ig-G antibodies were detected using ELISA micro-well method (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-Ig-G antigen coated micro-well plate was labeled calibrators, samples and blank appropriately. A quantity (100µl) of the calibrators and samples were dispensed in the wells, leaving the blank well empty. The strip was covered with adhesives film 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 300µl diluted wash buffer. A quantity (100µl) 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 300µl diluted wash buffer, the remaining liquid were aspirated off carefully. A quantity (100µl) of the substrate A/B was dispensed in all the wells including the blank respectively. The strip was covered with new adhesive and incubated at room temperature for 20min, protected from light and 100µl of stop solution was finally introduced in 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. Calibration curve was drawn using the optical densities (OD) of the six (6) calibrators against their units (IU/ml). Ig-G concentrations in the samples were calculated from the curve on a point-to-point fitting elaboration. In the follow-up of pregnancy, the cut-off has been set at 1IU/ml, value of CMV IgG concentration at which an individual is considered protected (Engvall and Perlmann, 1971).

Statistical analysis of the results

Chi-square (X^2) test was employed for the statistical analysis of the data obtained in the study.

RESULTS AND DISCUSSION

From the results obtained in this study, 88(97.8%) of the pregnant women tested had CMV Ig-G antibodies of less than 1 IU/ml, while 2(2.2%) had Ig-G antibodies of greater than <1 IU/ml. However, in the follow-up of pregnancy, the cut-off has been set at 1 IU/ml, value of CMV Ig-G concentration at which an individual is considered protected (Engvall and Perlmann, 1971). Therefore, 97.8% of these women were considered protected because they have Ig-G antibodies greater than 1 IU/ml, while 2.2% of these women were not having protective antibodies to CMV. The implication is that, infant born to these women are likely to have acute visceral disease with hepatitis, pneumonia purpura and severe thrombocytopaenia (Stagno *et al*., 1982). However, where they happen to be asymptomatic at birth, they will have late developmental problems like mental retardation, cerebral palsy, sensorineural hearing loss and vision impairment (Stagno *et al*., 1982; Istas *et al*., 1995; Ivarson *et al*., 1997).

The age groups of the women were also considered (Table 1). The results showed that 32(35.6%) were in age group of 16-20 years, out of which 2(2.2%) were unprotected, and they fall within the range at which infection is likely to occur. The base rate of CMV Ig-G sero-prevalence in pregnant women was shown to decrease with age and was consistently high in women less than 30 years of age (60-66%) as reported by Fowler *et al* (1993, 1997, 2003). Considering the demographic characteristics of the women (Table 2), 2(2.2%) attended tertiary institutions, 13(14.4%) went to secondary schools and 9(10.0%) went to primary schools. On the other hand, 64(71.1%) of them went to only Arabic schools, out of which 2(2.2%) of them were not protected. Fifty-five (61.1%) of the women reside in urban areas out of which 2(2.2%) were unprotected while 33(36.7%) reside in rural areas. Fourty-nine (54.4%) were unemployed out of which 2(2.2%) were unprotected, 37(41.1%) were self-employed while 2(2.2%) were civil servants. Demographic factors also play important role in the prevalence of CMV infection. This is because the risk factors for CMV have been significantly ($P > 0.05$) correlated with the socio-economic status within a community (Fowler *et al*., 1997, 2003).

Looking at the stage of their pregnancies (Table3), 30(33.3%) were in their third trimester, 56(62.2%) in their second trimester while 2(2.2%) were in their first trimester. 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 (Pass and Boppana, 1999). Therefore, these women having little or non-protective Ig-G antibodies are likely to have first contact with the virus (primary infection). So, they lack enough Ig-G antibodies to protect their fetus and since primary infection with CMV during pregnancy is much more likely than recurrent maternal infection to produce symptoms and sequelea in infants (Fowler *et al*., 1992; Stagno *et al*., 1986), their fetus are at high risk. Fowler *et al* (1992) and Stagno *et al* (1986) also reported that 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. This can be the case with these 2(2.2%) unprotected women, because both of them are in their second trimester (Table3), so the tendency of transmitting the virus to their fetus is high.

There was a significant (P > 0.05) correlation between the risk factors and the level of CMV Ig-G antibodies, stage of pregnancy as well as the symptoms of pregnancy complications observed while it was insignificant (P < 0.05) in the case of number of previous pregnancies.

Table 1: CMV-IgG of protected and unprotected pregnant women according to their age group

Age Group (Years)	Number sampled	Number protected (%)	Number of unprotected (%)
16 - 20	34	32 (35.6)	2 (2.2)
21 - 25	22	22 (24.4)	-
26 - 30	19	19 (21.1)	-
31 - 35	7	7 (7.8)	-
36 - 40	7	7 (7.8)	-
41 - 45	1	1 (1.1)	-
Total	90	88 (97.8)	2 (2.2)

Table 2: Demographic characteristics of the pregnant women

Variables	Number sampled	Number protected (%)	Number unprotected (%)	χ2 Value
Education				0.86
Primary	9	9 (10.0)	-	-
Secondary	13	13 (14.4)	-	-
Tertiary	2	2 (2.2)	-	-
Arabic	66	64 (71.1)	2 (2.2)	-
Total	90	88 (97.8)	2 (2.2)	-
Occupation				0.32
Civil servant	2	2 (2.2)	-	-
Self employed	37	37 (41.1)	-	-
Unemployed	51	49 (54.4)	2 (2.2)	-
Total	90	88 (97.8)	2 (2.2)	-
Types of Residence				0.28
Urban	57	57 (61.1)	-	-
Rural	33	33 (36.7)	-	-
Total	90	88 (97.8)	2 (2.2)	-

Table 3: CMV- IgG antibodies according to pattern of previous pregnancy

Variables	Number sampled	Number protected (%)	Number unprotected (%)	χ2 Value
Stage of pregnancy				0.57
1st trimester	2	2 (2.2)	-	-
2nd trimester	58	56(62.2)	2(2.2)	-
3rd trimester	30	30 (33.3)	-	-
Total	90	88(97.8)	2(2.2)	-
Number of pregnancy	-	-	-	0.00
Nil	2	-	2(2.2)	-
1	22	22(22.2)	-	-
2	21	21(23.3)	-	-
3	10	10(11.1)	-	-
4+	35	35(38.9)	-	-
Total	90	88(97.8)	2(2.2)	-
Symptoms observed	-	-	-	0.97
Under weight	7	7(7.8)	-	-
Poor head dev	1	1(1.1)	-	-
Jaundice	1	1(1.1)	-	-
None	83	81(90.0)	2(2.2)	-
Total	90	88(97.8)	2(2.2)	-
No. of death at birth				
1	7	7(7.8)	-	-
2	5	5(5.6)	-	-
3	1	1(1.1)	-	-
None	77	75(83.3)	2(2.2)	-
Total	90	88(97.8)	2(2.2)	-

Conclusions and Recommendations

From the results obtained in this work, antibodies to CMV had been demonstrated in 98.7% of the pregnant women who attended Specialist Hospital and Maryam Abacha Women and Children Hospital, Sokoto. The age group that was most at risk was 16-30 years and, the stages of pregnancy at risk were second and third trimesters. Therefore, there is need for women of child-bearing age to be properly diagnosed. Follow-ups and management of CMV infected pregnant women should be adopted.

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