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SEROPREVALENCE OF CYTOMEGALOVIRUS INFECTION AMONGST PREGNANT WOMEN IN KADUNA STATE, NIGERIA

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ABSTRACT

Cytomegalovirus (CMV) is a major public health problem throughout the world. It is the leading cause of congenital infections leading to neurological disabilities in children that result to severe sequelae such as sensor neural hearing loss, neuro-developmental delay and blindness. This study was conducted to determine the seroprevalence of human cytomegalovirus among pregnant women in Kaduna State, Nigeria. Three hundred and sixty three (363) blood samples were collected from 330 pregnant women attending antenatal clinics and 33 non pregnant women attending the Outpatient Department in three selected General Hospitals in Kaduna State. Serum obtained from the blood samples were examined for the presence of CMV IgG antibodies by IFA method. About 94.8% of pregnant women tested positive to CMV IgG antibodies while all (100%) of the non pregnant women tested positive. There was no statistically significant association between CMV and pregnancy ($\chi^2=1.784$, $df=2$, $p=0.182$). Infection with CMV was strongly associated with zone location ($\chi^2=15.381$, $df=2$, $p=0.000$) and employment status of the women ($\chi^2=5.519$, $df=1$, $p=0.018$). There was no significant association between CMV infection and age, gravida, gestation age, education, number of marriages and history of previous transfusion. The high prevalence of CMV infection observed in this study indicates that the virus is prevalent in the study area and it is therefore advisable that routine screening of CMV infection be implemented for all antenatal women in the State.

Keywords: Seroprevalence, Cytomegalovirus, IgG, Pregnant women, Kaduna, Nigeria

SEROPREVALENCE D'INFECTION A CYTOMEGALOVIRUS PARMIS LES FEMMES ENCEINTES D'ETAT DE KADUNA, NIGERIA

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RÉSUMÉ

Le cytomégalovirus humain est un problème majeur de santé publique dans le monde entier. Il est la principale cause des infections congénitales conduisant à des séquelles neurologiques chez les enfants résultant des séquelles graves tels que la perte sensorielle de neurones auditifs, le retard de développement neurologique, la cécité. Cette étude a été menée pour déterminer la séroprévalence de cytomégalovirus humain parmi les femmes enceintes de l'Etat de Kaduna au Nigéria. Trois cents soixante trois (363) échantillons du sang étaient collectés chez 330 femmes enceintes en consultation prénatale cliniques, 33 femmes non enceintes fréquentant le service de consultation externe de trois hôpitaux généraux sélectionnés dans l'Etat de Kaduna. Les sérums obtenus des échantillons sanguins ont été analysés par la méthode IFA pour déterminer la présence des anticorps IgG anti-CMV. Environ 94,8% de femmes enceintes ont été testées positives aux anticorps anti-CMV tandis que toutes les femmes non enceintes étaient positives (100%). Il n'y avait pas une association statistiquement significative entre le cytomégalovirus humain (HCMV) et la grossesse ($\chi^2=1,784$, $ddl=2$, $p=0,182$). Les infections au cytomégalovirus ont été fortement associées à l'emplacement de la zone ($\chi^2=15,381$, $ddl=2$, $p=0,000$) et le statut d'emploi de femmes ($\chi^2=5,519$, $ddl=1$, $p=0,018$). Il n'y avait pas d'association significative entre l'infection à cytomégalovirus humain (HCMV) et l'âge, gravida, l'âge de gestation, l'éducation, le nombre de mariages et l'antécédent de la transfusion précédente. La forte prévalence de l'infection à CMV observée dans cette étude indique que le virus est répandu dans la zone d'étude et il est donc souhaitable que le dépistage systématique de l'infection à cytomégalovirus doit être mis en œuvre pour toutes les femmes en consultations prénatales dans l'Etat.

Mots clés: cytomégalovirus (CMV), IgG, femmes enceintes

INTRODCUTION

Cytomegalovirus (CMV) is a member of a family of 8 human herpesviruses (HHV) designated as HHV type 5 [1]. It belongs to the subfamily *Betaherpesvirinae* and the family *Herpesviridae*. Other members of *Betaherpesvirinae* include HHV types 6 and 7 which share common clinical characteristics with CMV [2]. Cytomegalovirus shares many attributes with other herpesviruses including genome, virion structures and ability to cause latent and persistent infection. It has a double stranded DNA with 162 hexagonal capsomeres surrounded by a lipid layer [2].

Cytomegalovirus is mainly a problem for certain high risk groups which include unborn babies whose mothers become infected with CMV during pregnancy and children or adults whose immune systems have been weakened by disease or drug treatment such as organ transplant recipients or people infected with HIV [1,3]. It is a common opportunistic infection among Human immunodeficiency virus (HIV) infected individuals, a major source of viral complication among organ transplant recipients and a leading cause of hearing loss, vision loss and mental retardation among congenitally infected children. More children suffer disabilities caused by congenital CMV than by several better known childhood maladies such as Down's syndrome or foetal alcohol syndrome [4]. Each year about 1 in 150 babies is born with congenital CMV infection and about 8,000 children develop lasting disabilities caused by congenital CMV infection [5]. The virus is the leading cause of congenital infection affecting between 0.3-0.6% of all live births in Europe [6].

Cytomegalovirus is usually an asymptomatic infection. In immunocompetent individuals, symptomatic disease usually manifest as infectious mononucleosis. It is characterised by malaise, headache, sore throat and fatigue. Fever is also commonly present and may persist for weeks in 98% of cases of cytomegalovirus mononucleosis. Lymphadenopathy, pharyngitis and splenomegaly are each seen in 30% of CMV patients [6].

Other clinical abnormalities of CMV infection in normal host include Guillain-Barre, retinitis, thrombocytopenia, gastrointestinal ulceration, hepatitis and pneumonia [1,6]. In few cases, there are symptoms at birth which include premature delivery, being small for gestational age, jaundice, rashes and feeding difficulties [4].

Cytomegalovirus is spread through close personal contact with people who excrete the virus in body fluids (e.g. saliva, urine, breast milk cervico-vaginal secretions and semen), by vertical transmission, through organ transplantation or via blood transfusion [6-8]. Mother to child transmission occurs by three routes described by Pass [6] which include; transplacental, intra-uterine and breast milk transmission.

Serological surveys have shown CMV infections in virtually every population that have been tested [9], with seropositivity ranging from 40 - 100% in different parts of the world [8,10]. For example, a serological survey of over 20,000 women in London found 54.4% of these women were seropositive for CMV [11]. It has been reported that African Continent has the highest prevalence of CMV IgG antibodies. Seroprevalence of 72.2% and 96.0% have been reported in Western Sudan and Egypt [12]. In Asian countries such as Malaysia, the seroprevalence of CMV IgG antibodies among pregnant women was reported to be 84.0% [13]. In Nigeria, serological survey conducted in Bida, Lagos and Sokoto among pregnant women found a seroprevalence of 84.2%, 97.2% and 98.7% respectively [9, 14,15]. These results shows that CMV is on the rise in Nigeria.

The prevalence of CMV has been studied in relation to other causes of congenital infections such as *Toxoplasma gondii* and rubella (TORCH agents) among pregnant women. In one of these comparative studies conducted in Turkey, CMV was found to be leading in prevalence with a seroprevalence of 96.4% for IgG, 0.7% for IgM and 1.9% for IgG + IgM [16]. Similarly, a study in Turkey found higher CMV seroprevalence of 94.4% for IgG and 0.4% for IgM [17].

Cytomegalovirus infection has been studied in relation to other infections in Nigeria. In a serological study carried out in Ibadan, Nigeria using complement fixation test (CFT), a seroprevalence of 86.6% among tuberculosis (Tb) patients, 50.6% among patients other than Tb patients and 54.6% among healthy blood donors was recorded. In general, a seroprevalence of 68.3% was obtained among all patients, with Tb patients having the highest rate [18]. Seroprevalence of CMV among blood donors in Nigeria is also high. Seroprevalence of 92.0% CMV IgG antibodies has been observed among blood donors in Jos [19]. Similarly, seroprevalence of CMV among paid and unpaid blood donors in Tirana, Albania gave an overall prevalence of 83% [10].

A case was reported in 2004, of three siblings from a monogamous family in Osun State, Nigeria who presented with a history of visual impairment/blindness due to CMV [20].

Primary infections occur between 1-4% in seronegative women during pregnancy and may be transmitted to foetus in 40% of cases [21-23]. Infected infants can develop hearing, vision, neurological and developmental problems over time. 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 [3,7].

Nevertheless, there is little awareness of CMV among medical personnel and the general public [7]. Research indicates that fewer than half of the obstetricians in U.S talk to their pregnant women about CMV infection [5]. In the same vein, Nigerian medical personnel are hardly aware of the presence of this virus and the damage it cause to the unborn foetuses, nor do they talk to their pregnant women about this virus. So seroprevalence studies of CMV in Nigeria are necessary to raise awareness of CMV infection and inform on appropriate and rational interventions in the country.

MATERIALS AND METHODS

A cross sectional study was carried out amongst 330 pregnant women attending antenatal clinic in selected hospitals from each of the three geographical zones of Kaduna State. The hospitals were Hajiya Gambo Sawaba Hospital (HGSH), Zaria from the North zone, Yusuf Dan Tsoho Memorial Hospital (YDMH), Kaduna from the Central zone and General Hospital Kafanchan (GHK), from the South zone.

Thirty three (33) female patients visiting the hospitals other than pregnant women were used as control population. From each woman, five ml (5ml) of blood was collected in plain vacuutainers, centrifuged on same day and the serum stored at -20°C. All samples were screened for CMV IgG antibodies using Immunofluorescence Antibody (IFA) technique CMV IgG kit (Diagnostic Automation, Inc., Calabatas USA).

Principles of Test

The DAI fluorescent CMV-IgG antibody test system is designed to detect circulating CMV-IgG antibodies in human sera. The system employs CMV infected substrate cells and FITC-labeled goat anti-human IgG (γ chain specific) adjusted for optimum use dilution and free of nonspecific background staining. The reaction occurs in two steps: The first step is the interaction of CMV antibodies in patient sera with CMV infected substrate cells. The second is the interaction of FITC-labeled anti-human IgG (γ chain) with the CMV-IgG antibody attached to the CMV localized in the nucleus of the infected cells.

Data Collection and Analysis

Consenting participants were instructed to fill a questionnaire to obtain information on demography, risk factors, and reproductive characteristic. This information included age, town of residence, gravida, gestational age, educational status, occupation, marital status, number of marriages, history of blood transfusion and history of congenital deformity.

Result and data from questionnaires were analysed using SPSS version 16 and the Pearson Chi square

test at 95% confidence interval and a significance level of 0.05 was used to determine the relationships between the variables and seroprevalence rates. Consenting women were recruited between the months of January and April 2011. Ethical approval was obtained from the Ethical Committee of Kaduna State Ministry of Health.

RESULTS

The prevalence of Cytomegalovirus was found to be 94.8% (313/330) among pregnant women, and all (100%: 33/33) the non pregnant women had antibodies to CMV (Table 1). There was no significant difference in the prevalence of CMV infection between the pregnant and non pregnant women ($\chi^2=1.784$, $df=1$, $P=0.182$)

Further analysis of the data based on geographical zoning, showed that there was a very strong significant difference in the distribution of CMV among the pregnant women by zonal location in Kaduna State ($\chi^2=15.381$, $df=2$, $P=0.000$). Women attending HGSH, Zaria from the North zone had the highest prevalence (99.1%: 109/110) while those attending YDMH, Kaduna from the Central zone had the lowest prevalence (88.2% : 97/110) (Table 2).

Table 1: Seroprevalence of Cytomegalovirus among Pregnant and Non Pregnant Women in Kaduna State

Subjects	Positive (%)	Negative (%)	Total
Pregnant women	313 (94.8)	17 (5.2)	330
Non-pregnant women	33 (100.0)	0 (0.0)	33
Total	346 (95.3)	17 (4.7)	363

$\chi^2=1.784$, $df=1$, $p=0.182$

Table 2: Seroprevalence of Cytomegalovirus among Women in Selected Hospitals in Kaduna State

Hospital	Positive (%)	Negative (%)	Total
GHK	107 (97.3)	3 (2.7)	110
HGSH	109 (99.1)	1 (0.9)	110
YDMH	97 (88.2)	13 (11.8)	110
TOTAL	313 (94.8)	17 (15.5)	330

$\chi^2=15.381$, $df=2$, $p=0.000$

Key: YDMH = Yusuf Dan-Tsoho Memorial Hospital.
HGSH= Hajiya Gambo Sawaba Hospital.
GHK= General Hospital Kafanchan.

Age as a risk factors for CMV infection was considered in this study and classified into seven groups. The result shows that there was no significant difference between age group and CMV

infection ($\chi^2=8.790$, $df=6$, $p = 0.186$). All the women in both age group 15-19 and >44 years had highest IgG antibodies to CMV while women in age group 40-44 had the lowest prevalence (85.7%: 6/7) (Table 3).

Table 3: Seroprevalence of Cytomegalovirus among Pregnant Women in Kaduna State by Age Group

Age group (years)	Positive (%)	Negative (%)	Total
15-19	24 (100.0)	0 (0.0)	24
20-24	116 (98.3)	2 (1.8)	118
25-29	79 (90.8)	8 (9.2)	87
30-34	60 (93.8)	4 (6.3)	64
35-39	26 (92.9)	2 (7.1)	28
40-44	6 (85.7)	1 (14.3)	7
>44	2 (100.0)	0 (0.0)	2
Total	313 (94.8)	17 (5.4)	330

$\chi^2=8.790$, $df=6$, $p=0.186$

Analysis of the result based on gravida shows that there was no significant difference between CMV infection and gravida ($\chi^2=0.889$, $df=4$, $p= 0.927$). All women with gravida above 8 had antibodies to CMV (100%: 15/15) and the lowest prevalence rate was recorded among women with gravida 7-8 (94.3%: 33/35) (Table 4).

Table 4: Seroprevalence of CMV among Pregnant Women in Kaduna State by Number of Gravida

Gravida	Positive (%)	Negative (%)	Total
1-2	111 (94.9)	6 (5.1)	117
3-4	85 (94.4)	5 (5.6)	90
5-6	69 (94.5)	4 (5.5)	73
7-8	33 (94.3)	2 (5.7)	35
>8	15 (100.0)	0 (0.0)	15
Total	313 (94.8)	17 (5.2)	330

$\chi^2=0.884$, $df=4$, $P=0.927$

Further, analysis of risk factors for CMV infection among the pregnant women based on gestation period showed that there was no significant difference between gestation and CMV infection ($\chi^2=1.661$, $df=2$, $p=0.436$). Highest prevalence was however recorded in the second trimester (95.8%: 207/216) while the lowest was recorded in the third trimester (Table 5).

Analysis of the the prevalence obtained based on blood transfusion in this study also showed that there was no association between transfusion and CMV infection ($\chi^2=0.074$, $df=1$, $p=0.789$). A higher prevalence rate of 94.9% (299/315) was however recorded among women that had never undergone transfusion against those who had (93.3%: 14/15) (Table 6)

Further analysis of the results based on socio-demographic data such as employment status, educational status and number of marriages shows that there was no statistically significant association between the virus and educational status and number of marriages the women had been in ($p>0.05$). There was however a strong association between CMV infection and employment ($p=0.0180$). Highest seroprevalence of CMV was obtained among women with no formal education (95.9%: 70/73) and the lowest among women who reached tertiary levels of education (90.3%: 28/33) (Table 7)

Table 5: Seroprevalence of CMV among Pregnant Women in Kaduna State by Gestational Age

Gestational age (months)	Positive (%)	Negative (%)	Total
First trimester	22 (95.7)	1 (4.3)	23
Second trimester	207 (95.8)	9 (4.2)	216
Third trimester	84 (92.3)	7 (7.7)	91
Total	313 (94.8)	17 (5.2)	330

$\chi^2=1.661$, $df=2$, $P=0.436$

Table 6: Seroprevalence of Cytomegalovirus Infection in Pregnant Women in Relation to History of Blood Transfusion in Kaduna State

Transfusion History	Positive (%)	Negative (%)	Total
Not transfused	299 (94.9)	16 (5.1)	315
Transfused	14 (93.3)	1 (6.7)	15
Total	313 (94.8)	17 (5.2)	330

$\chi^2=0.074$, $df=1$, $P=0.789$

DISCUSSION

The results obtained from this study show that the seroprevalence rate of CMV infection among pregnant women in Kaduna State is high. A seroprevalence of 94.8% was obtained in this study. This result is similar to the seroprevalence of 84.2% among pregnant women reported in Bida, Nigeria, 97.2% among pregnant women reported in Lagos, Nigeria and 98.7% among pregnant women

reported in Sokoto State, Nigeria [9,14,15]. This prevalence agrees with the assertion of Saraswathy et al. [13], that African continent has the highest prevalence rate of CMV IgG antibodies.

There was no significant association between the seroprevalence of CMV in pregnant and non pregnant women. This could be due to the low sample size of non pregnant women compared to that of pregnant women. However the result is similar to that reported in Southeast Brazil [24]. The reason for the high prevalence of CMV infection among pregnant women in Kaduna State may not be unconnected to the depreciating socioeconomic standard, poor hygienic practices and low standard of education among these women.

High seroprevalence of CMV infection in Kaduna State may have serious consequences on children in Kaduna State. It has been reported, that the incidence of congenital CMV infection depends on

epidemiological characteristics of a population, in particular the maternal CMV seroprevalence [25]. High rates of congenital CMV infection have been consistently demonstrated in populations with high CMV seroprevalence [25]. Hence, the high prevalence rate of CMV infection among pregnant women in Kaduna state implies there might be a corresponding high incidence of congenital CMV infection among infants born in the state. In the study carried out by Mussi-Pinhata et al. [25], it was found that congenital CMV disease occurs in populations with high seroprevalence rates, with a similar incidence of CMV-related hearing loss. This also implies that there may be increased incidence of congenital CMV infection among infants in Kaduna state as a result of high seroprevalence of CMV infection in pregnant women; This might also lead to a similar increase in the incidence of CMV-related hearing and vision loss in children in the state.

Table 7: Seroprevalence of Cytomegalovirus among Pregnant Women in Kaduna State by Sociodemographic Data

Variable	No Positive (%)	No Negative (%)	Total	p-value
No of marriages				
1	290 (95.1)	15 (4.9)	305	
2	22 (91.7)	2 (8.3)	24	0.746
3	1 (100)	0 (0.0)	1	
Employment				
No	200 (97.1)	6 (2.9)	206	
Yes	113 (91.1)	11 (8.9)	124	0.018
Education				
No formal education	70 (95.9)	3 (4.1)	73	
Primary	79 (95.2)	4 (4.8)	83	0.682
Secondary	136 (95.1)	7 (4.9)	143	
Tertiary	28 (90.3)	3 (9.7)	31	

There was a strong association between the seroprevalence of CMV infection and zone of location of the women; with Zaria zone having the highest seroprevalence rate and Kaduna zone having the lowest. Most of the women enrolled in the study from Zaria zone were resident in Zaria City where there is overcrowding and congestion of settlement leading to poor hygiene and condition of living. It was also noticed that majority of women from Zaria zone have low education and are non employed which could possibly account for the highest seroprevalence [12]. The lowest prevalence rate obtained in Kaduna zone might be as a result of enlightenment as the women are more educated and have better hygienic environments and condition of living. This result agrees with the report of Ludwig and Hengel [7] who asserted that seroprevalence of CMV differs between countries and regions.

There was no association between women who had been transfused and those that were not and seroprevalence was higher among those that were not transfused than those that had been transfused. This result disagrees with the report of Matos et al. [8] where blood transfusion was shown to be a risk factor for transmission of CMV infection. The reason for the disagreement here could be as a result of the disproportionate size of women who were transfused to those who were not transfused enrolled in the study.

Analysis of the result by age shows that there was no significant association between CMV and age contrary to previous report [9, 26] that used ELISA method. There was no predictable pattern between seroprevalence and age, even though the youngest and oldest age gave the highest prevalence. The highest seroprevalence among the youngest age group could be explained by increase sexual activities of this age group as the virus can be transmitted sexually while longer duration of exposure to the virus might be responsible for the highest prevalence seen in the older age group.

Eventhough the seroprevalence of CMV was highest in the second trimester, there was no significant difference between the seroprevalence and gestation period. This result agrees with previous findings [9,15] and could be due to the fact that most pregnant women report for antenatal in their second trimester than in the first and third trimester.

Similarly, there was no significant association between gravida and CMV infection in this study and this agrees with the findings of Okwori et al. [9]. The pattern of change of seroprevalence with There is no licenced vaccine so far against Cytomegalovirus infection except those currently under investigation [12]. Hence, the only means to prevent transmission of CMV among pregnant

gravida was not clear in this study, though the highest prevalence rate was found in gravida >8. This result agrees with the findings of Hamdan et al. [12] that reported high gravida to be a significant risk factor for CMV infection. This could be so as increase in gravida could imply increase in parity which also implies increase in age, which according to previous reports [7, 26] is a significant predictor of CMV infection.

Seroprevalence of CMV infection by employment status which is one of the predictors of socioeconomic status was higher among the non employed. There was a strong association between employment status and CMV infection in this study. Socioeconomic status has been shown to be a risk factor for CMV infection [27]. The reason for this is probably because, high socioeconomic status implies ability to acquire education and afford better and healthy living conditions which decreases exposure to the virus.

The distribution of seroprevalence rates of CMV infection among pregnant women by number of marriages shows that women who had been involved in up to three marriages in their life time had the highest seroprevalence rate. However this difference was not statistically significant. This result disagrees with previous findings [26]. This observed non statistical difference may have arisen from the disproportionate composition of women in the various groups.

In this study, the seroprevalence rate decreased insignificantly with increase in education. The decrease in seroprevalence agrees with a previous report [12] that showed that illiterate women are at higher risk of CMV infection due to contact with contagious secretions from their own children and poor hygienic practice.

CONCLUSION

The seroprevalence of 94.8% obtained in this study shows that CMV is highly associated with pregnant women in Kaduna State. This implies that there might be a corresponding increase in the incidence of congenital CMV infection hence similar increase in the incidence of CMV-related hearing loss, vision loss, microcephaly and poor mental development among children in Kaduna State. Zone location and employment status were statistically associated with CMV infection. Other risk factors such as history of blood transfusion, gestation, parity, number of marriages and educational status did not show any significant association.

RECOMMENDATIONS

women is to create awareness through health talk during antenatal on the transmission, consequences of infection on foetus and its control and preventive measures.

Routine screening of pregnant women for CMV should be adopted in all health care facilities and blood should be properly screened for CMV before transfusion to pregnant women.

Babies born to seropositive mothers should be screened and examined immediately after delivery for possible signs of hearing and vision defects for early management.

More studies are required in order to ascertain the incidence rate of CMV infection among pregnant women in Kaduna State through determination of IgM and IgG avidity test.

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