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Seropositivity of Anti-CMV IgM Antibody among Women of Child-Bearing Age Attending Selected Hospitals in Kaduna State, Nigeria

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Abstract

Human Cytomegalovirus is one of the most common cause of congenital viral infections. The study was conducted to determine the seroprevalence of Human Cytomegalovirus among women of child-bearing age attending selected hospitals in Kaduna State, Nigeria. A total of 228 blood samples were obtained from the women and processed serologically using Enzyme Linked Immunosorbent Assay (ELISA). In addition, structured questionnaire was used to determine socio-demographic and risk factors associated with Human Cytomegalovirus infection. Out of the 228 women, 215 (94.3%) were positive for anti-CMV IgM. The highest seroprevalence was observed among women belonging to age group 15-24 (98.8%), those with secondary level of education (98%), those living in the urban areas (95%), the civil servants (100%), the married (94.2%) and those of medium socioeconomic status (94.9%). A statistical significant difference was observed between the age groups only (p = 0.000). With respect to risk factors, female patients; with no sex partners (100%), with history of blood transfusion (96.7%), who do not wash their hands with soap after changing baby's diaper (95.5%), who wash their hands after contact with children's urine (94.7%) and those in close contact with children had highest seroprevalence (94.4%). No statistical significant association was observed between Human Cytomegalovirus infection and the risk factors considered. Female patients should be educated on the transmission routes as well as preventive measures of Human cytomegalovirus infections.

Keywords

Seropositivity, Anti-CMV IgM, Child-bearing age women, Kaduna

Introduction

Congenital infections are significant cause of perinatal morbidity and mortality estimated to be responsible for up to 50% of stillbirths in low and middle income countries and 10-25% in high income countries [1]. Human Cytomegalovirus (HCMV), an ubiquitous herpes virus with the highest morbidity and mortality compared to other herpes viruses [2] is one of the most common cause of congenital anomaly. The incidence of congenital Human Cytomegalovirus infection has increased by nearly 300% since the early 2000s [3].

Maternal HCMV infection could result to bad pregnancy outcome including abortion, premature delivery, stillbirth and congenital malformation [4,5]. Sexual activity and contact with young children are the main sources of primary maternal HCMV infection (Fowler and Pass, 2006). Primary HCMV infection occurs in 0.7-4.1% of all pregnancies [6]. About 32% of women with primary infection during pregnancy, transmit the virus across the placenta to produce intrauterine infection [7].

Human Cytomegalovirus is the most common infectious cause of fetal malformations [8]. Congenital HCMV affects one in every 150 live births globally [9]. Approximately 7 to 10% of infants with congenital HCMV develop clinical manifestations. These include petechiae,

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jaundice, hepatosplenomegaly, chorioretinitis as well as neurological deficits, which consists of physical and mental retardation, deafness, and even death in about 10% of patients [10-12].

Factors associated with Human Cytomegalovirus infection include age, socioeconomic status, exposure to young children, race/ethnicity [8,13-15]. In addition, increasing number of live births, early sexual debut, ≥ 10 life time sexual partners, and herpes type II seropositivity also predispose one to HCMV infection [15].

Despite the fact that most women of reproductive age in developing countries are assumed to have immunity against HCMV because of the high seroprevalence, the prevalence of congenital HCMV is still higher in developing countries. Most previous studies on HCMV were conducted on pregnant women and that necessitate the need to include non-pregnant women of reproductive age since maternal HCMV infection can occur prior to or during pregnancy.

Objectives of the Study

1. To determine the seroprevalence of anti-CMV IgM using Enzyme Linked Immunosorbent Assay (ELISA).

2. To determine socio-demographic and risk factors associated with Human Cytomegalovirus infection using structured questionnaire.

Methodology

Study area

The study was conducted in Kaduna State, which is found in Northern part of Nigeria. Kaduna State is a cosmopolitan state owing to the various educational institutions, research institutes and other governmental and non-governmental parastatals situated which attract people from different parts of the country. The state is divided into three senatorial zones; North zone, Central zone and South zone. The hospitals selected include: Hajiya Gambo Sawaba General Hospital in Zaria from North zone, Yusuf Dantsoho Memorial Hospital in Kaduna metropolis from Central zone and Sir Patrick Ibrahim Yakowa General Hospital Kafanchan from South zone.

Study design

The study is a hospital-based, cross-sectional and descriptive research aimed at women of child-bearing age between the ages of 15-50 years attending Hajiya Gambo Sawaba General Hospital Zaria, Yusuf Dantsoho memorial Hospital, Kaduna and Sir Patrick Ibrahim Yakowa General Hospital, Kafanchan.

Ethical approval and informed consent

Ethical clearance was obtained from the Research Ethics committee of Kaduna State Ministry of Health. Permission to conduct research was sought from the Management of the respective study hospitals. In addition, informed consent of the patients was sought for participation in the study.

Sample size determination

The sample size of the study was determined using a previous prevalence of 94.8% [16] for Human Cytomegalovirus in Kaduna state. The formula described by Naing, et al. [17] was employed. Seventy-six samples were collected from each of the study hospital.

Questionnaire administration and sample collection

Structured questionnaire was administered to the patients to determine socio-demographic and risk factors associated with Human Cytomegalovirus infection. Two milliliters of blood were collected from the patients using a sterile syringe and transferred into plain tubes. The blood samples were transported in insulated cooler containing ice packs to the Laboratory of Department of Microbiology, Ahmadu Bello University, Zaria for analysis. The blood samples were processed according to the method described by Umeh, et al. [18]. Sera from the blood samples were separated by allowing the blood to clot at room temperature and centrifuged at 2000 rpm for 10 minutes. The sera were transferred into cryovials using clean Pasteur pipettes and stored at -20 °C until required for further analysis.

Detection of human anti-cytomegalovirus IgG using enzyme linked immunosorbent assay (ELISA)

All patients' sera were tested for anti-CMV IgM using commercially available ELISA kits (Diagnostic Automation, USA). The procedure was carried out according to manufacturer's instructions. Briefly, 1:40 dilutions were prepared by adding 5 µL of the test samples, negative control, positive control, and calibrators to 200 µL of sample diluent. It was mixed well. Hundred microliter (100 µL) of diluted sera, calibrator, and controls were dispensed into appropriate wells. Hundred microliter (100 µL) of sample diluent was added to the reagent blank well. The plate was incubated at room temperature for 30 minutes. The liquid was removed from all wells by decanting. This was followed by washing the wells with washing buffer and the washing was repeated three times. Hundred microliter (100 µL) of enzyme conjugate was added to each well and incubated for 30 minutes at room temperature. Enzyme conjugate was removed from all wells by decanting and washing was repeated three times with washing buffer. Hundred microliter (100 µL) of tetramethylbenzidine (TMB) chromogenic substrate was added to each well and incubated for 15 minutes at room temperature. This was followed by adding 100 µL of stop solution to stop the reaction. The optical density of each well was read at 450 nm with a microwell reader

and interpreted. Samples having CMV G index ≥ 1 were considered positive while those with CMV G index ≤ 0.9 were considered negative.

Data analysis

Odds ratio and Chi-square were used at 95% confidence interval to determine association and significant difference between the variables respectively. P-values ≤ 0.05 were considered as statistically significant.

Results

The overall seroprevalence of Human Cytomegalovirus was 94.3% (Figure 1). Out of the 228 patients examined in the three study hospitals, 215(94.3) were positive for anti-CMV IgM. The highest seroprevalence was observed in HGSGH with 100% (Table 1). Female patients in age-group 15-24 years, those with secondary level of education, those living in the urban areas, the civil servants and the middle class patients had the highest seroprevalence with 98.8%, 98%, 95%, 100% and 94.9% respectively (Table 2). The highest seroprevalence was observed in non-pregnant women (95.2%), pregnant women in their second trimester of pregnancy (95.1%), those with no child and one child (100%), women who had no history of miscarriage (94.7%), those with history of still-birth (100%), women with history of premature delivery (95.2%) and those without disabled child (94.2%) (Table 3). Patients having no sex partners, those with history of blood transfusion, women who do not wash their hands after changing baby's diaper, those who wash their hands after coming in contact with children's urine and those in close contact with children had higher seroprevalence with 100%, 96.7%, 95.5%, 94.7%, 94.4% respectively (Table 4).

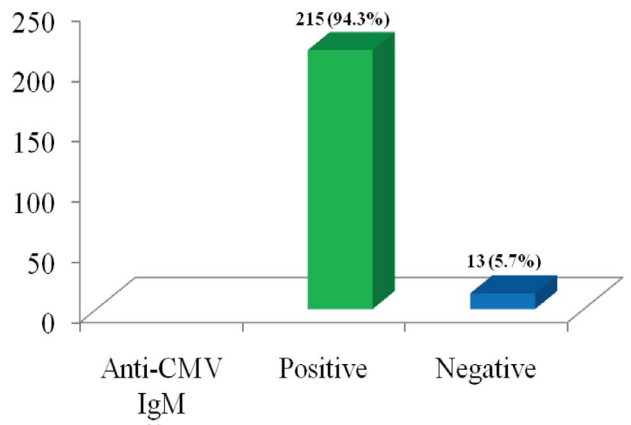

Figure 1: Overall seroprevalence of Human Cytomegalovirus.

Table 1: Distribution of Human Cytomegalovirus in relation to study hospitals.

Study hospital	No. analysed	No. (%)	χ^2-value	p-value
HGSGH	76	76 (100)	21.699	0.000
YDMH	76	75 (98.7)		
PIYGH	76	64 (84.2)		
Total	228	215 (94.3)		

Key: HGSGH- Hajiya Gambo Sawaba General Hospital; YDMH- Yusuf Dantsoho Memorial Hospital; PIYGH- Patrick Ibrahim Yakowa General Hospital; χ^2 - Chi-square

Discussion

A seroprevalence of 94.3% was obtained for anti-CMV IgM among women of reproductive age attending selected hospitals in Kaduna State, Nigeria. Our finding is similar to 94.3% IgM seroprevalence reported by Nahla, et al. [19] among neonates in Sudan. The high IgM seroprevalence observed in the study is a suggestion that an epidemic might have occurred

Table 2: Seroprevalence of Human Cytomegalovirus with respect to socio-demographic factors.

Socio-demographic factor	No. analysed	No. (%)	χ^2-value	p-value
Age group (yrs)			19.531	0.000
15-24	83	82(98.8)		
25-34	87	85(97.7)		
35-44	48	40(83.3)		
45 and above	10	8(80)		
Educational status			8.967	0.062
None	1	1(100)		
Informal	45	41(91.1)		
Primary	45	39(86.7)		
Secondary	102	100(98)		
Tertiary	35	34(97.1)		
Area of residence			0.368	0.832
Rural	41	38(92.7)		
Semi-urban	47	44(93.6)		
Urban	140	133(95)		
Occupation			1.984	0.371
Civil servant	9	9(100)		
Self-employed	135	125(92.6)		
Unemployed	84	81(96.4)		
Socio-economic status			1.187	0.276
Low	30	27(90)		
Medium	198	188(94.9)		
High	0	0(0)		
Marital status			0.309	0.958
Single	17	16(94.1)		
Married	206	194(94.2)		
Divorced	4	4(100)		
Widow	1	1(100)		

Table 3: Seroprevalence of Human Cytomegalovirus with respect to reproductive history.

Reproductive characteristics	No. tested	No. (%)	Statistics
Pregnancy			OR- 0.8611
Yes	164	155(94.5)	CI- 0.2254-3.2893
No	64	60(93.8)	p-0.8269
			χ^2- 0.050
			p- 0.824
Gestational age			
1st trimester	20	18(90)	χ^2- 0.946
2nd trimester	102	97(95.1)	p- 0.814
3rd trimester	43	41(95.3)	
History of miscarriage			OR- 0.8225
Yes	78	73(93.6)	CI- 0.2598-2.6042
No	150	142(94.7)	p- 0.7397
			χ^2- 0.111
			p- 0.739
History of stillbirth			OR-3.2961
Yes	23	23(100)	CI- 0.1897-57.2696
No	205	192(93.7)	p- 0.4129
			χ^2- 1.547
			p- 0.214
History of premature delivery			OR- 1.2308
Yes	21	20(95.2)	CI- 0.1520-9.9636
No	207	195(94.2)	p- 0.8457
			χ^2- 0.038
			p- 0.845
Congenitally infected child			OR- 0.1127
Yes	3	2(66.7)	CI- 0.0095-1.3320
No	225	213(94.7)	p- 0.0832
			χ^2- 4.317
			p- 0.038
No. of children			
None	39	39(100)	χ^2- 6.235
One	32	32(100)	p- 0.101
Two	60	55(91.7)	
Three and above	97	89(91.8)	

CI: Confidence Interval; OR: Odds Ratio

Table 4: Seroprevalence of Human Cytomegalovirus with respect to risk factors.

Risk factor	No. analyzed	No. (%)	Statistics
No. of sex partners			
None	11	11 (100.0)	χ^2- 0.703
Single	152	143 (94.1)	p- 0.703
Multiple	65	61 (93.8)	
History of Blood transfusion			OR- 1.8710
Yes	30	29 (96.7)	CI- 0.2344-14.9346
No	198	186 (93.3)	p- 0.0832
			χ^2- 0.360
			p- 0.540
Washing hands with soap after changing diaper			OR- 0.7078

Yes	161	151 (93.8)	CI- 0.1885-2.6574
No	67	64 (95.5)	P- 0.6087
			χ^2- 0.264
			p- 0.607
Washing hands with soap after coming in contact with children's urine			OR- 1.1302
Yes	94	89 (94.7)	CI- 0.3579-3.5688
No	134	126 (94.0)	p- 0.8348
			χ^2- 0.044
			p- 0.835
Close contact with children			OR- 1.5455
Yes	216	204 (94.4)	CI- 0.1840-12.9831
No	12	11 (91.7)	p- 0.6885
			χ^2- 0.163
			p- 0.686

during the time of the study. As symptoms of Human Cytomegalovirus infections mimic those of other viral infections, the outbreak might have gone unnoticed particularly in developing countries like Nigeria where outbreaks may not always be recognized.

Since studies have demonstrated high congenital Human Cytomegalovirus infections in population with high seroprevalence, the implications of this finding is that there may be a corresponding increase in congenital Human Cytomegalovirus infections among infants in the state. This may lead to rise in HCMV-related hearing and vision loss in children. Maternal HCMV infection is regarded as a predisposing factor for congenital HCMV infection and HCMV-associated hearing loss in children [20].

A sample positive for anti-CMV IgM is not always indicative of recent infection. It may be due to reactivation as a result of the same virus or re-infection with a new or different strain of HCMV. In addition, specific IgM antibody may still be detected for several months or years in low titers after primary infection [21].

However, lower seroprevalences of anti-CMV IgM of 6.0%, 8.9%, 10.5%, 38.5%, 43.6%, 11.8% and 29.3% have been reported in Sudan [22], Kano [23], Kafanchan [24], Ghana [25], Abakaliki [26], Kaduna [27] and Zaria [28] respectively.

In relation to study hospitals, female patients who attended Hajiya Gambo Sawaba General Hospital (HGSGH) had the highest seroprevalence for anti-CMV IgM 100%. This may be as a result of low socioeconomic status and poor hygiene of the women. This is similar to the findings of Yusuf, et al. [28] who observed difference in relation to location.

Women in age group 15-24 years had the highest IgM seroprevalence which is comparable to the findings of Aliyu, et al. [27] who reported highest seroprevalence among women between 16-20 years. The findings contradicts the reports of Khairi, et al. [22], Pathmavathy, et al. [29] and Yusuf, et al. [28] who observed highest seroprevalence among ≥ 40 years, 25-30 years and 21-30 years respectively. Most of the women in that age group are married and it may be as a result of increased sexual activity.

Women who had no congenitally infected child had higher seroprevalence than those with congenitally infected child. This shows that the child's anomaly could be as a result of other agents responsible for congenital abnormalities.

Conclusion and Recommendation

A seroprevalence of 94.3% was obtained for anti-CMV IgM among women of reproductive age attending selected hospitals in Kaduna State, Nigeria. Women in age group 15-24 years had the highest seroprevalence. Female patients should be educated on the transmission routes as well as preventive measures of cytomegalovirus infections.

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The authors declare no conflicts of interest.

Contribution of Authors

Anchau, Z.G.: Study design, literature search, data aquisation, data analysis, manuscript preparation; Suleiman, A.B.: Study design, manuscript editing, manuscript review; Olonitola, O.S.: Study design, manuscript editing, manuscript review; Kwanashie, C.N.: Study design, manuscript editing, manuscript review.

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