Prevalence and Risk Factors Associated with Human Cytomegalovirus and Human Immunodeficiency Virus Coinfection in Pregnant Women in Ilorin, Nigeria

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Abstract: Human Cytomegalovirus (HCMV) and Human Immunodeficiency (HIV) viral infection in pregnant patient is associated with high risk of maternal complications. This was a cross sectional study aimed to determine the prevalence and risk factors of HCMV and HIV mono-infections among 230 pregnant women attending ante-natal clinic of Sobi specialist hospital Ilorin, Nigeria. Data of consenting participant was collected via interviewer-administered questionnaire and clinical report form before collection of blood sample for analysis. The extracted serums were screened for HIV and CMV using rapid test-kits (Aleredetemine ^{TM}HIV1/2) and ELISA for detection of anti-CMV IgG and IgM respectively. All 230 participants were negative to HIV infection while 97.8% and 3.0% was CMV IgG and IgM positive respectively. High positivity was recorded across all age group with 15-19 having 100% anti-CMV IgG while the 25-29 and 30-34 years age group had highest anti-CMV IgM (P=0.907). 168(73.0%) of 171(74.3%) and 57(24.8%) of 59(25.7%) subjects within the parous and nulliparous group respectively were CMV-IgG positive while highest IgM positivity was noticed among the parous group (P=0.828). High CMV-IgG sero-positivity was also noticed across all level of education (P=0.700), marital status (P=0.668), types of marriage (P=0.008) and blood transfusion status (P=0.479). All the risk factors considered for HIV were very low while those for CMV had high sero-positivity for both current and past infections. The high level of CMV among pregnant women noticed in this study could be responsible for the congenital infection among newborns prevalent in the study area.

Keywords: Sero-prevalence, HIV, CMV, Co-infection.

Introduction

Human Cytomegalovirus (HCMV) is a member of the Betaherpesvirinae subfamily of herpersviridae with HIV being a member of the genus Lentivirus and family Retroviridae [1]. CMV is a common viral pathogen affecting the bulk of the world's populace by early middle age [2] with 50 to 80% of adults infected at 40 years [3]. Immuno-competent individuals typically display no symptoms of infection [4] but in individuals with compromised immune system (immature and/or weakened) such as organ transplant or Acquired Immunodeficiency Syndrome (AIDS) patients, morbidity and mortality due to HCMV as a significant pathogen is hardly avoidable [5]. These individuals show different symptoms which include; spiking fever, leucopenia (decrease in white blood cell), malaise, hepatitis, pneumonia, gastrointestinal disease and retinitis [4]. HCMV is also to blame for just about 8% of infectious mononucleosis cases [6] and is also implicated in birth defects often leading to deafness and mental retardation in the foetus if the woman is infected during pregnancy [7].

The virus also spreads in households and young children in day care centers [8] with person to person transmission requiring close intimate contact with a carrier of the virus either in saliva, urine, or other bodily fluids, it can also be transmitted sexually, via breast milk, organ transplant and blood transfusion [9]. Cytomegalovirus (CMV) persists in the host for life [10] and recurrent infections are common during pregnancy constituting the majority of congenital infections in women with high prevalence of CMV-IgG [11].

In different populations, the sero-prevalence of CMV-IgG among women of reproductive age ranges from 30% to 100% [12] and the main influencing elements of CMV-IgG variability are geographical location, socioeconomic status (SES), the woman's age and parity and most importantly hygiene level [13]. The highest CMV-IgG sero-prevalence in pregnant women of 70–100% has been observed in Africa, Asia and South America [14] while pregnant women of developed countries have demonstrated lower CMV-IgG sero-prevalence levels of about 30–50% [15].

In HIV patients, HCMV reactivations and re-infections are common, and they are a major cause of congenital infection in infants worldwide [16]. In most cases of CMV infection among healthy people, infection is usually asymptomatic, with few symptoms in some cases. Therefore, for the vast majority of individuals, CMV infection is harmless. In Lagos, Nigeria it was reported that 35(14.8%) patients: 10(4.2%) males and 25(10.6%) females were positive for HCMV infection [17]. In Maiduguri, Nigeria it was reported that the sero-prevalence of anti-CMV IgG was 100% among HIV-infected patients. Having multiple sexual partners, traditional practices such as tattooing and cupping, and blood transfusion were significant risk factor among these cases [1].

CMV infection in pregnant women is of great concern because of their immune-compromised state and risk of infection to the foetus whose immune system is not fully developed. The prevalence of CMV deduced from three locations in Nigeria namely Bida (84.2%), Lagos (97.2%) and Sokoto (98.7%) among pregnant women reveals that the infection is on the increase. This study aims to provide baseline information about HCMV and HIV co-infection among pregnant women attending ante-natal clinic in the study area which will

help in management of the infection and prevent secondary spread.

Materials and Methods
Study Setting
This research was conducted at the Ante-natal Clinic of Sobi Specialist Hospital Ilorin, Kwara State, Nigeria. Ilorin, the capital of Kwara State in Nigeria is located on 8^o30^oN 4^o33^oE /8.500^oN 4.550^oE.

Study Population and Sample Size
The target population were pregnant women attending ante-natal Clinic of Sobi Specialist Hospital, Ilorin. The sample size derived using Fishers formula [19] was 230.

Data and Sample Collection
Interested participants were administered an informed consent form and a structured close ended questionnaire before collection of blood samples for analysis.

Assay
The blood samples were kept in an Ethylene Diamine Tetra-acetic Acid (EDTA) anti-coagulant., after collection via needle and syringe. This was centrifuged at 3000 r.p.m. for 5 minutes; the serum was harvested into clean sterile plain bottles using Pasteur pipette and pipette tips and then frozen at -20°C for assay.

HIV assay
HIV screening of the samples was achieved by using the rapid test kits (Aleredetermine^{TM} HIV1/2) following the manufacturers instruction.

CMV assay
Enzyme Linked Immunosorbent Assay method (ELISA) was used and it has been shown to be a sensitive and reliable procedure for detection of CMV antibodies with diagnostic sensitivity and specificity of 98% and $\geq$ 98% respectively (Rapid Labs Limited, UK). All samples were assayed for CMV-IgG and positive ones were further assayed for CMV-IgM to confirm current infections.

The manufacturer's conditions for valid assay were met and all the entire process was according to the manufacturer's specific instructions (Rapidslab UK, 2014)

Data Analysis
The data from the questionnaire was analyzed using X^2 (chi-square) variable while test for statistical association was done using SPSS version 17.0.

Ethical Considerations
Approval for the study was obtained from Ethical Committee of Ministry of Health, Ilorin. Informed consents were obtained from patients and the study was at no cost to the subjects. Information from the patient was held confidential.

Results
Out of the 230 participants, no case of HIV infection was found and analysis of its risk factors showed that 7.83% had undergone surgery while most respondent (92.17%) had not, 4.8% have had tattoo as against those (95.2%) who did not, while few respondents (10.40%) had history of blood transfusion. Based on multiple sex partners, 22.10% of the population had multiple sex partners while 76.10% had single partners.

From a total number of 230 samples that were screened for CMV-IgG, 225 (97.8%) tested positive, 5 (2.2%) were negative and none was equivocal while IgM assay for CMV-IgG positive subjects results show that 7(3.1%) were positive, 217(96.4%) were negative and 1(0.4%) was equivocal.

All the 8(3.5%) subjects that were single and 217(94.3%) of 222 (96.5%) that were married tested positive CMV-IgG (P=0.668) while 6(2.7%) of 217(96.4%) married subject and 1(0.4%) of the 8(3.6%) single subject were positive when assayed for CMV-IgM antibodies (P=0.293). The only equivocal result was recorded among the married women.

The educational level of the respondent revealed that 9(3.9%) subjects without any form of education, 40(17.4%) of 42(18.3%) that attended only primary school, 79(34.3%) of 81(35.2%) with secondary education and 92(40.0%) of 93(40.4%) subjects that had tertiary education were all positive to CMV-IgG (P=0.700). Out of the 79 (35.1%) subjects in the secondary education

group, only 1(0.4%) was positive while 3(1.3%) of 40(17.8%) subjects in primary group and 3(1.3%) of 92(40.9%) subjects in tertiary group were positive to CMV-IgM antibodies (P=0.373).

The CMV IgG and IgM status of subjects in relation to the parity status revealed that 168(73.0%) out of 171(74.3%) subjects within the parous group and 57(24.8%) of 59(25.7%) subjects in nulliparous group, tested positive to CMV-IgG (P=0.458) while results from CMV-IgM assay revealed that 5(2.2%) out of 168(74.7%) subjects within the parous group and 2(0.9%) out of 57(25.3%) subjects that are nulliparous were positive. One equivocal result was recorded among subjects in the parous group (P=0.828).

Table 1: Cytomegalovirus IgG and IgM prevalence in relation to age

		15-19	20-24	25-29	30-34	35-39		40-44	NO INDICATION	Total (%)	P value	X²
						Age(yrs)						
IgG	POSITIVE (%)	6(2.6)	36(15.7)	102(44.3)	50(21.7)	21(9.1)	6(2.6)	4(1.7)	225(97.8)	0.883	2.371	
	NEGATIVE(%)	0(0.0)	1(0.4)	1(0.4)	2(0.9)	1(0.4)	0(0.0)	0(0.0)	5(2.2)			
	Total (%)	6(2.6)	37(16.1)	103(44.8)	52(22.6)	22(9.6)	6(2.6)	4(1.7)	230(100)			
IgM	POSITIVE (%)	0(0.0)	1(0.4)	3(1.3)	3(1.3)	0(0.0)	0(0.0)	0(0.0)	7(3.1)	0.907	6.172	
	NEGATIVE(%)	6(2.7)	35(15.6)	99(44.0)	46(20.4)	21(9.3)	6(2.7)	4(1.8)	217(96.4)			
	EQUIVOCAL(%)	0(0.0)	0(0.0)	0(0.0)	1(0.4)	0(0.0)	0(0.0)	0(0.0)	1(0.4)			
	Total (%)	6(2.7)	36(16.0)	102(45.3)	50(22.2)	21(9.3)	6(2.7)	4(1.8)	225(100.0)			

P value < 0.05 is statistically significant
X^2—chi square value

Table 2: Sero-prevalence of CMV IgG and IgM in relation to marriage type of respondent

		MARRIAGE TYPE			Total(%)	Pvalue	X^2
		MONOGAMY	POLYGAMY	NO INDICATION			
IgG	POSITIVE(%)	166(74.8)	48(21.6)	3(1.4)	217(97.7)	0.008	9.587
	NEGATIVE(%)	3(1.4)	1(0.5)	1(0.5)	5(2.3)		
	Total(%)	169(76.1)	49(22.1)	4(1.8)	222(100.0)		
IgM	POSITIVE(%)	5(2.3)	2(0.9)	0(0.0)	7(3.2)	0.967	0.565
	NEGATIVE(%)	160(73.7)	46(21.2)	3(1.4)	209(96.3)		
	EQUIVOCAL(%	1(0.5)	0(0.0)	0(0.0)	1(0.5)		
	Total(%)	166(76.5)	48(22.1)	3(1.4)	217(100.0		

P value < 0.05 is statistically significant
X^2—chi square value

Table 3: Sero-prevalence of cytomegalovirus in relation to blood transfusion of subjects

		BLOOD RANSFUSION		Total (%)	P value	X^2
		YES	NO			
IgG	POSITIVE(%)	23(10.0)	202(87.8)	225(97.8)	0.479	0.500
	NEGATIVE(%)	1(0.4)	4(1.7)	5(2.2)		
	Total(%)	24(10.4)	206(89.6)	230(100.0)		
IgM	POSITIVE(%)	1(0.4)	6(2.7)	7(3.1)	0.886	0.241
	NEGATIVE(%)	22(9.8)	195(86.7)	217(96.4)		
	EQUIVOCAL(%)	0(0.0)	1(0.4)	1(0.4)		
	Total(%)	23(10.2)	202(89.8)	225(100.0)		

value < 0.05 is statistically significant
X^2—chi square value

Discussion

From this study, no case of HIV infection was found among the subjects and this could be due to low prevalence rate of HIV patient and its risk factors in the study location. Low prevalence of 0.7% and 5.4% in other parts of Nigeria was reported [20] in Abeokuta (South western Nigeria) and [21] in Abakaliki (South eastern Nigeria). In

concurrence with this research, a study in Kabul [22] found no case of HIV infection in the 4,452 pregnant Afghan women and another in Malekan [23] also found no case of

HIV infection in the 680 pregnant Iranian women. There is a wide geographical variation in the sero-prevalence of HIV infection amongst pregnant women within and outside Nigeria. The variation may be a reflection of the differences in sexual practices and behaviour, awareness of HIV infection and status, socio-cultural practices, and accessibility to healthcare. The level of HIV awareness and status among this group is encouraging as risk of infection to the child is been checked. Among other risk factors to HIV transmission such as surgical procedure, tattoo practice etc, low blood transfusion level was also recorded and this is in contrary to a report [24] were pregnant women with history of blood transfusion had higher prevalence of HIV infection. Thus, it can be said that the transfusion in this case was thoroughly screened for HIV. Also, the population of respondents that share multiple sex partners was low which correlates to a report [25] that exposure to multiple sex partners has been found to be associated with increased risk of acquiring HIV infection. Another study [24] also reported that high prevalence of HIV among pregnant women can be linked to involvement with multiple sex partners.

The results obtained from this study show that the sero-prevalence of Cytomegalovirus (CMV) infection among pregnant women in Ilorin, Kwara state is high with IgG 97.8% and IgM 3.1% detected. The high prevalence rates could be due to lower

socioeconomic status and poor hygienic conditions of the respondents in the study location. This result is in agreement with a study [26] that had prevalence rate of 97.2% among pregnant women in Lagos, Nigeria. Also in two similar studies, [27], [28] prevalence of 97.2% in Benin and 96% in Egypt were recorded respectively among pregnant women. However, in some European countries, low CMV infection rates have been reported, Australia (56.9%) and France (46.8%) [29]. The low prevalence rates in these countries could be due to the inclusion of CMV screening among the antenatal profile tests and better hygienic standards. The detected 97.8% of CMV infections in the current study showed that these women were at higher risk of HIV infections since the risk factors are similar.

According to this study, the prevalence of CMV-IgG varied with age. The distribution of the age of the surveyed respondent showed that majority of respondent were aged 20-34years, accounting for 81.7% of the total participants and fewer percentages accounted for the older group. This is largely due to the fact that majority of pregnant women attending Ante-natal clinic belong to the child bearing age and are largely embedded in this age group. This statement conforms to study on changing pattern of cytomegalovirus sero-prevalence among pregnant women in Norway who reported that the highest CMV-IgG sero-positivity rate was detected in young women [30]. This is in contrast to other studies, reporting an increased CMV-IgG sero-prevalence with increase in age [31].

Evidence of current cytomegalovirus infection via IgM also supports that most infection occurs early in life

because from the seven subjects with current infection (i.e IgM positive) as at the time of this research work four were below age 30 while three were below 35. However, there was no statistical significant (P=0.907) between CMV infection and age of the respondent. From this study, it was observed that a larger percentage of study respondents were parous (73.0%) as compared to those that were nulliparous. However, there was no significant difference (P=0.828) to the risk of

acquiring CMV infection between parous and nulliparous in the study. The result is similar to that reported [6] in Lagos state, which reported that there was no significant relatedness to the risk of acquiring CMV infection in the parity status of respondents to his study. Conversely, this in contrast to another research [29] in Kenya, which reported that there was a statistical correlation (P=0.0001) to the risk of acquiring CMV infection in the parity status correspondent to his study. According to the present data, it can be concluded that the risk of infectivity increases as the educational level gets higher because contact rate also increases which can be due to the low cleanliness level common across the group. It was also noticed that majority of the respondents in tertiary level were positive to cytomegalovirus IgG which may be due to the contact at earlier stages of education. This statement conforms to the report that illiterate women are at higher risk of CMV infection due to contact with contagious secretions from their own children and poor hygienic practice [32]. However, analysis of the result by educational level shows that there was no significant association (P=0.373) disproportionate size of women who were transfused to those who were not

between CMV infection and educational status.

The percentage of marital survey shows that most of the respondents are married with only 3.5% of the population been single. Cytomegalovirus infection is higher in married women than in singles which suggest continuous circulation of the virus within the area and this conforms to a study that also recorded increased sero-positivity among married women than single ladies [26]. However, there was also no significant difference (P=0.294) in the transmission of CMV infection between those that were married or single in this study [17] which is in concordance with another report that there was variation in prevalence of HCMV in different marital status, but the variation was not statistically significant (P>0.05). The study showed (P=0.008) a significant association between Cytomegalovirus infection and type of marriage. With respect to the fact that majority of respondents claimed to be married, a vast majority of respondents were monogamous when compared to their counterparts whose marriage type was polygamous. The sero-prevalence of Cytomegalovirus was higher in women who had been involved in up to two marriages in their life time. This is in agreement with a research that states that the distribution of CMV sero-prevalence 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 sero-prevalence rate [33].

There was no association between women who had been transfused and those that were not (P=0.479). The reason could be as a result of the transfused enrolled in the study. This is in concordance with a research [26] that

stated that past history of blood transfusion was found to be insignificant to the risk of acquiring CMV infection in his study but this statement is in contrast to a report that pointed blood transfusion as a risk factor for transmission of CMV infection [34].

Although most of the considered risk factors were not of statistical significance and there was no recorded case of co-infections, this study however shows that CMV is highly associated with pregnant women in Ilorin implying that there might be a corresponding increase in the incidence of congenital CMV infection among infant born in the state in form of CMV-related hearing loss, vision loss and poor mental development among children in Ilorin, Kwara state. Thus, increased public awareness

about CMV and HIV infection is needed across Nigeria, and this can be done through primary health care channels such as antenatal clinics where the transmission, consequences of infection on foetus and its control and preventive measures can be discussed. Efficient blood screening for CMV and HIV before transfusion to pregnant women, routine screening of pregnant women for CMV should be adopted in all health care settings and babies born to sero-positive mothers should be screened and examined immediately after delivery for possible signs of hearing and vision defect for early management.

Aknowledgement

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