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Seroprevalence and Co-infection of Cytomegalovirus (IgG) and Human Immunodeficiency Virus in Antenatal Patients Attending Some Primary Health Centers in Parts of Kaduna State, Nigeria

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ABSTRACT

Human cytomegalovirus (HCMV) is a major public health problem throughout the world. In HIV infected persons, HCMV is considered an AIDS-defining infection, indicating that the T-cell count has dropped to low levels. This work was aimed at determining the seroprevalence of CMV and HIV in antenatal patients attending Primary Health Centers in parts of Kaduna State, Nigeria. Serological screening for CMV IgG antibodies was done using Enzyme Linked ImmunoSorbent Assay (ELISA) to detect IgG and HIV screening using Determine HIV1/2 kits. Three hundred and sixty samples were collected, out of which three hundred and fifty seven (99.2%) were positive for IgG and three (0.8%) for HIV. Co-infection of CMV (IgG) and HIV occurred in 0.8% of the total population and was found in age groups less than 30 years. Prevalence of CMV IgG increased with age with 100% prevalence in age groups of greater than 29 years and HIV in age groups less than 20 and 20-29. It is concluded that there was a high seroprevalence of CMV IgG among pregnant women investigated which means CMV is common in the study area and most women have been exposed to the virus and protective antibodies developed. Low HIV prevalence could be due to the effectiveness of HIV awareness programs which are beginning to yield result. Precautions should therefore be taken to avoid reinfection of this virus especially in the HIV positive patients which is a threat to the unborn child with a significant risk of mother to child transmission. It is hereby advocated that relevant vaccines should be made available to protect women of child bearing age and pregnant women.

INTRODUCTION

Human cytomegalovirus (HCMV) belongs to the viral family Herpesviridae. It is a double stranded DNA genome with an icosahedral capsid structure, surrounded by a lipid bilayer outer envelope. It is a lytically replicating virus that disrupts the cytoskeleton, thus causing massive cell enlargement, which is the source of the virus' name.

Although they may be found throughout the body, HCMV infections are frequently associated with the salivary glands [1]. HCMV infection is typically unnoticed in healthy people, but can be life-threatening for the immunocompromised, such as HIV-infected persons, organ transplant recipients, or new born infants [2]. Following infection, HCMV has an ability to remain latent within the body for the rest of the person's life. Eventually, it may cause mucoepidermoid carcinoma and possibly other malignancies [3]. Some develop a syndrome similar to infectious mononucleosis or glandular fever, with prolonged fever, and a mild hepatitis [4].

Human Immunodeficiency virus (HIV) on the other hand, is a lentivirus, a member of the retroviridae family that causes Acquired Immune Deficiency Syndrome (AIDS) [5]. The prevalence of HIV in Nigeria has dropped from 4.6% in 2008 to 4.1% in 2011 with the number of infected people estimated at 3.1 million (National Agency for Control of AIDS [6]. Human immunodeficiency virus (HIV) is increasingly recognized as a pathogen of the human embryo and fetus [7,8]. About 80% of children infected with HIV are infected prenatally or perinatally [9]. It is

estimated that 360,000 children are living with HIV in the country; most of whom became infected from their mothers [10], an increase from 220,000 in 2007 [11].

Since the beginning of the global HIV epidemic, intercurrent infection with human cytomegalovirus (CMV) has commanded considerable attention. Indeed, prior to the discovery of HIV, the ubiquitous presence of CMV infection in Kaposi Sarcoma patients suggested that CMV may have a causal association with AIDS [12]. Experimental and clinical studies have demonstrated that the interaction between CMV and HIV-1 furthers disease progression and leads to increased HIV-1 replication [13].

In patients with AIDS, the progressive loss of immune function, and, in particular, loss of cell-mediated immunity, permits CMV reactivation and replication. Asymptomatic excretion of CMV in urine can be detected in approximately 50% of HIV-infected individuals with a CD4 lymphocyte count <100 cells/µL [14]. Early in the AIDS epidemic, cytomegalovirus (CMV) was a major cause of morbidity and mortality in the patients in the United States [15]. Therefore there is the need to study the prevalence and the co-infection of CMV with HIV and also generate data for further action.

MATERIALS AND METHODS

This work was carried out in some selected primary Health centers (PHC) in Kaduna State, Nigeria they includes: PHC Samaru, PHC Muchia, PHC Jushi, PHC Badarawa, PHC Ungwa-Shanu and PHC Zakari-Isa. A simple random selection of the women used in this study was done to select a total of Sixty (60) women in each clinic. Ethical permit for the study was granted by the Local Government ethical board and informed consent was obtained from all the women on their willingness to participate.

Sample collection

A total of 2mls of venous blood was collected aseptically from each woman into plain bottles, allowed to clot at room temperature and serum separated for further analysis.

Sample analysis

CMV IgG antibody was detected using ELISA microwell method as follows: All samples and kit reagents were brought to room temperature (20-25°C) and mixed thoroughly by gentle swirling before used. Samples were numbered according to the microtitre well then 1 in 40 dilutions was done by adding 5µl of the test sample in 200µl of the sample diluents and mixed thoroughly. A quantity (100 µl) of diluted sera, calibrator and controls were dispensed into the appropriate wells. For the reagent blank, 100µl sample diluent was dispensed in 1A well position. Holder was tapped to remove air bubbles and also to mix properly. All the plates were incubated for 30mins at room temperature. The liquid from the wells were removed by empting the content into a waste container and wells washed three times with washing buffer after which it was drained and dried by blotting using tissue. A quantity (100µl) of the enzyme conjugate was dispensed into each well and the plates were incubated at room temperature for 30mins. Excess enzyme conjugate was removed and each well was washed using washing buffer three times, after which it was drained and dried by blotting using tissue. A quantity (100µl) of chromogenic substrate was dispensed into each well and incubated at room temperature for another 30mins, after which a quantity (100µl) of stop solution was dispensed into each well to stop the reaction, there was a colour change from blue to yellow and within 15 minutes the results were obtained at an optical density of 450nm using a microplate reader (WKEA MED SUPPLIES, CHANGCHUN, CHINA).

RESULTS

The prevalence of CMV IgG obtained in this study was 99.2%, co-seropositivity of CMV (IgG) and HIV was seen in 0.8% of the women (Figure 1). In relation to the clinics visited, had the highest prevalence (100%) in most of the clinics and the lowest prevalence of (96.7%) in Zakari Isa clinic. (Figure2).

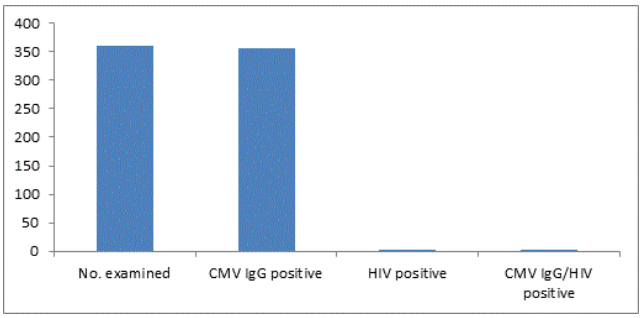

Figure 1: Seroprevalence of CMV and HIV among antenatal patients attending some PHC in parts of Kaduna State, Nigeria

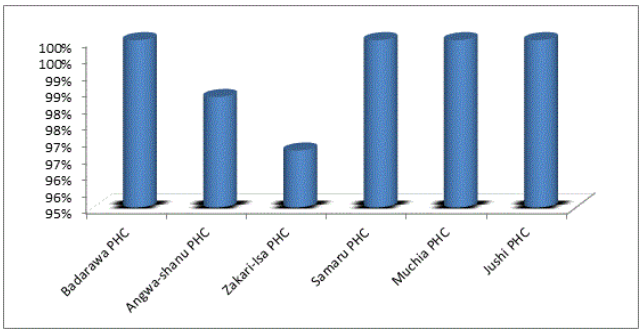

Figure 2: Seroprevalence of CMV in relation to the PHC in some parts of Kaduna State, Nigeria

The result was analyzed according to age and the result showed that women in the age group greater than 30 years old had the highest prevalence of 100% and the least prevalence is recorded in age group less than 20 years old with 98.8% prevalence. For HIV, age group less than 20 years old had the highest occurrence with 1.3% and the least occurrence were recorded in age group of 20-29 with 1.1%. (Table 1).

Table 1: Seroprevalence of CMV and HIV in relation to age among antenatal patients attending some PHC in parts of Kaduna State, Nigeria

Age Groups	No. Examined	CMV IgG positive (%)	P value	HIV positive (%)	P value
<20	80	79 (98.8)		1 (1.3)	
20-29	183	181 (98.9)		2 (1.1)	
30-39	91	91(100)	0.77	0 (0)	0.77
>40	6	6 (100)		0 (0)	
Total	360	357 (99.2)		3 (0.8)	

Result was analyzed according to risk factors; we had the highest prevalence (100%) of CMV IgG on the basis of marital status in single and separated women, with least prevalence among married women 99.1%. Highest prevalence of HIV was recorded among married women with values of 0.9% prevalence. Cytomegalovirus IgG seropositive cases were high in polyandry type of marriage and least occurrence was in monogamy type of marriage with 77 (100%) and 280 (98.9%) prevalence respectively. In addition, a high prevalence of HIV was recorded in monogamous type of marriage with 3 (1.1%). No data showed any significant difference between CMV-HIV co-infection and the different types of marriage (P>0.05).

The seroprevalence of CMV infection according to trimester in the study population showed that women in their first trimester had the highest prevalence of 100% and least among women in their second trimester 98.6%. Analysis according to gravida (i.e number of pregnancy), highest prevalence of CMV IgG was discovered among women who had 6-10 and greater than 10 pregnancies with a 100% prevalence. The seroprevalence of CMV infection according to number of children among antenatal patients revealed that women with greater than 5 children had the highest

CMV IgG prevalence of 100%. Though there is no significant difference from the result analyzed between CMV gestational age, gravid and number of children (P>0.05).

Seroprevalence of CMV in the study population on the basis of history of blood transfusion, there was a highest prevalence of 100% among women who had history of blood transfusion and those without history of blood transfusion having the least prevalence of 99.1%. For HIV, the highest occurrence occurred among women without history of blood transfusion with prevalence of 0.9%. These data showed no significant difference between CMV, HIV and history of blood transfusion (P>0.05)(Table 2).

Table 2: Seroprevalence of CMV and HIV in relation to some risk factors among antenatal patients attending some PHC in parts of Kaduna State, Nigeria

Risk Factors	No. Examined	CMV IgG Positive (%)	P value	HIV Positive (%)	P value
Marital Status					
Single	3	3 (100)		0 (0)	
Married	350	347 (99.1)	0.96	3 (0.9)	0.96
Separated	7	7 (100)		0 (0)	
Total	360	357 (99.2)		3 (0.8)	
Type of Marriage					
Monogamy	283	280 (98.9)		3 (1.1)	
Polyandry	77	77 (100)	0.36	0 (0)	0.36
Total	360	357 (99.2)		3 (0,8)	
Gestation age (months)					
1-3	30	30 (100)		1 (3.3)	
04-Jun	140	138 (98.6)		2 (1.4)	
>7	190	189 (99.5)	0.78	0 (0)	0.22
Total	360	357 (99.2)		3 (0.8)	
Gravid					
01-May	284	281 (98.9)		3 (1.1)	
06-Oct	72	72 (100)	0.67	0 (0)	0.67
>10	4	4 (100)		0 (0)	
Total	360	357 (99.2)		3 (0.8)	
No. of children					
0-5	326	323 (99.1)		3 (0.9)	
06-Oct	34	34 (100)	0.57	0 (0)	0.57
Total	360	357 (99.2)		3 (0.8)	
History of blood transfusion					
Yes	17	17(100)		0(0)	
No	343	340(99.1)	0.7	3(0.9)	0.23

Total	360	357(99.2)		3(0.8)	

DISCUSSION

This study indicates a high seroprevalence of CMV IgG of 99.2% in pregnant women which is not surprising as many studies from West African countries including Nigerian, Benin and Gambia have reported prevalence of 87% to 97% [16-18]. The high prevalence of IgG antibodies to CMV confirms the general perception that such viruses are common in developing countries including Africa.

However, in respect to HIV positivity, the study reported HIV prevalence of 0.8% which means that co-infection of CMV IgG and HIV had a prevalence of 0.8%, this could indicate that our patients harbour CMV virus in the latent form in their leucocytes and thus stand high risk of reactivation. They may therefore benefit from CMV prophylaxis with Ganciclovir to reduce incidence of active disease that may result from reactivation [19]. This result is in contrast with the findings of Mujataba et al., [20] whose work found a 32.4% incidence of HIV co-infection with CMV. The low prevalence of HIV could be due to early indication that the awareness being created by NACA is beginning to yield some results as many are aware of the infection and prevention measures are being taken to prevent it. However, further studies are recommended to establish the fact.

The highest CMV IgG prevalence in the Clinics sampled could be due to overcrowding noticed in the community studied. Similar observation has been reported by Krech [20].

The prevalence of CMV IgG antibody was found to increase with increasing age in this study, with the highest prevalence (100%) in the age groups ≥30 years. Longer duration of exposure to the virus might be responsible for the highest prevalence seen in the older age group. Although not statistically significant it agrees with the work of Dollard et al., [21] and disagrees with that of Fowler et al., [22]. Women who were less than 20 years and within the age group of 20-29 years were HIV positive and this could be attributed to increase sexual activities in these age groups.

On the basis of marital status, highest CMV IgG prevalence was recorded among women who were single and separated (100%) while HIV prevalence occurred only in married women (0.9%). This prevalence among these women may have been due to their sexual behavior and it could also be possible that the wives and also their husbands have concubines outside their homes and in the process might have been infected therefore transmitting the virus.

Based on types of marriage, the highest CMV IgG prevalence was obtained in polygamous women. This could be because the husband's have several other wives therefore the risk of dissemination among the wives is high. HIV was highest in those with monogamous marriage, the virus could possibly have been gotten from their partners or from other sources of transmission.

Looking at the stages of pregnancies of the patients in relation to CMV IgG seropositivity, the highest prevalence of 100% was among women in their first trimester. This may have been due to different behaviors prior to pregnancy (e.g. hygiene or sexual behavior). However, some research suggests that first-trimester infection produces greater fetal harm, but congenital infection can harm the fetus even in late pregnancy [23].

The result in relation to the number of pregnancies (gravid) showed a high prevalence among women who have had greater than five pregnancies being sero positive for CMV IgG. This indicates that women are at a greater chance of coming down with the infection as their number of sexual intercourse increases. Also the high prevalence here agrees with findings of Hamdan et al., [24], that reported high parity being significant risk factors for CMV infection. This could be so as increase in gravid could imply increase in age which according to Ludwig and Hengel, [25] and Stadler et al., [26], is a significant predator of CMV infection.

Blood transfusion is another risk factor in which CMV and HIV can be transmitted. From this study, women with such a history had the highest CMV prevalence which agrees with a previous study by Akinbami et al., [18]. This high prevalence could be an indication that these women may have been infected through this means which also agrees with the study by Akinbami et al.,[18]. Three (3) out of the 343 women with prevalence of 0.9% had no history of blood transfusion but where HIV positive while those with history of blood transfusion were all negative to HIV. The HIV positive patients here could have gotten the infection through other means such as their sexual behavior. From this study there was no significant difference ($P>0.05$) between blood transfusion and CMV infection. This disagrees with report of Matos et al., [27] and this could be due to the disproportionate size of women who were transfused to those who were not [28].

From the population of women with ≤5 children, three (3) were HIV positive with prevalence of 0.9%, this could be because of other means of HIV transmission other than sexual intercourse.

It is noteworthy in this study that the risk factors were not statistically associated with CMV and HIV positivity (P>0.05), which is consistent with the study by Akinbami et al., [18].

CONCLUSION

The present study has shown a high prevalence of CMV IgG (99.2%) and a low prevalence of HIV (0.08%) in antenatal patients attending some primary health centers in parts of Kaduna State. This high CMV prevalence shows that cytomegalovirus was common in the study area, which could be due to overcrowding or low hygiene of the study population, and most women must have come down with the infection and have developed protective antibodies to the virus and this antibody can be transferred to their unborn child. For the HIV patients there is a possibility that they are harboring the CMV virus in the latent form in their leucocytes and thus stand high risk of reactivation which could lead to a fetal outcome, therefore there should be proper follow up and they can benefit from CMV prophylaxis with Ganciclovir to reduce incidence of active disease that may result from reactivation.

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