International Journal of TROPICAL DISEASE & Health
4(9): 984-992, 2014

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Seroprevalence of Human Cytomegalovirus and Rubella Virus Antibodies among Anti-retroviral Naive HIV Patients in Lagos

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AAI, IOM, IAC and OAG were involved in the concept design. Authors AAS, SBB and IOM were involved in drawing up of protocol, collection and analysis of specimen. Authors OAO, AAI and TSA were involved in literature research, statistical analysis and drafting of the manuscript. All authors read and approved the final manuscript.

Original Research Article

Received 28th March 2014
Accepted 16th June 2014
Published 26th July 2014

ABSTRACT

Background/Aims: Opportunistic infections such as Cytomegalovirus (CMV) and Rubella virus that pose no threat to healthy individuals can be life threatening in those with impaired immune systems. The aim of this study was to determine the sero-prevalence of human Cytomegalovirus (CMV) and Rubella Immunoglobulin M and G (IgM and IgG) antibodies among anti-retroviral naive patients in Lagos.
Study Design: This is a cross sectional study.
Place and Duration of Study: AIDS Prevention Initiative in Nigeria (APIN) clinic in Lagos University Teaching Hospital (LUTH) between April 2011 and May 2012.

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Methodology: The study was carried out among 80 (28 males and 52 females) HIV infected adults attending the AIDS Prevention Initiative in Nigeria (APIN) clinic in Lagos University Teaching Hospital and the patients were aged between 18 and 60 years. IgG and IgM assay were performed using ELISA reagents (produced by Biotec laboratories, United Kingdom). Also, CD4+ cell counts were evaluated. Pearson's Chi-squared test was used for the analytic assessment.
Results: From our findings, twenty (25%) patients were positive for CMV IgM and sixty (75%) patients were positive for CMV IgG. Also, 59 (73.75%) patients were positive for Rubella IgG and only one (1.25%) patient was positive for rubella IgM. There was no significant statistical difference in seroprevalences of CMV-IgM, CMV- IgG, and rubella IgG with respect to sex, age, and CD4+ cell counts.
Conclusion: This study showed that the sero-prevalence of CMV and Rubella virus is high among anti-retroviral naive HIV patients in Lagos, Nigeria.

Keywords: Cytomegalovirus; rubella; seroprevalence; naive; anti-retroviral; HIV; APIN.

1. INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a disease of the human immune system caused by the human immunodeficiency virus (HIV). The virus attacks the cells of the immune system, particularly the CD4+ T lymphocytes depleting them from circulation thereby exposing the patient to a myriad of opportunistic infections [1]. In addition, infections that pose no threat to normal people can cause severe, even fatal disease in those with impaired immune systems [2].

Opportunistic Infections (OIs) have been recognized as common complications of HIV infection due to immune deficiency. OI is the main reason behind hospitalisation and substantial morbidity in HIV infected patients [3]. It necessitates toxic and expensive therapies and reduces the expected life span of such patients. Virtually all HIV-related mortality is preceded by opportunistic infection [4].

OIs encompass a wide variety of microorganisms that produce series of infections in immunocompromised HIV seropositive patients. Viral pathogens causing OI evoke a spectrum of illness ranging from asymptomatic to severe diseases in HIV infected individuals. Since the onset of the acquired immunodeficiency syndrome (AIDS) epidemic in 1980; human herpes viruses (HHV) have resulted in many of the secondary manifestations of human immunodeficiency virus (HIV) infection such as painful rash caused by Herpes Zoster [5,6].

OIs may be caused by bacteria, viruses, fungi and parasites that are normally controlled by the immune system [7]. However the primary causes of death from HIV/AIDS are opportunistic infections and cancer both of which are frequently as a result of the progressive failure of the immunes system [8,9].

Human Cytomegalovirus (CMV) 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 [10]. Cytomegalovirus is a common cause of blindness in AIDs patients and also causes hydrops fetalis in infants and spreads readily in day care centres [2].

Rubella virus is typically a mild, often unrecognized disease that is difficult to diagnose but infection of pregnant women can have tragic consequences [2]. HIV patients who are negative to Rubella IgG antibodies face risk of infection which may be critical especially amongst women of child bearing age. The primary symptom of rubella virus infection is the appearance of a rash (exanthem) on the face which spreads to the trunk and limbs and usually fades after three days. Other symptoms include low grade fever, swollen glands, joint pains, headache and conjunctivitis [11].

Considering the importance of human Cytomegalovirus and Rubella virus and their complications in immunocompromised individuals, it seems necessary to investigate the seroprevalence of these viruses among the anti-retroviral naive HIV patients. This will help medical personnel in making informed decisions on the control or prevention of opportunistic infections that can arise from these viruses and also in prescription of anti retro viral therapy to be used by the patients at the point of care. Hence, this study was aimed at determining the seroprevalence of human Cytomegagalovirus (CMV) and Rubella virus antibodies in anti-retroviral naive adult HIV patients attending the AIDS Prevention Initiative in Nigeria (APIN) clinic in Lagos.

2. METHODOLOGY

2.1 Study Design, Population and Setting

The research was carried out between April 2011 and May, 2012 at the AIDS Prevention Initiative in Nigeria (APIN) clinic located at the Lagos University Teaching Hospital LUTH, Idi-araba, Lagos-state. APIN is a non-governmental initiative jointly funded by Bill and Melinda Gates foundation and Presidential Emergency Programme for Aids Reduction (PEPFAR) to cater for people living with HIV by way of counselling, testing, care as well as anti-retroviral treatment. The enrolment of new patients at this clinic is estimated to be 30 per week. Simple random sampling technique was used to select eighty (28 males and 52 females) patients who had not started anti-retro viral therapy.

2.2 Eligibility Criteria

Patients between the ages of 18 and 60 years who had been previously screened at the clinic and confirmed to be HIV positive and had not started receiving anti- retroviral therapy were recruited into the study after their informed consent was sought and obtained. Structured questionnaires were also administered on them.

2.3 Screening of Patients

Individuals that presented themselves for retroviral screening at the APIN clinic, upon counselling were screened using the Alere Determine HIV 1 /2 test kit. Those that tested negative were given post test counselling and discharged while those that tested positive were retested using western blot. This testing was done by highly trained APIN staff.

2.4 Laboratory Methods

5ml of venous blood was drawn from each of the patients that had met the inclusion criteria into a plain vacutainer bottle that was properly labelled. The samples were allowed to stand for an hour, after which were spun at medium speed (2500 revolution per minute) of the

centrifuge. The resulting sera were transferred into another sterile plain vacutainer bottles for viral assay and stored at -20ºC till they were assayed. A total of eighty sera samples were collected from 28 males and 52 females.

The reagent used for Rubella Immunoglobulin M and G (IgM and IgG) antibody detection in the patients' sera was the rubella IgM and IgG fast EIA KIT manufactured by Biotec laboratories, United Kingdom. While the kits used for Cytomegalovirus IgM and IgG antibody detection in the patients' sera was the Cytomegalovirus IgM and IgG fast EIA KIT also manufactured by Biotec laboratories, United Kingdom. The kits detect antibodies based on the ELISA principle (Nester et al. [2]) which has been shown to be a sensitive and reliable procedure for antibody detection.

Briefly, the patients' sera were diluted based on the manufacturer's instructions and added to 82 micro wells (80 samples and 2 controls) previously coated with purified Rubella and CMV antigen. All unbound materials were washed away using the washing buffer. The enzyme conjugate was then added and the plate was incubated to allow the hydrolysis of the substrate by the enzyme. The intensity of the colour generated is proportional to the amount of antibody in the sample. The negative and positive control for each assay were run in duplicate and it was ensured that the mean Optical Density (O.D) values for the control falls within the range stipulated by the kit manufacturers. The patients CD4+ cell counts were determined using flow cytometry.

2.5 Statistical Analysis

The data obtained were subjected to descriptive statistical analysis using EPI info 7 and Minitab 16. Chi squared test was used to determine associations and the differences were considered to be statistically significant when the p value obtained was less than 0.05

3. RESULTS

A total of 80 anti-retroviral naive HIV patients participated in this study, and out of which 28(35%) were males and the remaining 52(65%) were females. Their ages ranged between18 and 60 years. Majority of the patients studied (51.25%) were in the 28-37 years age bracket while only 1(1.25%) patient was ≥ 58 years (table 1).

Table 1. Age and Gender Distribution of 80 HIV Patients attending APIN clinic in LUTH

Age group	No (%)	Male (%)	Female (%)
18-27	14(17.50)	1(1.25)	13(16.25)
28-37	41(51.25)	10(12.50)	31(38.75)
38-47	18(22.50)	11(13.75)	7(8.75)
48-57	6(7.50)	6(7.50)	0(0)
≥ 58	1(1.25)	0(0)	1(1.25)
Total	80(100)	28(35)	52(65)

A total of twenty patients (25%) were positive for CMV IgM. Twelve (15%) of them were females while 8 (10%) were males. Sixty patients (75%) comprising of 21 (26.25%) males and 39 (48.75%) females were positive for CMV IgG, However, 59 patients (73.75%) made up of 37 females and 22 males were positive for Rubella IgG while only one male (1.25%) was positive for Rubella IgM (tables 2 and 3).

Table 2. Seroprevalence of CMV and Rubella antibodies among the different age groups of the male and female HIV patients

Age (yrs)	CMV IgM Positive		CMV IgG Positive		Rubella IgM Positive		Rubella IgG Positive	
Range	Male	Female	Male	Female	Male	Female	Male	Female
18-27	0	3	1	9	0	0	1	9
28-37	2	8	8	25	0	0	7	22
38-47	5	1	6	4	0	0	9	5
48-57	1	0	6	0	1	0	5	0
≥ 58	0	0	0	1	0	0	0	1
Total	8(10%)	12(15%)	21(26.25%)	39(48.75%)	1(1.25%)	0(0%)	22(27.50%)	37(46.25%)

Table 3. CMV and Rubella antibodies status among different age groups

Age (Yrs) range	Total no of individual	CMV IgM+(%)	CMV IgG+(%)	Rubella IgM+(%)	Rubella IgG+(%)
18-27	14	3(21.43)	10(71.43)	0(0)	10(71.43)
28-37	41	10(24.39)	33(80.49)	0(0)	29(70.73)
38-47	18	6(33.33)	10(55.56)	0(0)	14(77.78)
48-57	6	1(16.67)	6(100)	1(16.67)	5(83.33)
≥58	1	0(0)	1(100)	0(0)	1(100)

4. DISCUSSION

Our findings revealed a seroprevalence of 25% for IgM anti-CMV representing the percentage of those that had active infection of CMV. However, previous studies reported a low prevalence of 6.6% [12] and 7.0% [13] among HIV patients in Lagos and Benin respectively. Also, another previous study reported a very high IgM anti-CMV prevalence of 42.9% among AIDS patients in Iraq [14].The disparity observed in the seroprevalence of IgM anti-CMV might be as a result of the different status of the immunocompromised study subjects: Ojide *et al.,* (2010) reported a sero-survey of CMV IgM antibodies among HIV-infected adults on HAARTS, while Ali et al. [14] reported sero prevalence of CMV IgM antibodies on AIDS patients and our study revealed a seroprevalence of CMV IgM antibodies among anti-retroviral naive HIV patients.

Also, a seroprevalence of 75% IgG anti-CMV was revealed in this study. This figure is quite low when compared to previous studies carried out in Nigeria with seroprevalence rate of 100% [12], 97% [15] and 98.8% [13]. However, a very low seroprevalence of CMV IgG antibody of 59.9% was reported among HIV infected patients in Ghana [16]. The disparities in the result might be as a result of different methodologies used for the studies.

The rubella IgM seropositivity in this study revealed a prevalence rate of 1.25% while that of the rubella IgG indicated a prevalence rate of 73.75%. Data on the seroprevalence of rubella IgM and IgG among HIV infected patients is extremely rare. However, a seroprevalence rate of 93.1% and 0.6% rubella IgG and IgM respectively was reported in a study on TORSH in Brazil [17], while a study on the seroprevalence of rubella infection in pregnancy, Benin City, Nigeria reported a prevalence rate of 53% rubella IgG and rubella IgM seropositivity of 9.7% [18].

Majority (41 out of 80) of the patients studied were within the age bracket of 28-37, this further reiterates the seriousness of the HIV infection among this age group as it is obvious

that this modal class represents the very active segment of the society. This was also reported by Nester et al. [2] which stated that AIDS is the leading cause of death among persons aged 25 to 44 years. Highest prevalence (33.33%) of CMV IgM was observed in the age group of 38-47 and lowest prevalence (0%) of CMV IgM was recorded in the age group ≥58. Highest prevalence (100%) of CMV IgG was observed in the ≥48 and lowest prevalence (55.56%) of CMV IgG was recorded in the age group 38-47.For rubella IgM, highest prevalence (16.67%) was observed in the age group 48-57 and highest prevalence (100%) of rubella IgG was observed in age group ≥58 while lowest prevalence (70.73%) of rubella IgG was recorded in age group 28-37 (table 3). There was no statistically significant association between age and the seroprevalences of IgM anti- CMV, IgG anti CMV and IgG rubella antibodies (P=0.894, P=0.495, P=0.692 respectively).

Nearly half (35 out of 80) of patients studied had CD4+ cell counts ≤200/µl and AIDS is defined as CD4 levels below 200 [19]. Seroprevalence of CMV IgM was highest (46.15%) among patients with CD4+ cell counts 401-600/µl and lowest seroprevalence (0%) of CMV IgM was recorded among patients with CD4+ cell counts ≥601/µl. Also, highest prevalence (80%) of CMV IgG was observed among patients with CD4+ cell counts between 1-200/µl and lowest prevalence (50%) of CMV IgG was recorded among patients with CD4+ cell counts ≥601/µl. Also, For rubella IgM, highest prevalence (7.69%) was observed among patients with CD4+ cell counts 401-600/µl and highest prevalence (100%) of rubella IgG was observed among patients with CD4+ cell counts ≥601/µl while the lowest prevalence (65.71%) of rubella IgG was recorded among patients with CD4+ cell counts 1-200/µl (tables 4 and 5). There was no statistically significant association between CD4+ cell counts and the seroprevalences of IgM anti- CMV, IgG anti CMV and rubella IgG antibodies (P=0.315, P=0.641 and P=0.354 respectively).

However, among the 80 patients investigated in this study, it was observed that 28 were males (35%) and 52 were females (65%), and it has been reported that incidence of HIV is more in females than in males [12]. In addition, seroprevalence of CMV IgM antibody was more in the female patients (15%) than in males (10%), while CMV IgG antibody sero-status was 48.75% for female patients studied against 26.25% recorded by the male patients. Also, the sero prevalence of Rubella IgG was more in females (46.25%) than in males (27.50%), on the other hand, this study revealed that the Rubella IgM sero-status was recorded only in one male (1.25%) and there was none in female (table 2). There was no statistically significant association between sex and the seroprevalences of IgM anti- CMV, IgG anti CMV and rubella IgG antibodies (P=0.787, P=0.787 and P=0.651 respectively).

Table 4. CD4+ counts range for CMV IgM and IgG and Rubella IgM and IgG positive samples

CD4+Range	CMV IgM+	CMV IgG+	Rubella IgM+	Rubella IgG+
1-200	7	28	0	23
201-400	7	21	0	24
401-600	6	10	1	10
≥601	0	1	0	2
Total	20	60	1	59

Table 5. CD4+ cell counts with CMV and Rubella antibodies status

CD4+ counts (/µl)	Total no of individual	CMV IgM+(%)	CMV IgG+(%)	Rubella IgM+(%)	Rubella IgG+(%)
1-200	35	7(20)	28(80)	0(0)	23(65.71)
201-400	30	7(23.33)	21(70)	0(0)	24(80)
401-600	13	6(46.15)	10(76.92)	1(7.69)	10(76.92)
≥601	2	0(0)	1(50)	0(0)	2(100)

In this study, the number of patients with CMV IgM antibodies (25%) indicating recent infection is worrisome, considering its effect in immunocompromised people and the scale of the CMV problem in developing countries (such as Nigeria) is still not known as most cases of CMV retinitis are never diagnosed. Yet it has been estimated that between 5% and 25% of all HIV infected patients in the developing world can be expected to develop CMV retinitis at some point during the course of their illness [20] leading to some to warn of a possible epidemic of blindness [21]. Consequently, treatment of opportunistic infection is an integral part of HIV management at the primary care level and treatment of CMV retinitis should be included [22].

5. CONCLUSION

The findings of our study indicated high prevalence of CMV and rubella seropositivity among anti-retroviral naive HIV patients in Lagos. Hence screening of cytomegalovirus and Rubella virus would be a vital diagnostic step for HIV patients. There was no significant statistical difference in seroprevalences of CMV-IgM, CMV- IgG, and rubella IgG with respect to sex, age, and CD4+ cell counts. However, further studies that would have more cases of Rubella IgM positivity would address the statistical association between the prevalence of Rubella IgM with respect to age, sex and CD4+ cell counts.

CONSENT

All authors have declared that written informed consents were obtained from the patients for publication of this work.

ETHICAL APPROVAL

Ethical approval for this study was granted by the research ethics committee and review board, Lagos University Teaching Hospital (LUTH), Idi-araba, Lagos state, Nigeria.

ACKNOWLEDGEMENT

We would like to acknowledge the support of the staff and management of the AIDS Prevention Initiative in Nigeria (APIN) Clinic. LUTH, Idi-Araba, Lagos, Nigeria

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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