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Cytomegalovirus retinitis in HIV patients attending Eye/Uveitis clinic in Korle-Bu Teaching Hospital, Accra -Ghana

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Cytomegalovirus (CMV) retinitis is an ocular manifestation of human immunodeficiency virus (HIV) infection especially in individual with low CD4 cells count. In Ghana, a country where CMV infection is hyperendemic, there is no data of CMV retinitis among HIV positive persons. This work was conducted to evaluate the prevalence of CMV retinitis among HIV positive persons attending eye clinic in Korle-Bu Teaching Hospital. Eye swab and vitreous samples were collected from HIV positive patients with retinal inflammation. The samples were assayed for DNA of Herpes Simplex Virus, Varicella Zoster, Cytomegalovirus and Epstein - Barr virus by using qualitative polymerase chain reaction (PCR) (multiplex PCR) to detect the presence or absence of herpes viruses. Sixty-two patients had retinal inflammation from ophthalmoscopy out of the total 404 that came into the clinic during the study period from July, 2010 to April, 2011. Forty six of these were HIV positive. Only 3 (6.5%) HIV positive patients had their swab or vitreous humour samples yielding DNA of CMV virus. These patients had their CD4 cell counts above 25 cells/µl. This is the first study to show the prevalence of CMV retinitis among HIV patients in Korle-Bu Teaching Hospital, Ghana. It is necessary for more comprehensive longitudinal study to evaluate incidence and cumulative risk of CMV retinitis among HIV positive individual.

Key words: Cytomegalovirus, human immunodeficiency virus, Ghana, Korle-Bu, retinitis, CD4, ophthalmoscopy.

INTRODUCTION

Herpetic retinitis is a retinal inflammation caused by human herpes viruses namely; Herpes simplex type 1

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and 2 (HSV1 and 2), Varicela Zoster virus (VZV), Epstein Bar virus (EBV) and Cytomegalovirus (CMV) (Guex-Crosier et al., 1997). CMV is mostly acquired during childhood and it remains latent for a long period until such a time when there is immunosuppression (Urwijitaroon et al., 1992; Liu et al., 1990). Its link with HIV/AIDS is well established. CMV is a common opportunistic infection in people with HIV/AIDS (Gallant et al., 1992). The common CMV manifestation in persons living with HIV is retinitis (Gallant et al., 1992), though there are possible extra ocular manifestations that can also be seen (Wallace and Hannah, 1987; Liestøl et al., 2002).

In developed countries, CMV retinitis was known to occur in approximately one third of AIDS patients and contributed to about 90% of HIV related blindness (Jabs et al., 1989; Yust et al., 2004). However, more recently because of the increased availability of highly active anti-retroviral therapy (HAART), CMV retinitis is now a rare occurrence (Drew, 2003). In developing countries the true picture of the prevalence of CMV retinitis is not well known as most cases are not diagnosed (Heiden et al., 2007). Diagnosis is a big issue in resource limited countries as routine retinal examination is not performed in HIV patients, but is only done in eye clinics when they have eye complaints (Heiden et al., 2007; Resnikoff et al., 2012). Indirect ophthalmoscopy by an experienced clinician to examine the retina through a dilated pupil is the gold standard for diagnosis of CMV retinitis. This is often a challenge in resource limited countries as experience and /or necessary equipment to carry out this task is not available (Kestelyn, 1999).

Despite this diagnostic difficulty some data suggest a low prevalence of CMV retinitis in Africa, with cross-sectional studies suggesting prevalence ranging from 0 to 8.5% (Katelyn, 1999). Another longitudinal study carried out in Togo which followed 200 patients over 20 months had an incidence rate of 21.4% (Balo, 1999). Aside the diagnostic difficulty probably being the cause of discrepancies in the prevalence recorded in various studies, the short duration from diagnosis to death can also play a role (Hodge et al., 2004) especially in cross sectional study which will obviously underestimate the cumulative risk of CMV retinitis.

The short duration of CMV retinitis and death in HIV/AIDS is due to the fact that CMV retinitis is reported to be linked to extreme immunosuppression seen as low CD4 count (Kuppermann et al., 1993). For years, the CD4 cell count proved a reliable predictor of the risk of ocular complications of HIV infection (Ghana AIDS Commission, 2016). According to Ghana AIDS Commission, the 2013 HIV sentinel survey reported 1.2% prevalence of HIV (Ghana AIDS Commission, 2016). There are about 189,931 adults leaving with HIV and about 7,826 deaths in the same period (Ghana AIDS Commission, 2016). However to the best of our knowledge there are no published data on ocular manifestations of HIV or CMV retinitis in Ghana. This work tends to look at the prevalence of CMV retinitis in HIV patients attending eye clinic in Korle-Bu Teaching Hospital.

MATERIALS AND METHODS

Study area

This was a cross sectional study done in the Eye Clinic of Korle-Bu Teaching Hospital (KBTH) in the Greater Accra-Ghana. The KBTH is the largest teaching Hospital and major referral health facility in the country. The study was conducted from July, 2010 to April, 2011.

Study subjects

Patients with HIV between the ages of eighteen and seventy years attending the eye clinic and suspected to have any sign of peripheral necrotizing retinitis, and/or retinal arteritis on retinal examination by a specialist ophthalmologist were recruited for the study after informed consent had been obtained. Other relevant data such as CD4 cell counts and HIV status were retrieved from patients' folders to aid in analysis of data from the study subjects.

Ophthalmic evaluation

This included visual acuity (VA) testing by an ophthalmic nurse using Snellen's chart at a distance of 6 m. For patients who were unable to see the letters at the closest test distance, the following test sequence was used: count fingers (CF) at 1 m, hand movement (HM) at 1 m, light perception (LP) and no light perception (NLP). Best corrected Visual Acuity (BCVA) with spectacles was recorded when possible. Patients were examined by a specialist ophthalmologist and all equipment transported to the Fevers unit for the purpose of the study. External eye and anterior segment examinations were done using a slit lamp (Topcon ATE-600 serial number 800175, 2004, Germany). Ocular surface and intraocular pressures assessed were with the aid of florescein and amethocaine. A patient was considered to have uveitis if they had keratic precipitates, cells in the anterior chamber with flare, posterior synechaie, and sometimes vitreous cells. Fundus examination was done using direct ophthalmoscope through dilated pupils (using tropicamide 1%, and or cyclopentolate eye drops 1% with phenylephrine 2.5% eye drops) biomicroscopy with the slit lamp plus a + 90D lens, and indirect ophthalmoscope with +20D lenses. Patients were termed to have retinitis if they had pale or whitish retinal lesions with or without haemorrhages or vasculitis. Patients with retinitis and uveitis were sent to the main eye clinic where fundus photos were taken and vitreous tap done.

Sample collection

Under sterile condition, vitreous taps were done in the mini theatre. Uncontaminated vitreous humour was aspirated or tapped with a tuberculin syringe connected to the disposable needle. After tapping, the air in it was expelled carefully without causing aerosols and the needle was capped with a sterile rubber bung and sent to laboratory immediately. Approximately 100 µl (0.1 ml) of vitreous humour sample was collected from each patient and stored at -20°C until use for PCR. Eye swabs were taken in patients who refused vitreous humour sample collection. Eye swabs were taken

Table 1. Primers and predicted sizes of amplification.

Cat No. / ID No.	Sequence (5´- 3´)	Predicted product sizes (bp)
HSV-1:10336-022/ S5500A09	F: GCCAAGAAAAAGTACATCGGCGTCATC	292
HSV-1:10336-022/ S5500A10	R: TGAGGACAAAGTCCTGGATGTCCCTCT	
VZV: 10336-022/ S5500A11	F: TCCGACATGCAGTCAATTTCAACGTC	161
VZV: 10336-022/ S5500A12	R: GGTCGGGTAGACGCTACCACTCGTTT	
EBV: 10336-022/ S5500B01	F: CTTAGAATGGTGGCCGGGCTGTAAAAT	229
EBV: 10336-022/ S5500B02	R: ATCCAGTACGTCTTTGTGGAGCCCAAG	
CMV: 10336-022/ S5500B03	F: GCGCGTACCGTTGAAAGAAAAGCATAA	131
CMV: 10336-022/ S5500B04	R: TGGGCACTCGGGTCTTCATCTCTTTAC	

with the aid of sterile swabs and transported in a viral transport medium. This was also stored at -20°C until use for PCR.

DNA extraction from the samples

DNA extraction from the samples was done using the high pure viral nucleic acid kit from Roche (Roche Diagnostics Deutschland GmbH). DNA was extracted according to the manufacturer's instructions. Briefly, about 200 µl of the samples were lysed by incubation with binding buffer (supplemented with carrier RNA) and proteinase K at 72°C for 10 min. The nucleic acids present in the samples bind to the glass fibers pre-packed in the high pure filter tube during the DNA extraction process. Bound nucleic acids were washed with a special inhibitor removal buffer to get rid of PCR inhibitory contaminants. This was followed by washing of bound nucleic acids, purified from proteins and other impurities .Purified nucleic acids were recovered using elution buffer and stored at -70°C.

Multiplex PCR for four herpes viruses

The PCR reaction was directed at the detection of the genomic DNA of Herpes Viruses per the primer designs in Table 1.

Specific steps taken were as follow

DNA was amplified in a unit reaction of 50 µl of a reaction mixture. The master mix included 50 mM $MgCl_2$ (Invitrogen), 10× PCR buffer (Invitrogen), 10 mM dNTPs (Invitrogen), 5U Taq polymerase (Invitrogen), 15 to 24 nmol primers (Invitrogen) and extracted DNA as template- 5 µl (Table 3). An aliquot of 45 µl of master mix was despensed into 0.2 ml sterile PCR tubes and 5 µl of template added to each tube. Sterile PCR water was used as negative control and HHV-6 control DNA from Advanced Biotechnologies Inc. (USA) was used as positive control. The sample was amplified through 40 cycles in a DNA thermal cycler with a 5 min hot start at 95^0C, followed by 30 sec denaturation step at 94°C, 30 s annealing step at 55°C, 1 min elongation step at 72°C and a final extension at 72°C for 10 min.

Detection of amplified product for the mPCR

The amplified products with a 1 kb DNA marker (Invitrogen) were electrophoresed (100 V for 40 min) by agarose gel (2%w/v) stained in 0.5 µg/ml ethidium bromide. The fractionated bands were visualized under UV light and photographed for the records. The expected size for CMV positive amplification product was about 131 bp shown in Table 1.

Control

The PCR reaction was directed at the detection of the large tegument protein (LTP) gene in the SIE strain of the HHV-6. PCR Primers used were as follows:

SIE-1 5'GATCCGACGCCTACAAACAC3'
SIE-2 5'TACCGCATCCTTGACATATTAC3'

Statistical analysis of data

Data was analyzed using Microsoft Access and Statistical Package for Social Sciences (SPSS) V12. Differential analysis of clinical data was done and various sample proportions were compared using 95% confidence interval.

Ethical consideration

The proposal was submitted to both the Ethical Committee of the College of Health Sciences and Institutional Review Board of Noguchi Memorial Institute for Medical Research for approval. Ethical approval number NMIR-IRB CPN 061/11-12. Written informed consent was obtained from subjects before collection of sample.

RESULTS

Figure 1 shows that a total of 404 patients attended the eye clinic during the study period from July, 2010 to April, 2011. Sixty two patients were discovered to have some form of retinal inflammation from retinal examination by the ophthalmologist. Forty six of these patients were HIV positive and sixteen were HIV negative. PCR analysis detected CMV DNA from three HIV positive patients (6.5%) (Figure 2) while no DNA for EBV and HSV were detected. Table 2 shows the characteristics of the 62

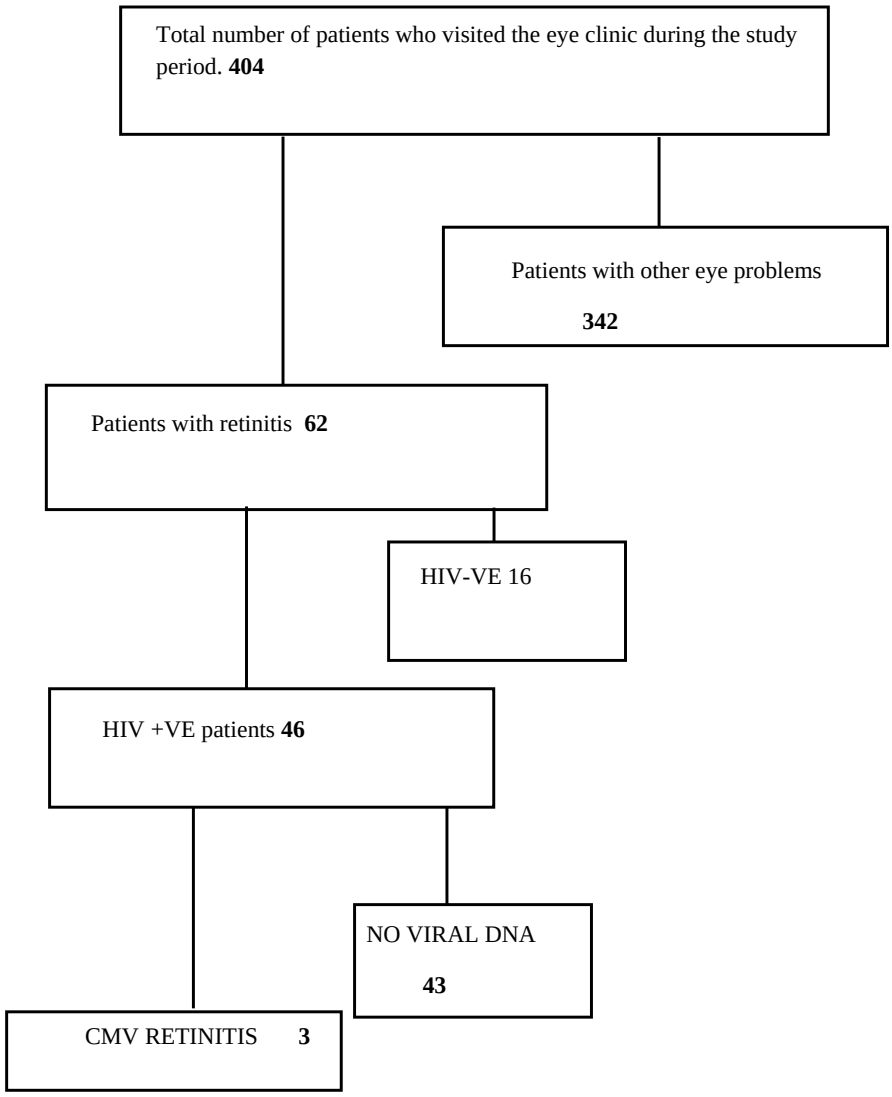

Figure 1. Flow chat of study selection process and results.

patient with retinitis that attended the eye clinic. There were more females than males in the study with 71.7% of them HIV positive. The highest age group was 20 to 30 years with 28.3% HIV positive. Majority of samples collected were the eye swabs constituting 65.2% among HIV positive patients. The 3 patients with CMV retinitis (2 females and a male) (Table 3) had CD4 lymphocyte count levels above 250 per µl, which was the modal CD4 count (63%) among the HIV positive patients (Table 4). Eye swab sample was used in one of the patient while vitreous humour was used for the other two (Table 3).

DISCUSSION

This was a cross sectional study aimed at evaluating the prevalence of CMV retinitis among HIV patients with retinitis. This study recorded a 6.5% prevalence for CMV retinitis which was relatively higher than most cross-sectional studies done in Africa (Pathai et al., 2011). However the result was within the range of a reported prevalence rate (Kestelyn, 1999). The result was far lower than reported prevalence of a longitudinal study done in Togo (Balo et al., 1999). This was very much probably due to inherent deficiency in cross sectional study design in measuring the cumulative risk of CMV retinitis in HIV patients compared to longitudinal studies (Hodge et al., 2004; Heiden et al., 2007).

This study had more female in contrast to some studies that had more male (Pathai et al., 2011; Tran et al., 2003). This trend of female preponderance was seen among HIV positive patients and HIV patients with CMV retinitis in this study. This was probably due to higher number of females in the national prevalence of adult (15 to 49

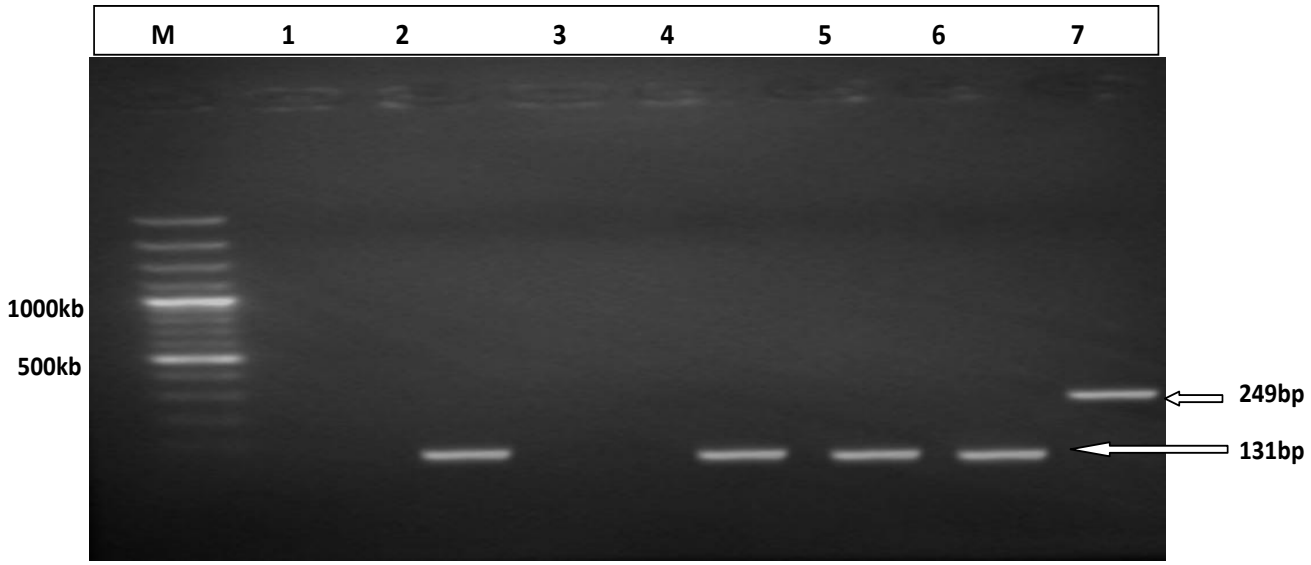

Figure 2. a 2% gel electropherogram for positive CMV group. Key: M= Molecular marker. Lane 1=Negative control; Lane 2= CMV; Lane 3= vtm23;Lane 4 = 4 CMV. Lane 5 = CMV (repeat of lane4); Lane 6= CMV; Lane 7=positive control (HHV6)

Table 2. Characteristics of patients attending eye clinic with retinitis.

Characteristics	HIV+VE (n = 46)
Sex	
Male	13
Female	33
Age (years)	
<20	3
20-30	13
31-40	10
41-50	8
51-60	8
>60	4
Specimen collection technique	
Vitreous humour	16
Eye swab	30
Herpes virus	
CMV	3
EBV	0
HSV	0

years) patients in Ghana (Ghana AIDS Commission, 2013). CMV retinitis was only seen among HIV positive patients and none seen in HIV negative patients. Increase risk of CMV retinitis among HIV has been seen in some studies (Hodge et al., 1998; Bowen et al., 1997; Moosa and Coovadia, 1997). However, most individuals are seropositive to CMV, being exposed to the virus at an early age (Urwijitaroon et al., 1992; Liu et al., 1992; Adjei, et al., 2006). More so in Ghana, CMV is hyperedemic (Adjei et al., 2006). Therefore, routine eye and/or retinal examination by a trained health care giver or specialist ophthalmologist should be practiced so as to treat this and any other ocular complications of HIV at an early stage.

CMV retinitis has been seen in HIV positive persons with CD4 counts below 50 cells/µl (Kuppermann et al., 1993). The 3 patients in this study with CMV retinitis had CD4 count above 250 cells/µl. CMV retinitis has been reported in HIV positive patients with CD4 count above 50 cells/ µl in a clinical trial study (Jacobson et al., 1997). This clinical trial study noticed that despite commencement of highly active antiretroviral therapy (HAART) and improvement of CD4 counts, CMV retinitis still occurred. Our present study has the limitation of not providing the details of HAART in the HIV positive patients.

Conclusion

This study provided the prevalence of CMV retinitis among HIV positive patient. This was however a prevalence obtained in Korle-Bu Teaching Hospital of Ghana. This might not be a true representation of CMV retinitis among HIV patients as only a longitudinal study taking into account the dynamics of HAART and CD4 counts in these patients can give the true incidence of CMV retinitis.

Table 3. HIV positive patients with CMV retinitis.

Gender	Age	Sample collected	CD4 Cell count/µl
Female	30	Eye swab	455
Male	28	Vitreous humour	295
Female	45	Vitreous humour	513

Table 4. CD4 cell counts in 46 HIV/AIDS Patients.

CD4+ lymphocyte count (per µl)	Frequency	Percentage
≥ 250	29	63.0
150 – 200	10	21.7
100 – 150	2	4.4
50 – 100	4	8.7
≤ 50	1	2.2

Limitation

The use of both Vitreous humour and Eye swab samples was a limitation of this study.

Conflict of Interests

The authors have not declared any conflict of interests.

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