

## Prevalence and Molecular Detection of Human Cytomegalovirus among HIV Patients in Khartoum State, Sudan

Omer S Mohammed<sup>1</sup>, Abdel Rahim M El Hussein<sup>2</sup>, Isam M Elkhidir<sup>3</sup> and Khalid A Enan<sup>2\*</sup>

<sup>1</sup>Department of Medical microbiology, Faculty of Medical Laboratory Science, El Nellin University, Khartoum, Sudan

<sup>2</sup>Department of Virology, Central Laboratory- The Ministry of Higher Education and Scientific Research, P.O. Box 7099, Khartoum, Sudan

<sup>3</sup>Department of Microbiology and Parasitology, Faculty of Medicine, University of Khartoum, Khartoum, Sudan

**\*Corresponding Author:** Khalid A Enan, Department of Virology, Department of Virology Central Laboratory, The Ministry of Higher Education and Scientific Research, P.O. Box 7099, Khartoum, Sudan. Email: Khalid.enan@gmail.com

Mobile No: +249912651103.

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### Abstract

**Background:** Human Cytomegalovirus (HCMV) is one of the opportunistic infections associated with significantly high morbidity and mortality among patients living with immunodeficiency syndrome.

**Aim:** The aim of this study was to determine the prevalence and to molecularly detect HCMV among HIV patients in Khartoum State Sudan, during the period 2016.

**Methods:** The study was carried out in Omdurman Teaching Hospital, Khartoum's Teaching Hospital and Bashaer Hospital, Khartoum State, Sudan. A total of 81 HIV sero-positive cases were included. HIV infection was confirmed by Enzyme linked Immunosorbent Assay (ELISA). HCMV IgG and IgM antibodies were detected using ELISA and HCMV DNA was detected using Loop mediated Isothermal Amplification PCR (LAMP PCR).

**Result:** Among 81 HIV positive samples 100% were found positive for HCMV- IgG while (24%) were positive for HCMV- IgM and (60%) samples were positive for HCMV DNA.

**Conclusion:** In Sudan, the existence of HCMV in patients with HIV infection was confirmed by using serological and molecular techniques. These findings indicate that the LAMP PCR system is an accurate and quick diagnostic system for HCMV.

**Keywords:** HIV; HCMV; ELISA; LAMP PCR; Sudan

### Introduction

Human Cytomegalovirus (HCMV) is a large encapsulated double stranded DNA virus. It belongs to the beta – herpes virus group. Most likely it is one of the most common latent infections known to humans [1]. HCMV infection is defined as isolation of the HCMV virus or detection of viral proteins or nucleic acid in any body fluid or Tissue specimen (e.g., plasma, serum, whole blood, peripheral blood leucocytes, CSF, urine, or tissue) [2].

Normally it is controlled by the cellular Immune response and hence characterized as a self- limiting infection in healthy individuals. In contrast, the HCMV in Immunosuppressed individuals as in case of HIV infection carries a risk of high morbidity and mortality [3]. Clinical disease due to HCMV has been observed in up to 40% of the patients with advanced HIV disease [4].

In HIV infected individuals, opportunistic viral infections are one of the major cause of morbidity and mortality. These agents cause infections which could be asymptomatic or mildly symptomatic in immunocompetent individuals, and it is often self- limiting. However, in immunosuppressed individuals and individuals with malignancy, infection with these agents leads to severe life-threatening diseases [5,6].

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The primary target of HIV during infection is CD4+ cells that lead to reduction in these cells and thereby cause immunosuppression. During HIV disease progression, the infected individuals could succumb to AIDS related opportunistic viral infections when CD4 cells are less than 200cells/ $\mu$ L [7]. The important opportunistic viruses that can cause infection in HIV infected individuals are herpes viruses like herpes simplex virus (HSV), varicella zoster virus (VZV), *Epstein-Barr virus* EBV and cytomegalovirus (CMV). Herpes viruses infections are seen in HIV infected individuals and can manifest as meningitis, encephalitis, lymph proliferative disorders, retinitis [8,9]. One another important herpes virus that can cause Kaposi's sarcoma associated lesions in HIV infected individuals is the human herpes virus-8 (HHV8) [10].

The variation in sero-prevalence of CMV IgM observed in several studies may probably be due to epidemiological and methodological differences. Cytomegalovirus is a ubiquitous and infection caused by this virus has become endemic throughout the world, with prevalence ranging from 40 -100% [11].

## **Material and Methodology**

### **Study design**

This is a Cross sectional study carried out in Khartoum state's hospitals.

### **Clinical samples**

Plasma samples from 81 patients with HIV seropositivity who were clinically suspected of acquiring HCMV were included in the study. The study was conducted in three Khartoum Hospitals (Basher Hospital, Khartoum Hospital and Omdurman Hospital) during period January to June 2015. All participating patients were given a written informed consent.

Blood samples were collected in EDTA tubes and centrifuged at 3000 RPM for 5 mins. Obtained plasma used for rapid Enzyme Linked Immunosorbent assay (ELISA) and DNA extraction for LAMP PCR.

The viral DNA was finally eluted in 30 $\mu$ l of elution buffer and stored at -20°C. The DNA of CMV strain AD169 was used as a positive control.

All the patient samples were tested by both the ELISA and LAMP assay to address the reproducibility of the LAMP PCR assay.

### **Serology**

Commercial ELISA kits (Chemux BioScience, INC, San Fransicsco, USA) were used to detect HCMV IgG and IgM according to the procedure described by the manufacturer.

### **DNA extraction kits**

Commercial DNA extraction kits (analytikjena, Germany) are used to detect DNA of HCMV according to procedure described by the manufacture.

### **LAMP PCR**

#### **LAMP primers Mix**

Primers mix that contains four primers (FIP, BIP, F3, B3, FLP and BLP) which were derived from the sequence of the HCMV glycoprotein B (gB) gene (12). Final concentration of FIP and BIP was 40 pmol each, FLP and BLP was 20 pmol each, F3 and B3 5 pmol each. The sequences of these primers are shown in (Table 1).

#### **LAMP condition**

The reaction was performed in a final volume of 25  $\mu$ l using LAMP regents (Mast, Reinfeld, Germany) which contained 12.5  $\mu$ l 2x LAMP reaction buffer, 1  $\mu$ l of Bst DNA polymerase, 2  $\mu$ l primer mix (PM) included (40 pmol each FIP and BIP primers, 5 pmol each F3 and B3 primers), 1  $\mu$ l fluorescence dye (FD), 6.5  $\mu$ l H<sub>2</sub>O and 2  $\mu$ l of target DNA. The mixture was incubated in a real-time PCR at 64°C for 60 minutes and visualized the results using FAM channel.

F3	TTCGCGCATGATCTCTTCG
B3	GAGGAATGTCAGCTTCCCAG
FIP	ATCGACCCGCTGGAAAATACCGTTTTGTTGCTGGAACGCAGCTCTT
BIP	ACTGCTGAGGTCAATCATGCGTTTTATCTTCATCGCCGGGAATC
LPF	GGTACTGGAACCTTACTCGCAGA
LPB	TTGAAGAGGTAGTCCACGTACTC

**Table 1:** LAMP Primers used in this study.

## Results

The results of ELISA IgG, IgM and LAMP PCR for the diagnosis of HCMV in plasma samples collected from HIV patients in Khartoum State are shown in Table 2 and 3.

Test	Positive	Negative	Total tested
CMV IgG	81 (100%)	0 (0%)	81
CMV IgM	25 (30%)	56 (70%)	81
LAMP PCR	49 (60%)	32 (40%)	81

**Table 2:** Frequency of CMV IgG and IgM antibodies and CMV DNA in HIV seropositive patients in Khartoum State.

Age group	♂	♀	Ophthalmic	Dermatitis	CMV IgM	CMV IgG	LAMP PCR
1 - 18	3	1	3	0	3	4	3
19 - 30	11	12	14	5	15	23	10
> 30	33	21	34	18	7	54	36
Total No. (%)	47 (58%)	34 (42%)	51 (62%)	23 (28%)	25 (30%)	81 (100%)	49(60%)

**Table 3:** Seroprevalence and LAMP PCR of CMV among HIV infected patients regarding their age and gender with ophthalmic and lesion.

## Discussion

Susceptibility of HIV seropositive individuals to CMV positive is a controversial issue [12-14]. The result of investigations from endemic parts of the world showed higher CMV seroprevalence rates in HIV-infected individuals and also its association with late stage of infection. It is not clear whether this is due to an opportunistic infection or a common method of transmission [8]. This study was designed to determine the seroprevalence of CMV antibodies and DNA in HIV infected patients in Khartoum.

LAMP was developed for the detection of CMV with high specificity and sensitivity [12]. Our present study represents the first report on using this technology for detection of CMV in HIV patients in Sudan.

Our above results for LAMP PCR for CMV is higher than [12] by using multiplex PCR to detect opportunistic viruses. The authors concluded that about 5 (7%) samples were positive for cytomegalovirus which is far below our present results for CMV in LAMP PCR (60%).

Our present results for anti-CMV IgG (100%) and IgM (30%) were higher than an earlier report stating 89.4% IgG antibodies and 10.6% IgM antibodies in HIV positive patients in north India [13].

The same study [13] reported that (32.4 %) of HIV positive patients had CMV co-infection and that part of the symptoms may be attributed to CMV in India [13]. The results are far lower than our present study (100%) but similar to in another study in India showing 93% of HIV patients were CMV IgG positive [14]. Patients who had ophthalmic manifestation or retinal detachment were reported to be 70% in HIV positive patients in India. Our present study showed that 62% of HIV patients with ophthalmic manifestation were associated with CMV co-infections.

In addition CMV infection in 16% patients with minor lesions has been implicated as contributory cause of death in these patients [15], and that 59% HIV positive patients indicated histological evidence for CMV infection [16]. Also our study showed the increase in CMV IgG sero-positivity with age (table 3) which agrees with the findings made in a similar study in Iran [17].

Results of one study reports CMV IgM antibody sero-prevalence to be 8.4% - 9% among HIV infected Thai children [18]. In contrast our study concludes that among 30% of CMV IgM positive patient there were 4.9% IgM positive children. However, about 2.3% IgM positive children were recorded in USA [18] which is lower than in our study.

## Conclusions

Our findings show high seroprevalence of CMV IgG, IgM and DNA among the study group. Our study reveals the need for further investigations in different parts of the country to highlight the severity of the problem. This will help in better management of the HIV cases by early diagnosis of CMV antibodies and DNA in the patients. Our study also represents the first report on using LAMP to detect CMV DNA.

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