

Seroprevalence of Human Cytomegalovirus Infection among HIV Patients in Khartoum State

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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. CMV has been reported to enhance HIV replication and accelerate the progression of HIV infection to AIDS.

Aim: The aim of this study was to determine the prevalence of HCMV among HIV patients in Khartoum State, Sudan, during the period April to July 2018.

Methods: The study was carried out in Khartoum State, Sudan. A total of 92 HIV sero-positive cases were included. HCMV IgG and IgM antibodies were detected using Enzyme Linked Immune Sorbent Assay.

Results: Among 92 HIV positive samples, 91 (99%) were found positive for HCMV- IgG while 3 samples (3.2%) were positive for HCMV- IgM.

Conclusion: In Sudan, the existence of HCMV in patients with HIV infection was confirmed by using ELISA. These findings indicate that CMV is hyper-endemic in HIV seropositive patients in Khartoum, Sudan.

Keywords: HIV; HCMV; ELISA; 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 [1,2].

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) [3].

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 [4]. Clinical

disease due to HCMV has been observed in up to 40% of the patients with advanced HIV disease [5].

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 [6,7].

The ELISA is the most common test available for measuring CMV IgG (past exposure to CMV) and CMV IgM (recent or reactivation of CMV infection) [8]. CMV infection among HIV patients has been reported in Sudan [9]. But still the surveillance of CMV infection in immunocompromised cases needs to be investigated to meet the future health challenges.

Cytomegalovirus is a ubiquitous and infection caused by this virus has become endemic throughout the world, with prevalence ranging from 40 -100% [10].

Materials and Methods

Study Design

This was a cross sectional study that was carried out in Khartoum state, Sudan, during period from April to July 2018.

Study Population

The study population constituted of 92 HIV sero-positive patients both males and females. The age group was between 20 to > 50 years.

Data Collection

The data was collected through personal interview using structured questionnaire to provide information regarding age, sex, residence, educational level and treatment.

Clinical Samples

A 5 ml of whole blood was collected from each HIV infected patient by vein puncture. Serum was separated after centrifugation of blood at 3000 rpm for 5 minutes and then stored at -20°C. All the sera were later analyzed by using ELISA.

Serology

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

Principle of the Test

Purified CMV antigen is coated on the surface of microwells. Diluted patient serum was added to the wells and the CMV specific antibody, if present binds to the antigen. All unbound materials are washed away. Excess enzyme conjugate was washed off and Tetramethylbenzidine (TMB) chromogenic substrate was added. The enzyme conjugate catalytic reaction was stopped after a specific time. The intensity of the color generated is proportional to the amount of specific antibody in the sample. The results are read by a microwell reader and compared in a parallel manner with calibrator and controls.

Assay Procedure

The desired number of CMV-antigen coated strips of microtitre wells were placed into the holder and 1:40 dilution of each negative control, positive control and calibrator was prepared by adding 200 µl of sample diluents to 5 µl of each of the reagents and mixed properly. One hundred µl of the diluted sera, calibrator and controls were dispensed into appropriate wells. For the reagent blank, 100 µl sample diluents were dispensed into the well in position A1. The holder was tapped gently to remove air bubbles from the liquid and also to mix the contents of each well. The test strips were incubated for 30 minutes at room temperature. After incubation, the liquid content was removed and further dapped onto tissue paper pad. During each wash, 100 µl of washing buffer was dispensed into the test wells and poured off. After the third wash, 100 µl of TMB chromogenic substrate was dispensed into each well and incubated again for 30 minutes at room temperature after which 100 µl of stop solution was added to stop the reaction. A microwell reader was used to read the optical density at 450 nm.

Interpretation of the Results

This was carried out according to the manufacturer's recommendations.

Negative result: CMV index of less than 0.90 for IgM and IgG antibodies.

Equivocal result: CMV index between 0.91-0.99 for IgM and IgG antibodies, and the sample should be retested.

Positive result: CMV index of 1.0 or greater for IgM and IgG antibodies.

Statistical Analysis

Data obtained from the study were analyzed by Chi-Square using software Statistical Package for Social Science (SPSS version 16) to determine the association between prevalence of infection and the studied parameters. Values obtained were considered statistically not significant (P value > 0.05).

Ethical Consideration

A written consent was obtained from participants after carefully explaining the concept of the study to them. Ethical clearance was sought and obtained from the University of Khartoum, Khartoum, Sudan.

Results

Out of 92 cases, IgG antibodies against CMV were detected more than IgM antibodies. The results of ELISA IgG and IgM for the diagnosis of HCMV in serum samples collected from HIV patients in Khartoum state are shown in Table 1.

Test	Positive	Negative	Total tested
CMV IgG	91 (99%)	1 (1%)	92
CMV IgM	3 (3.3%)	89 (96.7%)	92

Table 1: Frequency of CMV IgG and IgM antibodies in HIV seropositive patients in Khartoum State.

We found that CMV IgG positive result was higher in age between 30-50 years (71 out of 91 positive patients), 12 patients less than 30 years old, while only 8 positive patients above 50 years. IgM was equal in each age group as shown in Table 2.

Age group	CMV IgG (Positive)	CMV IgM (Positive)
<30	12	1
30-50	71	1
>50	8	1
Total No. (%)	91 (99%)	3 (3.2%)
P value	0.1	0.652

Table 2: Seroprevalence of CMV among HIV infected patients regarding their age.

49 males and 42 females were positive for CMV IgG. Two males and only one female were shown positive result for CMV IgM as seen in Table 3.

Test	Male	Female	Total. No (%)	P value
CMV IgG (Positive)	49	42	91 (99%)	0.615
CMV IgM (Positive)	2	1	3 (3.2%)	0.385

Table 3: Seroprevalence of CMV among HIV infected patients regarding their gender.

Discussion

Susceptibility of HIV seropositive individuals to CMV positive is a controversial issue [11-13]. The result of investigations from endemic parts of the world showed higher CMV seroprevalence rates in HIV-infected individuals. This study was designed to determine the seroprevalence of CMV antibodies in HIV infected patients in Khartoum state.

In this study, the seroprevalence of CMV IgG was 99% among our HIV-infected patients. This is in agreement with report from the Khartoum state, south west Nigeria and Burkina Faso by Khalid A Enan *et al.* [9] at and Ledru, *et al.* [14] respectively that documented a prevalence of anti-CMV IgG antibody of 100% among HIV-infected patients. The high CMV IgG seropositivity rate in this study is suggestive of widespread past exposure to infection.

However, lower prevalence rates have also been reported in other countries like India (10.4%) [15] and 95.6% among HIV positive Malawian children [16]. These differences in prevalence maybe related to characteristic of the study population and the sensitivity of the screening tests.

Results of one study reports CMV IgM antibody seroprevalence to be 8.4%-9% among HIV-infected Thai children [17]. Whereas higher than our study (3.2%) and other recorded in USA (2.3%) [17]. The variation in seroprevalence of CMV IgM observed may probably be due to epidemiological and methodological differences. There was no children in our study, the smallest age was 20 years old.

There is need to further investigate the prevalence of CMV infection immuno-compromised cases in other part of the country to have a better picture of the extent of this problem in Sudanese scenario.

Conclusions and Recommendations

Our findings show high seroprevalence of CMV IgG and low seroprevalence of IgM among the study group. This 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 in the patients.

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