Original Article

Prevalence of IgG and IgM antibodies to human cytomegalovirus among Sudanese renal transplant recipients and haemodialysis patients

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Abstract

Introduction: Human cytomegalovirus (HCMV) is a member of the genus Herpes virus and belongs to the family Herpesviridae. **Objective:** The aim of this research work was to study the prevalence of human cytomegalovirus (HCMV) infection in renal transplant and haemodialysis patients. Blood samples were collected randomly from 52 renal transplant patients and 41 haemodialysis patients. The sera were tested with an enzyme linked immunosorbent assay (ELISA) for HCMV IgG antibodies and additional ELISA test for HCMV IgM antibodies. **Results:** Renal transplant screening revealed that 98% of patients have IgG for HCMV antibodies and only 6% have IgM antibodies. In haemodialysis patients 95% showed the presence of IgG antibodies to HCMV and non of patient revealed the presence of IgM antibodies.

Key words: Human cytomegalovirus, renal transplant patients, serology

INTRODUCTION

Human cytomegalovirus (HCMV) is a member of the genus herpes virus and belongs to the family *Herpesviridae*. As with all members of the family, the virus has the ability to persist in the host in a latent state after primary infection.[1] It is a ubiquitous virus, the seroprevelance of which varies between 30% and 100% in different countries. Acquisition of the virus in the general population mainly occurs early in life. Transmission of the virus can occur vertically or horizontally through direct contact with infectious body fluids or blood. The virus can also be transmitted by blood products or transplanted organs. After the primary infection, the virus will remain in a latent state in the host life-long, but may reactivate later. Although rarely pathogenic in immunocompetent individuals, the virus causes a significant health threat to immunocompromised individuals and is a significant cause of morbidity and mortality especially in organ allograft and bone marrow transplant patients.[2,3]

HCMV is a significant pathogen in organ transplanted patients causing symptomatic infections and end-organ disease. Many risk factors for the development of symptomatic infection have been suggested. Viral load has been shown to be a major factor in the development of HCMV disease.

The first surveillance of HCMV infection in Sudan was conducted in 2004, in blood donors and antenatal women.[4] The second surveillance was carried out in 2006, in candidate recipients, kidney candidate donors and blood donors.[5]

In the present study, we detected HCMV immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies in kidney transplant and hemodialysis patients.

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MATERIALS AND METHODS

Sample collection and processing

A total of 93 blood samples were collected from two patient groups in Khartoum State. These include renal transplant recipients group ($n = 52$) and hemodialysis patients ($n = 41$) at the Sudanese kidney transplanted association (SKTA) Center in Khartoum North. Venous blood samples were collected using vacutainer and needles. The serum was separated and placed in sterile 1.5 ml eppendorf tubes and the tubes were centrifuged at 1000 rpm for 5 min at 4°C. The serum was then removed without disturbing the pellet and put in other tubes and stored at −20°C till used.

Enzyme linked immunosorbent assay (ELISA) for detection of HCMV IgG and IgM antibodies

This was done according to the manufacturer's instructions (Equipar Diagnostici Co., Ltd, Italy). The results were read against 450 nm absorbance using a microplate reader (Awarenss Technology, Palm City, USA). In brief, 100 µl of diluted patients serum (1:100 with serum diluents), one well negative and two wells positive controls were pipetted in duplicates into wells of microtiter plates precoated with HCMV antigen. After incubation for 15 min at 25°C, the plates were rinsed 5 times with 300 µl diluted washing solution to remove residual serum. 100 µl of enzyme-labelled antibodies to human IgG conjugate were added and incubated as above. Then well washed 5 times (300 µl washing solution) to remove unbound material. Then 100 µl of substrate solution (tetramethylbenzidine) was pipetted and incubated for 15 min to induce development of colour. The reaction was terminated by the addition of stop solution and the resulting dye was measured in a spectrophotometer (Awareness Technology, Palm City, USA) at a wave length of 450 nm against the substrate blank. The results were interpreted according to the manufacture instruction. For IgG ELISA, a sample was considered to be negative and positive when the absorbance of individual values was found <1.0 and >1.1, respectively. Test results were labeled as equivocal means when the absorbance value was found to be between 1.0 and 1.10. For IgM ELISA, a sample was considered as negative and positive when the absorbance of individual values was found <0.90 and >1.1. Samples were considered equivocal when absorbance of individual values was between 0.90 and 1.10.

RESULTS

ELISA results for cytomegalovirus (CMV) IgG and IgM

51 out of 52 blood samples collected from renal transplant recipients (98%) gave positive results and 39 out of 41 blood samples (95%) collected from hemodialysis patients gave positive results [Table 1]. 3 out of 52 blood samples collected from renal transplant recipients (6%) gave positive results and no samples out of 41 blood samples (0%) collected from hemodialysis patient gave positive results [Table 2].

DISCUSSION

Cytomegalovirus infection remains a major cause of morbidity and mortality in immunocomparamised patients, and most serious problem in organ allograft recipients. The possibility of using specific antiviral therapy to treat CMV infection makes a timely diagnosis imperative. [6]

We studied the prevalence of CMV antibodies among renal transplant and haemodialysis patients in Khartoum state using ELISA. IgG antibody prevalence was 98% and 95% in renal transplant and the haemodialysis patients, respectively, suggesting high incidence of previous infection in all groups tested. Since no vaccination program is practiced against CMV in Sudan our results illustrate for previous infections with the virus. Eldowma[4] detected IgG antibodies of CMV among blood donors and antenatal women in Sudan with 77% and 95%, respectively. Bushra[5] detected 96% seropositivity for IgG in pretransplant kidney recipients, 17% in healthy candidate donors, and 84% in blood donors. Taken together, our results and the findings of Eldowma[4] and Bushra[5], it is obvious that CMV is endemic in the Sudan. The present high CMV seroprevalence in the two groups studied points to high

Table 1: Detection of CMV immunoglobulin G antibody in kidney patient's groups in Khartoum state by ELISA

Group tested	No. of screened	No. of positive	No. of negative	No. of positive %	No. of negative %
Renal transplant	52	51	1	98	2
Haemodialysis patient	41	39	2	95	5
Total	93	90	3	97	3

CMV = Cytomegalovirus; ELISA = Enzyme linked immunosorbent assay

Table 2: Detection of CMV immunoglobulin M antibody in kidney patient's groups in Khartoum state by ELISA

Group tested	No. of screened	No. of positive	No. of negative	No. of positive %	No. of negative %
Renal transplant	52	3	49	6	94
Haemodialysis patient	41	0	41	0	100
Total	93	3	90	3	97

CMV = Cytomegalovirus; ELISA = Enzyme linked immunosorbent assay

spread of the infection in the population as a whole. This high prevalence of CMV may be attributed to the poor socioeconomic status and hygienic practice known to play an important role in transmission of CMV due to over-crowdness. the prevalence of infection varies with socioeconomic status, living condition, and hygienic practices, antibody prevalence may be moderate (40-70%) in adults in high socioeconomic groups in developed countries in contrast to prevalence of 90% in children and adults in developing nations and in low socioeconomic groups in develop countries according to Jawetz *et al*.[7] Further studies are needed to determine the exact prevalence of CMV infection in other parts of the Sudan.

Furthermore, IgM ELISA was used to establish whether or not infection in the normal host has occurred recently. The prevalence of HCMV IgM, in the present study was 6% in renal transplant and 0% in haemodialysis patients. It is well documented that renal transplanted patients receives high doses of anti inflammatory agents (e.g., corticosteroids). These anti-inflamatory agents cause significant suppression of the immune system with subsequent result of high prevalence rate of the HCMV infections. These might reflect an alarming picture of the disease in the population and indicate that seroconversion is an ongoning process in renal transplant group in the Sudan.

The presence of HCMV IgM antibodies mostly indicates active infection as has been reported previously by Farrell *et al*.[8] ELISA can detect HCMV antibodies of both primary CMV and reactivation of latent infection. Accordingly, high titer of CMV IgM antibodies by ELISA suggests recent exposure or reactivation of latent infection.

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