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RESEARCH ARTICLE

PCR DETECTION OF HUMAN CYTOMEGALOVIRUS IN PLASMA AND URINE SAMPLES FROM RENAL TRANSPLANT PATIENTS

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ABSTRACT

Background: This study was carried out to detect human cytomegalovirus (HCMV) in renal transplant patients in Khartoum state, Sudan, to show if urine gives a reliable result in HCMV PCR detection as plasma samples. Randomly, a total of 50 plasma and urine samples were collected from renal transplant patients at hospital of Sudanese renal transplant society during April to May 2012
Results: Urine and plasma together gave positive CMV result in 4 patients, while 2 patients were detected by plasma only, and one patient was detected by urine sample only. Analysis of result give a significant relationship between the two samples (p =0.00).
Conclusions: The results of present study indicated that urine can be used for PCR detection of CMV as plasma sample, but further study need to be done for more validation.

INTRODUCTION

Cytomegalovirus (CMV) is a major cause of disease in organ and cell transplant recipients. The virus belongs to the *herpesviruses* family and the *Betaherpesvirinae* subfamily. The species that infects humans is commonly known as human CMV (HCMV) or human herpesvirus-5 (HHV-5), and is the most studied of all cytomegaloviruses [(1)]. HCMV is an ubiquitous virus, with seroprevalence that varies between 30 to 100 % in different countries [(2)]. The greatest seroprevalence and also early acquisition of the virus have been associated with lower socioeconomic status and in developing countries with crowded population [(3)]. In Sudan only two published studies were reported, Sero-reactivity to human cytomegalovirus [(4)]; this study was done on renal transplant patients and the results showed that all patients (100%) had I gG antibodies, while only 6% of the patients had IgM antibodies. The other study compared Real-time PCR to ELISA for the detection of human cytomegalovirus infection [(5)]; from 98 renal transplant patients IgG antibodies were detected in all patient's plasma (100%) and IgM antibodies were detected in 6.1%, HCMV is transmitted from person to person via close contact with an individual who is excreting the virus. It can be spread through the placenta, blood transfusions, organ transplantation and breast milk. It can also be spread through sexual transmission [(6, 7)]. It infects wide range of tissues and cell types, and has been found in salivary glands, lung, liver, pancreas, kidney, ear, eye, placenta, alimentary tract, heart, ovaries, pituitary, brain, skin, thyroid, esophagus, prostate, testes, and adrenals [(8)]. The immune system of healthy individuals is usually able to prevent CMV from producing clinical manifestations; but some individuals experience a mononucleosis-like syndrome with symptoms including malaise, persistent fever, myalgia, cervical lymphadenopathy, and, less commonly, pneumonia and hepatitis[(9)]. The immune system very rarely completely eliminates CMV from the body - the viral genome remains in a latent stage waiting for reactivation [(8)]. HCMV infection can be life-threatening for the immunocompromised, such as HIV-infected persons, organ transplant recipients, or new born infants.[(1)] Diagnosis of HCMV is based on clinical symptoms, however, the symptoms of HCMV are confused with Epstein-Barr virus (EBV), and this may lead to difficulties in diagnosis [(10)]. Laboratory diagnosis can be done by several procedures; via cell culture. Despite it's a high specificity (89% to 100%), the sensitivity of these tests is quite low (45% to 78%). The other disadvantages are the long incubation period, the insufficient virus quantity and the high rate of false negativity [(11)]. Direct detection of antigens with a monoclonal antibody against the CMV matrix protein pp65, which has rates of sensitivity and specificity between 60%to 100% and 83% to 100%, respectively, has proved particularly useful [(12,13)].

Due to the high seroprevalence, diagnostic value of serologic tests is limited for the determination of an active infection in the adult population. However, the use of CMV specific antibodies may be beneficial in the diagnosis of the new onset infections (CMV IgM positivity (+), elevation in titration of IgG). Cases in the risk group for CMV reactivation can be identified by this method [(12, 14)]. In the tissues, histopathological detection of CMV can be provided by routine H&E staining of typical intranuclear and intracytoplasmic inclusion [(15)]. Polymerase chain reaction (PCR) has provided a very sensitive technique for detection of CMV [(16)]. Detection of CMV DNA in blood plasma by PCR may correlate with disease better than assays using leukocytes or whole blood. Other studies have found that CMV DNA detected by PCR in plasma correlated more closely with the CMV pp65 antigenemia assay [(17)].

MATERIAL AND METHODS

Study design: This study was carried out on patients admitted to Hospital of Sudanese Renal Transplant Society during April to May 2012.

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Patients

Fifty renal transplant patients were included in this study.

Ethical consideration

This study was approved by Al-Neelain Ethical Committee, and the Hospital's Ethics Committee. Informed written consent was obtained from all patients in the study.

Clinical specimens

Urine samples were randomly collected in clean urine containers, and Plasma samples were collected at the same time of urine specimens using EDTA anticoagulant. Data were collected using a structural interviewing questionnaire that covered general information (name, and duration of transplant).

DNA extraction and PCR

DNA was extracted from patient's materials using commercial Qigene QIAamp Blood Kit Cat No (51104) according to manufacture instructions. The extracted DNA was stored at -20 ^{0} C. The PCR was performed by processing the extracted DNA from urine and plasma with primers that are specific for the gB gene of (HCMV), the primer consisting of 5' TGG AAC TGG AAC GTT TGG C 3' and 5' AAA CGC GCG GCA ATC GG 3' (gB1319 and gB 1604) [18]. The reaction was performed in 20µl volume using Solis Biodyne master mix. The volume included : 4 µl master mix, 2 µl forward primer, 2 µl reverse primer,5 µl extracted DNA and 7 µl distilled water. The mixture amplified in thermo- cycling conditions using PCR machine Techne (TC-14) as follow: initial denaturation at 95ºC for 3 minutes, followed by 35 cycles of denaturation at 95ºC for 40 seconds, annealing at 57ºC for 1 minute and amplification at 72ºC for 4 minute, with a final extension 72ºC for7 minutes. 10µl of the amplified product was subjected to direct analysis by Gel Electrophoresis in 2% agarose, the gel was prepared by adding 0.7g of agarose to 35 ml 5X Tris Borate EDTA buffer. The product was visualized by staining with 0.15 % ethidium bromide using UV gel documentation system INGeNius. A 280 bp product was amplified with above gB specific primer.

Statistical analysis

Collected data were analyzed using the Statistical Package for Social Science (SPSS); Cross tabulation & chi-square tests for CMV detection in urine & plasma were l used.

RESULTS

Conventional qualitative PCR detected CMV DNA in 6 (12%) of plasma and in 5 (10%) urine samples. In 7 (14%) of the patients, HCMV was detected in both urine and plasma samples. Cross tabulation of the results indicated high correlation between urine and plasma sample for PCR detection of HCMV, *p* value (0.00), Table (1). There is no significant relationship between duration of the transplantation and positive urine and plasma samples, *p* value (0.276), (0.376) respectively.

Table 1. Cross tabulation of positive and negative PCR results of the plasma and urine samples

		Urine		Total	P. value
		negative	positive		
Plasma	Negative	43	1	44	
	Positive	2	4	6	0.000
Total		45	5	50	

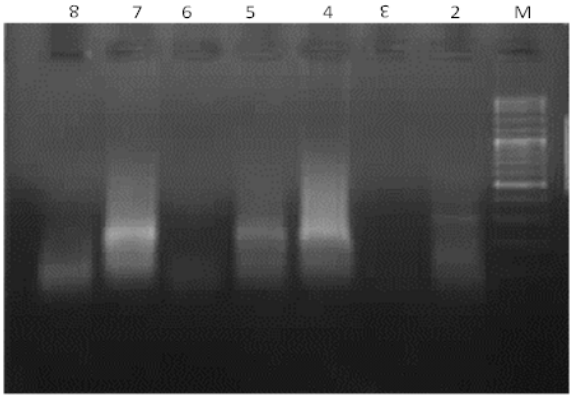

Fig.1. HCMV PCR results (298-300 bp) on 1.5% agarose gel. Lanes (2, 3, 4, 5 and 6) show PCR results in sex patients; Lane 8 shows negative, Lane 7: Positive control, M: 100 bp DNA size marker.

DISCUSSION

HCMV in immunocompermised patients causes direct systemic organ effect and indirect effect such as enhancing opportunistic infection, and it plays a major role in allograft rejection so it continues to be significant cause of morbidity and mortality in those patients [19]. For these patients, the early diagnosis of CMV is of vital importance for management of the disease. PCR which can be used to selectively amplify and detect specific DNA sequences, is known to be a rapid, specific and sensitive method for detection of HCMV DNA in various kinds of specimens, and also in cell free body fluid as serum and plasma [20]. In recent years many studies were carried out to establish urine as a suitable sample for PCR detection of HCMV, because it is easy to collect and can give reliable result, while blood collection is an invasive method and may need to repeat it many time to obtain blood, and need care and training especially when the patient is child, also the side of puncture may be consider source of infection mainly in those immunocompermised patients. In the current study the qualitative PCR procedure used urine as sample to detect HCMV DNA and compare with plasma sample.PCR was applied in specimens obtained from renal transplant patients as mentioned above. The results of urine samples had minor but insignificant differences from the plasma samples results, which is constant in 43 negative samples and 4 positive samples, and there were only 3 samples give different result between plasma and urine. We assumed, the duration of the transplant may lead to these differences, but the analysis showed there was no significant relationship between duration of the immunosuppressive therapy and CMV viuria and viraemia.

The positive HCMV that was detected by plasma only may be due to that the virus execrates firstly in plasma, so need more time to detect by the two samples, or may be the virus excreted in urine for a period of time and no detect in urine after that period. But the unusual result when the virus detected in urine only. Many previously published studies from epidemiological used urine as a sample to detect CMV; such as (Jutte *et al.*, 2012) [21], who use real time PCR verses viral culture on urine as gold standard method to detect CMV, (Mewara, *et al.*,2009) [22] detected CMV in a urine of infants by extract HCMV DNA for PCR technique, and also (Schlesinger, *et al.*, 2003)[23] they reached that Urinary CMV PCR is a reliable, rapid, and convenient method, and thus may serve as a screening tool for the detection of congenital CMV infection. Based on the results of our study and those of above previously studies, we reached that urine is a reliable and in invasive sample for PCR detection of HCM, so can use instead of plasma sample.

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