

Full Length Research Paper

Seroprevalence and Viremia of HHV-6 in Saudi Renal Transplant Recipients

Obeid E Obeid

Department of Microbiology, College of Medicine, University of Dammam, P.O. Box 2114, Dammam # 31451, Saudi Arabia.

Email: oobeid@yahoo.com. Phone: 00966509929487

Accepted 20 October, 2024

Human herpesvirus 6 (HHV-6) is a β -herpesvirus that is characteristically T-lymphotropic. It is regarded to be a major pathogen in transplant recipients. Very little is known about the prevalence of HHV-6 in Saudi transplant recipients. The aim of the current study was to determine the HHV-6 antibodies (IgM and IgG) and viral DNA in Saudi renal transplant recipients. The study was conducted at a tertiary hospital in Eastern Saudi Arabia over a period of 12 months. All kidney transplant recipients who regularly attended the nephrology clinics were included (n=150) (blood donors). Randomly selected control individuals, age and sex matched were recruited to serve as a control group in this study (n=158). Seropositivity for HHV-6 was determined by testing for IgG and IgM antibodies employing HHV-6 EIA and IFA. HHV-6 viral DNA was assessed by real time PCR (Sacace Biotechnologies, Italy). Of the 150 Saudi kidney transplant recipients included in this study, 145 had detectable level of Anti-HHV-6 IgG antibodies (96.7%). Anti-HHV-6 IgG antibodies were detected in 122 out of 158 blood donors (control group; 77.2%). Of the 145 HHV-6 IgG positive transplant recipients, only 4 were found to have detectable levels of anti-HHV-6 IgM antibodies (2.7%). None of the HHV-6 IgG positive blood donors had detectable anti-HHV-6 IgM antibodies. HHV-6 viral DNA was detected by real time PCR in all the 4 HHV-6 IgM-positive individuals (100%). There is a high frequency of detectable HHV-6 in the studied population of renal transplant recipients. Further investigation to monitor HHV-6 primary infection reactivation in graft recipients may prove to be important in improving the outcome for these patients.

Key words: HHV-6, transplant recipients, antibodies, DNA

INTRODUCTION

Human herpesvirus 6 (HHV-6) is a β -herpesvirus that is further classified into the molecularly, epidemiologically and biologically distinct variants A and B (HHV-6A and B) (Akhyani et al., 2000; Prober, 2011). HHV-6 is characteristically T-lymphotropic (by the cellular receptor CD46), although it can infect other cell types (Alcami, 2003). Almost all children are HHV-6 seropositive by 2 years old and primary infection with HHV-6B causes exanthem subitum (roseola or 6th disease). HHV-6A has not yet been firmly associated with any disease. After first infection, HHV-6 persists for life. Salivary glands are a potential site for HHV-6 persistence and saliva is a vehicle for transmission of the virus, either from mother to

child or between children (Abdel Massih et al. 2009). HHV-6 is increasingly being recognized as a pathogen in immunocompromised hosts, including transplant recipients (Alcami, 2003; Melne et al., 2000). HHV-6 infection after transplantation is believed to result from viral reactivation. Primary HHV-6 infection is likely to occur in the pediatric transplant population, especially in children less than 2 years of age, who have not been exposed to infection. Several clinical syndromes have been associated with HHV-6 infection after organ transplantation. These have been classified as either direct or indirect effects of HHV-6. The direct clinical manifestations due to HHV-6 include a febrile illness with

or without rash, myelosuppression, hepatitis, pneumonitis and neurological diseases. The indirect effects attributed to HHV-6 include an exacerbation of cytomegalovirus (CMV) disease, an increased severity of hepatitis C virus (HCV) recurrence, an increased risk of other opportunistic infections, allograft dysfunction, and acute cellular rejection. (Abdel Massih et al. 2009; Alcamì,

2003; Melne et al., 2000).

The antibody responses to HHV-6A and B cannot be differentiated by the methods currently available (Suga et al., 1992). Evaluation of antibody development (specially IgM) and titers in sequential serum samples as well as antibody avidity tests can identify recent primary as opposed to long-standing infection: if antibody avidity is low, this confirms recent primary infection but if the avidity is high, primary infection must have occurred at least 6 weeks previously (Zerr et al., 2000).

PCR on small amounts of PBMCs has been suggested to discern active from latent infection, as well as serum-plasma PCR and reverse transcription-PCR, which allow detection of circulating viral genomes and viral mRNAs, respectively. More recently, however, quantitative PCR analysis, which allows fast, sensitive, and absolute quantitation of viral load, has become the method of choice (Quereda et al., 2000; Blumberg et al., 2000).

HHV-6 is widespread throughout the world, with geographic differences in HHV-6 prevalence varying between 70 and 100% (Abdel Massih et al. 2009; Cristopher et al. 2009). Post-transplantation HHV-6 infection is frequently due to reactivation of HHV-6B and the incidence peaks at 2 to 4 weeks. The incidence varies between 48% (range, 28 to 75%) for bone marrow transplant (BMT) recipients and 32% (range, 0 to 82%) for solid organ transplant (SOT) recipients (Ogata, 2009; Shiley & Blumberg, 2010).

Very little is known about the prevalence of HHV-6 in Saudi transplant recipients or general population. The aim of the current study was to determine the HHV-6 antibodies (IgM and IgG) and viral DNA in Saudi renal transplant recipients.

MATERIALS AND METHODS

Study population

The study was conducted in a tertiary hospital in Eastern Saudi Arabia over a period of 12 months (2008-2009). All kidney transplant recipients who regularly attended the nephrology clinics were included (n=150) (see results below). The assessment was done 1-12 years after transplantation (average is 4.6 years). Randomly selected control individuals, age and sex matched were recruited to serve as a control group in this study

(n=158). There was clear description of the objectives of the present study. A written consent was obtained.

Antibody measurements

Peripheral blood samples were collected from the subjects by venepuncture in plain tube, left to coagulate and fresh serum samples was isolated and immediately frozen prior to analysis. Seropositivity for HHV-6 was determined by testing for IgG antibodies employing HHV-6 IgG EIA kit (Biotrin, The Rise, Mount Merrion, Co. Dublin, Ireland). All positive samples were then tested for HHV-6 IgM antibodies using HHV-6 IgM IFA kit (Biotrin, The Rise, Mount Merrion, Co. Dublin, Ireland) according to the manufacturer's recommendations.

HHV-6 Viral DNA measurement

HHV-6 viral DNA was assessed on blood samples collected in EDTA tubes on smartcycler system (Cepheid, USA) by Real Time Polymerase chain reaction (PCR) using HHV6 Real-TM Quant (Sacace Biotechnologies, Italy) using the primers provided by the kit (specific for the pol-gene of HHV-6) and according to the manufacturer's recommendations.

RESULTS

Characteristics of kidney transplant recipients

The present study used the same study population of kidney transplant recipients that were used in a previous study (Alzahrani et al., 2005). This cohort of patients comprised 93 males and 57 females, of mean age 42 years (range 19–62). Standard clinical care, including medical checkups and tests for renal function, liver function and hematology parameters, was given to the renal transplant recipients in the stable post-operative stage. The main inclusion criterion for participation in this study was that patients should have reached the clinically stable stage following renal transplant surgery, usually 6 months post-operatively. Control group included blood donors from the same population.

Assessment of HHV-6 antibodies and viral DNA in kidney transplant recipients

Of the 150 Saudi kidney transplant recipients included in this study, 145 had detectable level of Anti-HHV-6 IgG antibodies (96.7%). Anti-HHV-6 IgG antibodies were detected in 122 out of 158 blood donors (control group; 77.2%). The anti-HHV-6 IgG positivity is significantly

higher in kidney transplant recipients than in blood donors ($p=0.001$).

All anti-HHV-6 IgG positive samples were tested for anti-HHV-specific IgM antibodies. Of the 145 HHV-6 IgG positive transplant recipients, only 4 were found to have detectable levels of anti-HHV-6 IgM antibodies (2.7%). All 4 IgM-positive patients were asymptomatic. None of the HHV-6 IgG positive blood donors had detectable anti-HHV-6 IgM antibodies. HHV-6 viral DNA was detected by Real Time PCR in all the 4 HHV-6 IgM-positive individuals (100%).

DISCUSSION

The incidence of post-transplantation HHV-6 infection varies and ranges between 0 to 82% in solid organ transplant (SOT) recipients. The aims of this work are to determine the HHV-6 antibodies (IgM and IgG) and viral DNA in Saudi renal transplant recipients.

The anti-HHV-6 IgG antibodies were detected in 96.7% of the kidney transplant recipient included in this study, while only 2.7% have anti-HHV-6 IgM antibodies. IgM antibodies usually indicate recent or current infection whereas IgG antibodies are indicative of past infection. The HHV-6-specific IgG and IgM positivity is significantly higher in transplant recipient than in blood donor. This could well be due to the immunosuppressive therapy. Alternatively, the transplanted organ may have contributed to the infection of the transplant recipients.

Deborska et al (2003) reported HHV-6 seroconversion after organ transplantation. Ninety-one percent of 120 recipients were HHV-6 IgG-positive before transplantation. One hundred seven of 120 patients were anti-HHV-6 IgM-negative before transplantation. Primary/secondary HHV-6 seroconversion occurred in sera of 46.6% of these 107 patients.

Deborska-Materkowska et al (2006) performed a seroepidemiological survey to test the relation between seroprevalence among donors and recipients for HHV-6. IgM antibodies to HHV-6 appeared in four of five seronegative patients who received allografts from IgG seropositive donors.

Given the pharmacologically induced state of immunosuppression in transplant recipients, HHV-6 is likely to reactivate from latency and act as an opportunistic pathogen. On the other hand, as HHV-6 is harbored by many tissues such as mononuclear cells, bone marrow, lung, and liver, there is also the possibility of graft-induced (re-)infection with HHV-6, the virus being transferred from donor to recipient. Of special interest is the observation that the rate of HHV-6 reactivation is higher among transplant recipients receiving anti-CD3 (antithymocyte globulin), which is used for the treatment of rejection (Ogata, 2009; Shiley & Blumberg, 2010).

All the 4 anti-HHV-6 IgM antibody positive transplant

recipients were positive by RT-PCR. None of the four IgM-positive DNA-positive patients develop symptoms suggestive of HHV-6 infection. Cervera et al (2006) evaluated patients undergoing solid organ transplantation with negative IgG antibodies against HHV-6 by means of HHV-6 polymerase chain reaction. Among 193 recipients, seven were HHV-6 seronegative (prevalence 3.6%). A positive HHV-6 viral load was detected in only one patient, and four patients seroconverted after one year posttransplantation. Their findings showed a low incidence of symptomatic primary HHV-6 infection among seronegative solid-organ transplant recipients.

The presence of anti-HHV-6 IgM or a fourfold rise in anti-HHV-6 IgG supports a clinical diagnosis. An alternative more rapid method requires a single acute serum which is tested for viral IgG antibody and DNA; if DNA is found in the absence of antibody, this is interpreted as the transient viraemia that occurs in the acute phase of HHV-6 primary infections before antibodies are generated. Antigen detection is an alternative rapid method for the recognition of active HHV-6 infection and avoids the problem of distinguishing the DNA of latent or integrated virus from that present due to actual replication.

Altay et al studied HHV-6 IgM and IgG antibodies in 36 peritoneal dialysis (PD) patients, 35 hemodialysis (HD) patients, and 20 healthy subjects. HHV-6 IgM antibody were positive in 9 HD patients (25.7%), 8 PD patients (22.2%), and 2 control subjects (10.0%, $p > 0.05$). More HD patients (20.0%) than PD patients (5.6%, $p = 0.07$) or control subjects (0.0%, $p = 0.03$) were positive for HHV-6 IgG antibody. In HD patients, HHV-6 IgG seropositivity and duration of dialysis were positively correlated.

Dziedziatkowski et al assessed HHV-6 antibodies in 26 adult recipients of allogeneic stem cell transplant. Specific IgM antibodies were present in the serum of 2 (8%) patients, while the others were negative (92%). Twenty of the 26 persons (77%) had IgG antibodies against HHV-6 before HSCT and the other 6 (23%) were negative.

The association between viral infection and allograft rejection is controversial. Okuno *et al.* (1990) suggested that HHV-6 can infect renal tissues and that the infection may be linked to rejection or to immunosuppressive therapy. In another study, vascular adhesion molecule expression was observed in liver tissues infected with HHV-6 after liver transplantation (Lautenschlager et al. 1999). The authors suggested that this immunologic stimulation caused by the virus may be involved in or be the trigger of the rejection cascade. Wade *et al.* [8] reported that children rejecting their kidneys after the appearance of anti-HHV6 IgM antibodies with no prior immunity to HHV-6 appear to have extremely high risk for a virus-associated rejection, approaching 100% (Deborska et al 2005).

Assessment of HHV-6 infection in transplant recipients may help in early detection of the infection and may provide evidence for possible management of these patients. Future work will include inclusion of other transplant patients (e.g. bone marrow transplantation) and correlation between immunosuppressive regimen and clinical picture of HHV-6 infection.

ACKNOWLEDGEMENT

This study was financed by a grant from The Deanship of Scientific Research of The University of Dammam. The author is grateful to Prof. Alhusain J Alzahrani and Prof. Elharith E. Elharith.

REFERENCES

- Abdel Massih RC, Razonable RR (2009). Human herpesvirus 6 infections after liver transplantation. *World J. Gastroenterol.* 15: 2561-2569.
- Akhyani N, Berti R, Brennan MB, Soldan SS, Eaton JM, McFarland HF, Jacobson S (2000). Tissue distribution and variant characterization of human herpesvirus (HHV)-6: increased prevalence of HHV-6A in patients with multiple sclerosis. *J. Infect. Dis.* 182:1321-1325.
- Alcami A (2003). Viral mimicry of cytokines, chemokines and their receptors. *Nat. Rev. Immunol.* 3:36-50.
- Altay M, Akay H, Unverdi S, Altay F, Ceri M, Altay FA, Cesur S, Duranay M, Demiroz AP (2011). Human herpesvirus 6 infection in hemodialysis and peritoneal dialysis patients. *Perit Dial Int.* 31:320-324.
- Alzahrani AJ, El-Harith A, Milzer J, Obied OE, Stuhrman M, Al-Dayel A, Mohamed EA., Al-Egail S, Doud M, Chowdhury A, Guella A, Aloraifi I, Schultz TF (2005). Increased seroprevalence of human herpes virus-8 in renal transplant recipients in Saudi Arabia. *Nephrol. Dialysis Transplantation* 20: 2532-2536
- Aritaki K, Ohyashiki JH, Suzuki A, Ojima T, Abe K, Shimizu N, Yamamoto K, Ohyashiki K, Hoshika A (2001). A rapid monitoring system of human herpesviruses reactivation by LightCycler in stem cell transplantation. *Bone Marrow Transplant.* 28:975-980.
- Blumberg BM, Mock DJ, Powers JM (2000). The HHV-6 paradox: ubiquitous commensal or insidious pathogen? A two-step in situ PCR approach. *J. Clin. Virol.* 16:159-178.
- Cervera C, Marcos MA, Linares L, Roig E, Benito N, Pumarola T, Moreno A (2006). A prospective survey of human herpesvirus-6 primary infection in solid organ transplant recipients. *Transplantation.* 15:979-982.
- Christopher Vinnard C, Barton T, Jerud E, Blumberg E (2009). A Report of Human Herpesvirus 6-Associated Encephalitis in a Solid Organ Transplant Recipient and a Review of Previously Published Cases. *Liver Transplantation* 15:1242-1246.
- Deborska D, Durlík M, Sadowska A, Nowacka-Cieciura E, Pazik J, Lewandowski Z, Chmura A, Galazka Z, Paczek L, Lao M (2003). Human herpesvirus-6 in renal transplant recipients: potential risk factors for the development of human herpesvirus-6 seroconversion. *Transplant Proc.* 35:2199-2201.
- Deborska-Materkowska D, Sadowska A, Wesolowska A, Perkowska-Ptasinska A, Durlík M (2005). Human herpesvirus 6 variant A infection in renal transplant recipient. *Nephrol. Dial. Transplant.* 20:2294.
- Deborska-Materkowska D, Lewandowski Z, Sadowska A, Nowacka-Cieciura E, Chudziński W, Czerwiński J, Paczek L, Durlík M (2006). Fever, human herpesvirus-6 (HHV-6) seroconversion, and acute rejection episodes as a function of the initial seroprevalence for HHV-6 in renal transplant recipients. *Transplant Proc.* 38:139-143.
- Dzieciatkowski T, Przybylski M, Torosian T, Tomaszewska A, Luczak M (2008). Prevalence of human herpesvirus 6 antibodies and DNA in allogeneic stem cell transplant patients: two-year single centre experience. *Arch. Immunol. Ther. Exp. (Warsz).* 56:201-206
- Milne RS, Mattick C, Nicholson L (2000). RANTES binding and down-regulation by a novel human herpesvirus-6 beta chemokine receptor. *J Immunol.* 164:2396-2404.
- Ogata M (2009). Human herpesvirus 6 in hematological malignancies. *J. Clin. Exp. Hematop.* 49:57-67.
- Okuno T, Higashi K, Shiraki K et al (1990). Human herpesvirus-6 in renal transplantation. *Transplantation.* 49: 519-522
- Lautenschlager I, Hockerstedt K, Linnavuori K, Taskinen E (1999). Human herpesvirus 6 infection increases adhesion molecule expression in liver allografts. *Transplant Proc.* 31: 479-480
- Prober CG (2011). Human herpesvirus 6. *Adv. Exp. Med. Biol.* 697:87-90
- Quereda C, Corral I, Laguna F (2000). Diagnostic utility of a multiplex herpesvirus PCR assay performed with cerebrospinal fluid from human immunodeficiency virus-infected patients with neurologic disorders. *J. Clin. Microbiol.* 38:3061-3067.
- Shiley K, Blumberg E (2010). Herpes viruses in transplant recipients: HSV, VZV, human herpes viruses, and EBV. *Infect. Dis. Clin. North. Am.* 24:373-393.
- Suga S, Yoshikawa T, Asano Y (1992). IgM neutralizing antibody responses to human herpesvirus 6 in patients with exanthem subitum or organ transplantation. *Microbiol. Immunol.* 36:495-506.
- Wade AW, McDonald AT, Acott PD, Lee S, Crocker JF (1998). Human herpesvirus-6 or Epstein-Barr virus infection and acute allograft rejection in pediatric kidney transplant recipients: greater risk for immunologically naïve recipients. *Transplant Proc.* 30: 2091-2093.
- Zerr DM, Huang ML, Corey L (2000). Sensitive method for detection of human herpesviruses 6 and 7 in saliva collected in field studies. *J. Clin. Microbiol.* 38:1981-1983.