International Journal of Infectious Diseases 16 (2012) e124–e129

Contents lists available at SciVerse ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Human herpesvirus 6 in donor biopsies associated with the incidence of clinical cytomegalovirus disease and hepatitis C virus recurrence

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ARTICLE INFO

Article history:
Received 15 July 2011
Received in revised form 17 October 2011
Accepted 22 October 2011

Corresponding Editor: Mark Holodniy, California, USA

Keywords:
Liver transplantation
Cytomegalovirus
Human herpesvirus 6
Hepatitis

SUMMARY

Background: Reactivation of cytomegalovirus (CMV) and human herpesvirus 6 (HHV-6), as well as the recurrence of hepatitis C virus (HCV), occurs in the post liver transplantation period. However, their correlations remain questionable. The objectives of this study were to analyze the presence of CMV DNA and HHV-6 DNA in pre-transplant and post-transplant liver graft biopsies and to determine any correlations with CMV disease and HCV recurrence.

Methods: Forty-one liver transplant recipients were followed up in the post-transplant period. The presence of CMV DNA and HHV-6 DNA was detected by nested PCR.

Results: Four patients (4/41, 9.8%) were positive for CMV DNA in pre-transplant biopsies and three of them remained positive after transplantation; 11 patients became positive in the post-transplant biopsies ($p = 0.06$). Fifteen (15/41, 36.6%) patients were positive for HHV-6 DNA in pre-transplant biopsies and 11 of these remained positive after transplantation. Another 11 patients became positive after the surgery ($p = 0.05$). CMV disease occurred in 17 recipients; 10 of these 17 (58.8%) patients were positive for HHV-6 DNA in pre-transplant biopsies and they continued positive after transplantation ($p = 0.0128$). Twenty-eight patients were transplanted due to hepatitis C; 12 of these patients had recurrence of the virus, and HHV-6 was positive in nine of the 12 (75%) patients ($p = 0.049$).

Conclusions: Recipients with HHV-6 DNA in pre-transplant graft biopsies remained positive post transplantation, showing a possible risk for post-transplant allograft loss because there was an association between HHV-6 and recurrent HCV and CMV disease.

1. Introduction

Herpesvirus reactivation is a common occurrence after liver transplantation, and cytomegalovirus (CMV) and human herpesvirus 6 (HHV-6) infections cause severe diseases in these recipients.[1] Seronegative recipients of liver allografts from CMV-seropositive donors are at risk of CMV disease. Secondary infection by reactivation or superinfection may also lead to CMV disease during immunosuppression,[2] and HHV-6 reactivates from latency during the first post-transplant months, often together with CMV infection.[3,4]

These viruses are classified in the B-herpesvirus family. Infection occurs in childhood and then subsequently results in life-long latency, such that the seroprevalence rate in adults is over 90%.[5,6] Therefore, in adult solid organ transplant recipients, reactivation of endogenous latent viruses seems to occur very frequently, with reported infection rates of 30–50%.[7,8] Both viruses can cause several human diseases either as a consequence of reinfection or reactivation of latent infection.[9,10] CMV is reactivated during periods of stress and cytokine release, which happens in liver transplant recipients, wherein the pharmacologic-induced impairment of the immune response to 'endogenously reactivated' or 'allograft-transmitted' CMV leads to febrile and tissue invasive diseases.[11,12] HHV-6 reactivation and infections after liver transplantation are asymptomatic, although the risk factors are not completely defined. Nonetheless, clinical syndromes have been associated with HHV-6 infection after liver transplantation, and these have been classified either as direct or indirect effects,[13] so HHV-6 may cause clinically relevant indirect effects such as CMV disease and early fibrosis caused by the recurrence of hepatitis C virus (HCV).[6,7,14]

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doi:10.1016/j.ijid.2011.10.008

Cirrhosis caused by HCV has emerged as a leading indication for liver transplantation.[15] The recurrence of the disease after transplantation is universal and many factors have been associated with it, including CMV infection.[16] The interaction between herpesviruses and HCV has been proposed to be clinically important in HCV-infected liver transplant recipients,[17] including more severe forms of HCV recurrence. Patients with HCV infection have been shown to be more susceptible to infections that are associated with defects in cell-mediated immunity, such as CMV and fungal infections.[11,18–21]

Our study analyzed the presence of CMV DNA and HHV-6 DNA in liver biopsies from the donor patients collected at bench surgery and from the post-transplanted period, and correlated these viruses with the occurrence of CMV disease and HCV recurrence.

2. Patients and methods

During the period January 2005 to December 2007, a total of 41 liver transplant patients, transplanted at the Liver Transplant Unit, Clinical Hospital of the State University in Campinas (Unicamp), were enrolled in this study. This was an observational, analytic, longitudinal cohort and prospective study. The following patients were excluded from the study: children and cases of fulminant hepatitis, retransplantation, or death occurring during the first month after transplantation. The 41 patients who were enrolled met the following inclusion criteria: (1) survival in the post-transplantation period for >1 month; (2) indication for at least one liver biopsy post transplantation; (3) sufficient liver biopsy samples for DNA virus analysis by nested PCR method.

The first liver biopsy was collected from the organ at the bench before transplantation (pre-transplant graft biopsy). The other liver biopsies (post-transplant graft biopsies) were collected during the 6-month period after transplantation and when clinically indicated (due to elevated liver enzyme levels). All samples were formalin-fixed and paraffin-embedded until the moment of analysis. They were analyzed by nested PCR for CMV DNA and HHV-6 DNA. Liver biopsies were also monitored for recurrent HCV.

All patients included in this study had been given prophylaxis for herpes simplex infection with 200 mg of acyclovir twice a day for 60 days. The patients with CMV disease were treated intravenously with ganciclovir 5 mg/kg twice a day for 2 weeks.

All recipients and donors had positivity for CMV IgG before transplantation and there was no serology for HHV-6 before transplantation.

All patients underwent standard immunosuppressive therapy based on calcineurin inhibitors: cyclosporine A (CyA; 4–8 mg/kg) and FK506 (0.1 mg/kg) and steroids until the 180th day after the transplant, with tapering and withdrawal. The initial CyA blood level was 200–400 ng/ml in the first 3 months and then 150–250 ng/ml for a further month. The FK506 blood level used was 8–12 ng/ml in the first 3 months and 5–10 ng/ml for a further month.

2.1. Clinical definitions

Hepatitis C recurrence and disease severity were classified with a METAVIR score.[22] All HCV liver transplant patients had HCV RNA detected in the serum by conventional molecular methods prior to liver transplantation.[23–25] We analyzed the presence of CMV DNA and HHV-6 DNA in the same biopsies that were diagnosed with the recurrence of HCV after transplantation.

CMV disease was defined according to previously published criteria[9] and required the presence of clinical symptoms accompanied by the detection or isolation of CMV in blood by virological tests and/or histological detection in a biopsy specimen.

2.2. Molecular tissue testing

Viral DNA was extracted from the liver biopsies using the Qiagen Tissue Kit (Biotecnologia Brasil, SP, Brazil) in accordance with the manufacturer's instructions, with some modifications performed in our service, and final elution volumes of 30 μl.

2.3. CMV and HHV-6 nested PCR amplification

Five microliters of extracted DNA were used for the nested PCR for CMV and HHV-6 using a mixture containing specific primers for CMV (CMV primers; Invitrogen, São Paulo, Brazil), by the technique previously described, with some modifications,[26,27] and specific primers for HHV-6 (HHV-6 primers; Invitrogen, São Paulo, Brazil), by the technique previously described, with some modifications.[28]

The amplifications were carried out on a Peltier Thermal Cycler (MJ Research, Waltham, MA, USA). The nested PCR product was analyzed under UV light after electrophoresis in agarose gel (Gibco-BRL Life Technology, Carlsbad, CA, USA) in Tris–ethylenediaminetetraacetic acid buffer (TEB) stained with ethidium bromide.

All primer sequences and PCR products were analyzed using the GenBank database before initiating the study. PCR with primers for beta-globin gene amplification was performed on the samples to detect possible false-negative results, which were not included in this study.[29]

2.4. Statistical analysis

The variables were compared with non-parametric tests (Gamma correlation and Chi-square test or Fisher's exact test). For all tests, $p < 0.05$ was considered statistically significant.

This study was approved by the Institutional Ethics Committee of the Faculty of Medical Sciences, Unicamp (CEP number 030/2006).

3. Results

Forty-one transplant recipients were studied (32 men and nine women), with a median age of 48 (range 18–67) years. The main indication for the transplant was HCV (16 patients); other indications were HCV and alcohol in 12, cryptogenic cirrhosis in two, Wilson's disease in one, alcoholic liver disease in three, autoimmune hepatitis in two, primary biliary cirrhosis in one, primary sclerosing cholangitis in one, and hepatitis due to the hepatitis B virus in three.

3.1. CMV DNA and HHV-6 DNA in liver biopsies

Four patients (9.8%) were positive for CMV DNA in pre-transplant graft biopsies. After transplantation three of them remained positive in the post-transplant graft biopsies. From the 37 negative (pre-transplant graft biopsies) only 11 became positive (Table 1) in post-transplant graft biopsies ($p = 0.06$; Fisher's exact test; Table 2).

Fifteen patients (36.6%) were positive for HHV-6 DNA in pre-transplant graft biopsies, and 11 of these patients remained positive in post-transplant graft biopsies. Of the 26 negative patients, 11 became positive (Table 1) for this virus after the transplantation. The patients who were positive for HHV-6 DNA in the pre-transplant graft biopsy had a tendency ($p = 0.05$; Fisher's exact test; Table 2) to remain positive in the post-transplant graft biopsies.

3.2. Herpesvirus infection and CMV disease

CMV disease occurred in 17 of the 41 liver transplantation patients (41.5%), and they were treated with ganciclovir. Two of