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Original

Plasma Epstein-Barr viral load measurement as a diagnostic marker of lymphoma in HIV-infected patients

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ABSTRACT

Background and objectives: To assess the use of the Epstein-Barr virus (EBV) viral load as a marker for lymphoma diagnosis in HIV-infected patients. We also aimed to identify the relationship between EBV viral load in plasma and the presence of EBV in lymphoma cells.

Patients and methods: Retrospective observational study of two HIV-infected populations: one of patients diagnosed with lymphoma and a control group. Thirty-nine patients with AIDS-related lymphoma (ARL) (32 non-Hodgkin's and 7 Hodgkin's lymphomas) and 134 HIV-positive individuals without neoplasia or opportunistic infections were studied. Blood samples were collected before lymphoma treatment in ARL patients. EBV viral load was measured in plasma by real-time quantitative PCR and the presence of EBV-EBER mRNA in lymphoma tumor was investigated by *in situ* hybridization.

Results: Patients with ARL had higher EBV viral loads than those without lymphoma: 24,180.5 ($\pm$73,387.6) copies/mL versus 2.6 ($\pm$21.6) copies/mL ($p < 0.001$). HIV-infected patients without lymphoma had negative or very low EBV load values. Among ARL patients, no correlation was found between EBV viral loads and CD4+ lymphocyte counts or between EBV and HIV RNA loads, or any other clinical or biological parameter. Cases with an EBV-EBER-positive lymphoma had higher EBV viral loads than those with EBER-negative tumors.

Conclusions: EBV viral load is a useful marker of lymphoma in HIV-infected patients, and may be a useful tool for early diagnosis and treatment.

Carga viral del virus de Epstein-Barr como marcador diagnóstico de linfoma en pacientes infectados por el VIH

Palabras clave:
VIH
Virus de Epstein-Barr
Carga viral
Linfoma no hodgkiniano
Linfoma de Hodgkin

RESUMEN

Fundamento y objetivo: Evaluar el uso de la carga viral del virus de Epstein-Barr (VEB) como marcador para el diagnóstico de linfomas en pacientes infectados por el VIH. Identificar la relación entre la carga viral de VEB en plasma y la presencia del virus en las células del linfoma.

Pacientes y método: Estudio observacional retrospectivo de dos poblaciones de pacientes VIH: una de pacientes diagnosticados de linfoma y un grupo control. Se estudiaron 39 pacientes con linfoma asociado a infección por el VIH (32 linfomas no hodgkinianos y 7 de Hodgkin) y 134 individuos con infección por el VIH sin neoplasia ni infecciones oportunistas. Las muestras de plasma de los pacientes con linfoma fueron obtenidas en el momento del diagnóstico. La carga viral en plasma del VEB fue realizada mediante una PCR cuantitativa en tiempo real y la presencia de RNAm VEB-EBER en los tumores fue investigada por hibridación *in situ*.

Resultados: Los pacientes con linfoma asociado a infección por el VIH tenían cargas virales de VEB más elevadas que los pacientes sin linfoma: 24.180,5 ($\pm$73.387,6) copias/ml frente a 26 ($\pm$21,6) copias/ml ($p < 0{,}001$). Los pacientes con infección por el VIH sin linfoma presentaron carga viral muy baja o negativa. En los pacientes con linfoma no se halló correlación entre la carga viral del VEB y el recuento de linfocitos

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CD4+ ni la carga viral de VIH, ni con otros parámetros clínicos o biológicos. Los casos de linfomas VEB-EBER-positivos tuvieron una carga viral de VEB más elevada que la de los linfomas EBER negativos.
Conclusiones: En pacientes infectados por el VIH la carga viral del VEB es un marcador de linfoma, potencialmente útil para el diagnóstico y tratamiento precoz.

Introduction

The mechanisms of lymphomagenesis of AIDS-related lymphomas (ARL) are still not completely known, but a number of factors have been identified: infection by transforming agents such as some herpesvirus, oncogenes or suppressor genes, and persistent B-cell stimulation by high levels of certain cytokines.[1] The Epstein-Barr virus (EBV) is a human lymphotropic herpesvirus involved in the pathogenesis of lymphoma. It persists in latent form inside B lymphocytes as episomes in the cell nucleus and replicates during cellular division. In HIV-infected patients, the profoundly defective response of cytotoxic T lymphocytes may reactivate EBV infection, leading to uncontrolled lymphoproliferation and favouring the development of lymphoma.[2,3] However, the precise role of EBV in ARL lymphomagenesis and its preference for specific anatomic sites and association to specific histopathology remain unclear.

The presence of EBV has been demonstrated in tumor cells from both non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma (HL). The EBV genome can be detected in 60% of ARL cases, depending on the histological subtype of the lymphoma and its localization.[4] HIV-related HL is almost always associated with EBV.[5] Approximately 30–50% of AIDS-related systemic diffuse large B-cell lymphomas (DLBCL) contain EBV. In contrast, almost all cases of AIDS-related primary central nervous system lymphomas (PCL) contain EBV.[6] Therefore, the pathogenesis of AIDS-related systemic NHL and PCL may be different. In Burkitt's lymphoma, EBV is present in around 30% of cases, and lymphomagenesis includes, in addition to EBV infection, activation of the C-*MYC* gene and inactivation of *P53*.[7]

Titration of EBV antibodies against early and nuclear EBV antigen is not a useful diagnostic tool to detect virus reactivation of EBV-related diseases in immunosuppressed individuals (e.g., transplant recipients, AIDS patients) because of the insufficient humoral response in these patients. Thus, simple unquantified molecular detection of EBV is poor, while quantification of plasma EBV DNA is a useful tool to detect EBV-associated diseases in immunosuppressed patients.[8] The EBV virus apparently plays a role in ARL lymphomagenesis and EBV viral load might be elevated among HIV-infected patients concurrently developing lymphoma. Furthermore, high EBV viral loads may be associated with the presence of EBV in malignant cells. A few studies have been carried out to explore EBV viral loads in patients with ARL.[9–11] Several investigators have shown that a rapid increase of EBV viral loads in peripheral blood or plasma is predictive of lymphoproliferative disorders in transplant recipients, nasopharyngeal carcinoma and other malignancies.[9,12] Moreover, the therapeutic efficacy has been monitored from sequential measurements of EBV viral load in these disorders.[13,14] From a technical standpoint, EBV viral load assays have been shown to be sensitive and specific. New commercially available kits based on real-time PCR technology are less time-consuming and reduce the risk of contamination by amplicons.[15,16] These kits allow the sequential measurement of EBV viral loads on a high number of samples and patients.

The aim of this study was to assess the usefulness of EBV viral load as a marker for lymphoma diagnosis. EBV viral loads were compared in HIV-infected patients with and without lymphoma. We also identified the relationships between EBV viral loads and presence of virus in the tumor cells.

Methods

Patients

This retrospective study included 173 adult individuals coinfected with HIV and EBV (positive for VCA IgG antibodies).

Between 1998 and 2006, forty-nine consecutive cases of ARL (38 LNH and 11 LH) were diagnosed in a single institution. Ten ARL patients were excluded from the study because no plasma frozen samples were available. Thus, thirty-nine patients with ARL were included (32 NHL and 7 HL) in this study. The number of males and females was 28 (72%) and 11 (28%), respectively, and mean (SD) age was 40 (8) years. Twenty-five out of 32 NHL patients and 7 with HL were on highly active antiretroviral therapy (HAART) at the time of lymphoma diagnosis. The median duration of HAART was 45 months [range, 9–109]. Lymphoma diagnosis was performed by histological study of tissue biopsies. The following clinical and biological variables were measured: age, sex, risk activity, HAART, CD4 lymphocyte count, HIV viral load, histologic subtype of lymphoma, B symptoms and serum LDH. Following the guidelines of the Centers for Disease Control and Prevention (CDC), 4 ARL patients were classified as being in stage A2, 2 as in stage A3, 6 as in stage B3, 4 as in stage C2 and 12 as in stage C3.

A control group of 134 HIV-infected individuals without neoplasia or opportunistic infections was collected from the outpatient population of the HIV Unit of the Hospital Germans Trias i Pujol. Control cases were selected according to age, clinical and immunological status. There were 94 males (70.1%) and 40 females (29.9%), and the mean age was 43.6 years. From this population, 25 had a CD4 count $\leq 200/\mu L$ (24 on HAART); 54 had a CD4 count between 200 and $500/\mu L$ (25 on HAART) and 55 had a CD4 count $\geq 500/\mu L$ (25 on HAART).

In this study, we followed the rules of good clinical practice of the Germans Trias i Pujol University Hospital.

Biological samples

A specimen of blood with EDTA was collected after consent from all patients and controls included in the study. The specimens from ARL patients utilized for the current study were originally collected after the diagnosis of lymphoma and before starting the lymphoma treatment. The plasma samples of the control group were obtained at the time of routine clinical HIV follow-up visits, and were retrospectively assayed for EBV viral load. Plasma was separated by centrifugation and stored at $-80\,^{\circ}C$ until processing. Tissue samples were routinely fixed, paraffin-embedded, and cut into sections (5–6 μm thick).

EBV detection in tissue samples

The EBV-encoded RNA (EBER) in the tumor was studied in 17 of 39 lymphoma cases. The EBER was detected by *in situ* hybridization with the *INFORM EBER PROBE* assay (Ventana Medical Systems, Tucson, USA) and the *ISH IVIEWBLUE* detection system (Ventana Medical Systems) in a *BenchMark LT* automated instrument (Ventana Medical Systems).