Internal Medicine: Open Access

Diamantopoulos et al., Intern Med 2018, 8:3
DOI: 10.4172/2165-8048.1000273

Research Article Open Access

Lack of Association of Human Herpesvirus 6 (hHV-6) with Chronic Lymphocytic Leukemia and Leukemic Low-Grade B-Cell Lymphomas

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Received date: May 01, 2018; **Accepted date:** May 07, 2018; **Published date:** May 11, 2018

Abstract

Background: The oncogenicity of Human Herpesvirus 6 (HHV-6) is a matter of continuous interest and several studies have tried to define its pathogenetic role in malignancy with conflicting results.

Patients and Methods: We investigated the presence of HHV-6 DNA in peripheral blood samples of patients with immunephenotypically confirmed chronic lymphocytic leukemia and low-grade B-cell lymphomas by quantitative real-time PCR for the *U57* gene of the virus.

Results: None out of 48 patients was found positive for HHV-6 (CLL, 60.4%; splenic marginal zone lymphoma, 25.0%; hairy cell leukemia, 4.2%; mantle cell lymphoma, 8.3%; follicular lymphoma, 2.1%).

Conclusion: The seroprevalence and PCR detection rate of HHV-6 is relatively high in healthy individuals. This is the lowest percentage of HHV-6 DNA detection reported to date in patients' samples, supporting a lack of contribution of the virus in the malignant transformation in CLL and leukemic low-grade B-cell lymphomas.

Keywords: Human herpesvirus 6; Chronic lymphocytic leukemia; Low-grade B-cell lymphoma

Introduction

Human herpesvirus 6 (HHV-6) is a member of the herpesviridae and was first isolated and characterized from patients with lympho-proliferative disorders. Like other members of the family, following primary infection, latency is established in certain tissues, such as the salivary glands, T-cells and hematopoietic stem cells, and the virus can be reactivated in immunecompromised hosts. Its DNA integrates into the human genome, and due to this property, the oncogenicity of the virus was speculated early after its first detection [1]. HHV-6 harbors telomeric repeats (TMRs) identical to host telomeres at both ends of its linear genome. TMRs facilitate viral genome integration into host telomeres, a step important for establishment of latency and lympho-magenesis [2].

The pathogenetic role of HHV-6 in lympho-proliferative diseases has been of continuous interest for the last 20 years. Several studies have been performed to shed light on this correlation. Their conflicting results are explained by the different methods used to detect the virus, the diversity of specimens used for this purpose, as well as the wide spectrum of the diseases under study. Moreover, the presence of the virus in the bone marrow, lymph nodes or blood of the patients is interpreted by the investigators either as a result of immunosuppression, as an incidental finding, or as a causal for the under-study disease correlation. Currently, there are no definitive correlations of HHV-6 with any lympho-proliferative disorders.

Two studies on pediatric acute lymphoblastic leukemia (ALL) failed to reveal a causal relationship of the virus with ALL [3,4]. In one study, infection with HHV-6 and Epstein-Barr virus (EBV) was found to be correlated with histological progression of angio-immunoblastic T-cell lymphoma (AITL) [5], while another study did not confirm a correlation of the virus with AITL [6]. No correlation of HHV-6 with Hodgkin's and non-Hodgkin's lymphomas was found by a study published in 2005 [7]. HHV-6 was not detected in any of 47 biopsy samples of children with Hodgkin's lymphoma [8]. Finally, HHV-6 DNA was detected in 29% of a total of 35 biopsy samples of patients with Hodgkin's lymphoma, but the authors assumed that there was no direct role of the virus in the development of the disease due to the comparable rates of detection in healthy individuals [9].

To our knowledge, there are no data about HHV-6 in CLL and low-grade B-cell lymphomas. The aim of the present study is to detect HHV-6 in blood samples of patients with chronic lymphocytic leukemia (CLL) and leukemic low-grade B-cell lymphomas, namely splenic marginal zone lymphoma (SMZL), hairy cell leukemia (HCL), mantle cell lymphoma (MCL), and follicular lymphoma (FL), that were diagnosed and treated in our center, and to investigate a postulated causal correlation.

Materials and Methods

Diagnoses were confirmed by hematologic, histologic and immunephenotypic criteria. The individual diseases were categorized according to the 2008 World Health Organization (WHO) classification of lympho-proliferative disorders [10]. The presence of

the neoplastic clone in the peripheral blood of all patients at the time of sample collection was verified by immunephenotypic analysis of the lymphocytes. Peripheral whole blood samples were collected in ethylenediaminetetraacetic acid (EDTA), and genomic DNA was extracted using the QIAamp® DNA Blood mini kit (Qiagen) following the manufacturer's instructions. A quantitative real-time polymerase chain reaction (RT-PCR) was performed for the *U57* gene of HHV-6 using the *CMV HHV6, 7, 8* R-gene™ Quantification COMPLETE kit (Argene, bioMérieux SA, Marcy l'Etoile, France). U57 is located at the 5th major core gene block of the unique segment of the viral genome. It is a conserved gene coding for the major capsid protein of the virus with approximately 95-97% nucleotide homology between HHV-6A and HHV-6B [11].

The kit uses internal quantification standards to generate a standard curve based on which the HHV-6 viral load is calculated and sensitivity controls in order to validate the performance of the assay over time. Positive and negative controls are provided within the kit. The RT-PCR steps are as follows: Taq polymerase activation at 95°C for 15 minutes followed by 45 cycles of denaturation at 95°C for 10 seconds, hybridization and annealing at 60°C for 40 seconds. For each reaction 15 μL of buffer and 10 μL of DNA were used. All reactions were carried out twice. The study was performed on LightCycler® 2.0 (Roche, Mannheim, Germany). The kit amplifies a fragment of 116 base pairs of the *U57* gene and the detection limit of the technique is <156 copies/mL, as reported by the kit manufacturer. The samples had already been tested for EBV with real-time PCR for the purposes of a previous study. The quantification of the viral load was based on the detection of the *BXLF-1 thymidine kinase* gene of the virus using DNA from whole blood samples. For this purpose, the EBV R-gene™ Quantification COMPLETE kit (Argene, bioMérieux SA, Marcy l'Etoile, France) was used according to the manufacturer's instructions. Quantification was performed on LightCycler® 2.0 (ROCHE, Mannheim, Germany).

Results and Discussion

Forty-eight (48) patients were included in the study and their main epidemiologic and hematologic characteristics are shown in detail in Table 1. None of the examined samples was found positive for HHV-6, while 19 (39.6%) were found positive for EBV, a result that has been already published [12]. No correlations could be made with the epidemiologic, clinical and prognostic characteristics of the patients, or with EBV detection, due to the null percentage of HHV-6 DNA detection.

Characteristic	Result
N (%)	48 (100)
Age (years), median (range)	74.5 (51-87)
Sex (male/female)	1.09
Lymphoproliferative disorder (WHO 2008 classification), N (%)	
CLL	29 (60.4)
SMZL	12 (25.0)
HCL	2 (4.2)
MCL	4 (8.3)
FL	1 (2.1)
Previous treatment, N (%)	19 (39.6)
Number of previous treatment lines	0.5 (0-5)
Rituximab containing regimen, N (%)	6 (12.5)
EBV positivity	19 (39.6)
HHV-6 positivity	0 (0)

Table 1: Patient characteristics and results: Chronic Lymphocytic Leukemia (CLL); Splenic Marginal Zone Lymphoma (SMZL); Hairy Cell Leukemia (HCL); Mantle Cell Lymphoma (MCL); Follicular Lymphoma (FL)

Conclusion

The seroprevalence of HHV-6 in healthy individuals from Greece has not yet been determined. Studies in healthy individuals from other parts of the world show a high prevalence ranging from 20% to 100% [13-16]. A wide range of HHV-6 DNA detection by PCR in the whole blood of healthy individuals has been reported so far (2.9% in the United Kingdom [17], 17% in Italy [8], 40% in Spain [18]). HHV-6 DNA was detected in the blood of 11% and the bone marrow of 28% of 18 otherwise healthy individuals undergoing total hip arthroplasty [19]. The reported rates in healthy individuals are much higher than those of our study.

None of our patients was found positive for HHV-6, and this is the lowest percentage of HHV-6 DNA detection reported to date in healthy individuals or patient samples. Thus, in comparison to the above-mentioned studies on lympho-proliferative disorders that all have a comparable sample size, our finding is far more definite, and supports a lack of contribution of HHV-6 in the malignant transformation in CLL and leukemic low-grade B-cell lymphomas. This is the first report in the literature about HHV-6 in this group of diseases that, although relatively heterogeneous, comprises lympho-proliferative disorders that share several significant common characteristics. Moreover, no correlation could be found between the prevalence of the two viruses, since although 39.6% of the patients were EBV positive, none of them was HHV-6 positive, and although EBV is implicated in the pathogenesis or progression of CLL, the same does not apply for HHV-6. The accumulation of such data can help implicate or absolve HHV-6 for its lympho-magenic potential with a higher degree of certainty.

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