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ORIGINAL RESEARCH REPORT

Plasma Epstein Barr viral load in adult-onset Hodgkin lymphoma in South India

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Abstract
Objective/background: Epstein Barr Virus (EBV) DNA load is increasingly being used as a non-invasive biomarker for detecting EBV association in lymphomas. Since there is a need of data from India, we undertook to prospectively evaluate plasma EBV DNA load as a marker of EBV association in newly diagnosed adult-onset Hodgkin lymphoma (HL).
Methods: EBV DNA was quantified using real-time polymerase chain reaction. In a subset of patients, an assay was validated qualitatively with EBV latent membrane protein-1 (LMP1) immunohistochemistry (IHC). Wherever possible, follow-up plasma samples post three cycles of chemotherapy were obtained.
Results: Over a period of 10 months, 33 newly diagnosed adult-onset HL were enrolled in the study. Pretherapy plasma EBV DNA was detectable in ~49% (16/33) patients (viral loads range, 1.0–51.2 $\times$ 10^3 copies/mL) and undetectable in 30 voluntary blood donors. LMP1 IHC was positive in 56% of cases tested (14/25). Sensitivity and specificity of plasma EBV DNA with respect to LMP1 IHC were 86% and 100%, respectively. Of the eight patients in whom follow-up plasma was available, in five EBV baseline-positive patients EBV load reverted to negative postchemotherapy and corroborated with clinical remission.

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Conclusion: Plasma EBV DNA load estimation may be useful in detecting EBV-association and possibly monitoring the response to therapy in EBV-related HL especially in our country where EBV association of HL is higher than in developed nations.

Introduction

Epstein Barr Virus (EBV) is associated with a variety of human malignancies: Burkitt's lymphoma, lymphomas associated with immunosuppression, nonHodgkin lymphomas, Hodgkin lymphoma (HL), and nasopharyngeal carcinoma [1,2]. Seroepidemiologic studies indicate that EBV infects >95% of the human population by adulthood and is maintained latently in a small fraction of memory B cells [1—3]. While EBV DNA can be detected in the serum or plasma of most patients with EBV-associated malignancies, it remains undetectable in healthy individuals even though they are latently infected [4—6]. Hence, a serum/plasma EBV DNA detection assay can be used as a marker of EBV-associated malignancies. In recent years, plasma EBV-DNA assays are increasingly being used as a noninvasive biomarker for EBV-association in lymphomas [5,7—14].

In order to validate its utility in determining EBV association, there is a need for studies from different geographical regions. In overcrowded living and unsanitary conditions of developing countries like India, primary infections occur very early in life [2]. This is partly responsible for greater EBV-associated HL in developing countries than in developed countries [2]. Since studies on EBV load in malignancies and HL from India are limited, the aim of this study was to investigate the utility of plasma Epstein Barr viral load as a marker of EBV association in adult-onset HL patients.

Materials and methods

Type of study

This was a prospective study carried out at a tertiary care cancer center in south India. The study was approved by the institutional ethics committee and was performed as per the Helsinki Declaration 2000. A written, informed consent was obtained from all patients enrolled in the study.

Patient selection

The target population included patients with a diagnosis of HL following a detailed histopathological examination and immunohistochemistry (IHC). Staging evaluation included a complete history, thorough physical examination, imaging (computed tomography [CT] scan of the thorax, abdomen, and pelvis), and bone marrow aspiration and biopsy. Data regarding demography, clinical staging, International Prognostic Score (IPS), chemotherapy administered, and clinical outcome were recorded. Response assessment was done based on standard criteria [17,18]. Positron emission tomography-CT scan was not done on any patient as it was not available at our center due to the poor affordability of the patients we cater to.

Sample collection

Peripheral blood samples were collected before initiation of anticancer chemotherapy in newly diagnosed HL patients and in healthy voluntary blood donors. In HL patients, follow-up plasma samples at the end of three courses of chemotherapy were collected whenever possible. In order to validate the performance characteristics of the plasma viral DNA assay, EBV latent membrane protein-1 (LMP1) IHC was also performed in a subset of cases.

Laboratory protocol

All samples were handled with sterile precautions. Plasma was separated from all blood samples (pretherapy, posttherapy, or blood donors) within a few hours of collection by centrifugation at 1200*g* for 2 min and stored frozen at −20 °C until DNA extraction. All samples were found to be seronegative for human immunodeficiency virus and hepatitis B virus.

Real-time polymerase chain reaction

Nucleic acid was extracted from 250 μL of plasma with a silica based manual extraction protocol, and eluted with 50 μL elution buffer. The manual method was chosen over commercial extraction columns as it gave better and more consistent results (data not shown) [16]. Plasma EBV DNA in samples was quantified using real-time polymerase chain reaction (PCR) as per previously published protocol [13]. The standard curve was constructed using EBV DNA of known concentration diluted 10-fold (a kind gift from Professor Y.L. Kwong, University Department of Medicine, Queen Mary Hospital, Pok Fu Lam, Hong Kong). As optimized and validated in other major studies, EBV DNA load more than 500 copies/mL was considered as positive [8,14]. In order to validate the performance characteristics of the plasma viral DNA assay, in addition to routine IHC for confirmation of lymphoma diagnosis, EBV LMP1 IHC could be performed in 25 HL cases.

Results

Over a period of 10 months, 130 histologically confirmed cases of adult-onset lymphomas were diagnosed of which 33 were HL, while the remaining were nonHodgkin lymphomas. Thirty age-matched healthy blood donors (all men) were included as controls. Of the 33 patients with HL, 27