Epstein-Barr Virus Latent Membrane Protein 1 (LMP-1) in Hodgkin's Lymphoma Patients in Turkey

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ABSTRACT

Epstein-Barr virus (EBV) has been implicated as a contributing factor in the development of Hodgkin's lymphoma. The aim of this study was to elucidate the association of Hodgkin's lymphoma with EBV in a Turkish population using immunohistochemical detection of LMP-1. We studied a total of 21 consecutive cases of Hodgkin's lymphoma from Turkey. LMP-1 protein was detected in 9 of 21 (42.8%) cases. LMP-1 was positive in 4 of 7 (57%) mixed cellularity and 5 of 13 (38.4%) nodular sclerosis subtype. The results of the current study suggests a strong association of Epstein-Barr virus with Hodgkin's lymphoma in Turkey and, together with those reported previously showed that Epstein-Barr virus correlated with mixed cellular type, with a slight male predominancy while there was no correlation with age.

Key Words: Epstein-Barr virus, Hodgkin's lymphoma, Latent membrane protein 1.

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INTRODUCTION

Epstein-Barr virus (EBV) is a human B-lymphotropic herpes virus. It is the causative agent of infectious mononucleosis (IM). In developing countries, EBV infection occurs early in life and nearly entire population of a developing country becomes infected before adolescence. In such countries symptomatic infectious mononucleosis is uncommon. EBV infection delays until adulthood with the increased standards of hygiene[1]. EBV has been implicated in the etiology of diffe-

rent malignancies. The virus is well known for its association with Burkitt's lymphoma, B-cell lymphomas in immunocompromised individuals, and undifferentiated-nasopharyngeal carcinoma[2,3]. Recent studies suggest that EBV can infect many cell types such as Hodgkin's disease, various T-cell lymphomas and some carcinomas[4,5].

EBV latent membrane protein 1 (LMP-1) is considered to be a viral oncogene because it can transform rodent fibroblasts in vitro and render them tumorigenic in nude mice[6]. In human B cells, LMP-1 is essential for B-lymphocyte growth transformation and immortalization[7]. Also it induces the expression of activation markers and adhesion molecules[4,5]. It has also been demonstrated that LMP protects against apoptosis by increasing expression of the bcl-2 protooncogene[7-10,20].

The aim of this study was to elucidate the association of Hodgkin's lymphoma with EBV in a Turkish population using immunohistochemical detection of LMP-1.

MATERIALS and METHODS

In the present study we investigated 21 Hodgkin's lymphoma patient who admitted to Gazi University Medical Faculty Department of Pathology over a period of 2 years from 1996 to 1998. They aged between 23 and 61 (Median: 37, mean: 36.6 ± 9). There were 13 male and 8 female patients giving a male:female ratio of 1.6:1. Biopsies had been obtained from patients admitted to the adult hematology clinic of Gazi University Medical Faculty and Oncology Hospital. The diagnosis and classification of Hodgkin's lymphoma were based on the presence of Hodgkin and Reed-Sternberg cells according to the criteria of the Rye classification[11]. There were 13 cases of noduler sclerosis, 7 cases of mixed celluler and 1 case of lymphocyte depletion histological type. They were diagnosed from biopsy material from lymph nodes alone. Paraffin-embedded tissue blocks were used for immunohistochemical studies.

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LMP-1 was detected by using monoclonal mouse antibody (Mabs) (CS 1-4, Dako, Glostrup, Denmark). 4 µm tissue sections were deparaffinized, stored at 56°C for two hours and then a procedure of xylene, 75% alcohol and distilled water were performed. After the procedure of 0.3% H_2O_2 for 10 minutes sections were digested in phosphate-buffered saline (PBS) for 5 minutes and then stored at microwave with Antigen Retrieval Citra (HK087-5K) for 15 minutes. Sections were waited at room temperature for 30 minutes. The tissues are then sequentially incubated with primary antibody, secondary antibody and streptavidin -enzyme reagent (Kwik Kits Cat No 404000, Lipshaw, Pittsburgh). Slides were rinsed with PBS for 5 minutes and then stained with AEC chromogen and rinsed with distilled water. Slides were counterstained with hematoxylin, rinsed with distilled water and mounted.

RESULTS

The cases analysed included 13 cases of nodular sclerosis (61%), 7 cases of mixed cellularity (33%), and 1 case of lymphocyte-depleted subtypes (4.7%).

EBV in H-RS cells was detected by immunohistochemistry for LMP-1 protein in 9 of 21 (42.8%) patients. LMP-1 was positive in 4 of 7 (57%) mixed cellularity and 5 of 13 (38.4%) nodular sclerosis subtype. The 1 lymphocyte-depleted subtype was negative for LMP-1 protein. The age of EBV positive cases was 35 ± 5, negative cases was 35.2 ± 4. Three out of 8 female patients was positive (37%) while positivity was 6/13 in males (46%).

DISCUSSION

This study was carried out to assess the role of EBV in a group of patients with Hodgkin's lymphoma in Turkey. Since its earliest description, infectious nature has been a topic of discussion in Hodgkin's lymphoma epidemiological studies. Epidemiological studies

have shown that people who have had IM have an increased incidence of Hodgkin's lymphoma[12]. Increasing number of serological and epidemiological data suggests an association between EBV and Hodgkin's lymphoma[8]. In Hodgkin's lymphoma patients elevated antibody titers to EBV antigens have been shown at the time of presentation and also in serum samples taken some years before the onset of the disease[13]. EBV genomes have been detected in tumor material in 19% to 50% of Hodgkin's lymphoma cases with in situ hybridization[14], whereas PCR has shown it to be present in 70% of patients[15]. Immunohistochemical studies have detected the LMP-1 produced by EBV in infected Reed-Sternberg and Hodgkin cells[16-21]. These accumulating data suggest that EBV may play a role in the pathogenesis of Hodgkin's lymphoma.

In a previous study of Beyan et al. EBV genome has been studied by using PCR in 31 Turkish patients with Hodgkin's lymphoma[22]. EBV genome has been detected in 45% of patients. They have found no association between EBV-DNA positivity and age, gender, histological subtype.

In developing countries it has been shown that Hodgkin's lymphoma patients indicates a higher ratio of EBV positivity than in the United States and European countries[13,23,25]. Many studies have reported variation in the pattern of association of this virus with the histological subtype of Hodgkin's lymphoma. In many studies, EBV gene products have been shown in approximately 75% of mixed cellular and only 25% of nodular sclerosing histological subtype of Hodgkin's lymphoma. Histological subtype was the strongest risk factor for Hodgkin's lymphoma being EBV positive[7]. In this detailed evaluation of EBV patterns ethnicity affects risk of EBV associated Hodgkin's lymphoma independent of age, sex, histological subtype and nationality. EBV-positive Hodgkin's lymphoma occurred in a large proportion of Hispanic patients and also elevated in other nonwhite groups other than blacks. This difference may be the result of individual socioeconomic status or variation in genetic susceptibility. EBV-associated Hodgkin's lymphoma was more common in children and older adults[4]. LMP positivity showed a predominant association with mixed cellularity Hodgkin's lymphoma[4,12,15, 21,26-28].

The results of the current study suggests a strong association of Epstein-Barr virus with Hodgkin's lymphoma in Turkey and, together with those reported previously, revealed that Epstein-Barr virus correlated with mixed cellular type.

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