

A HISTOPATPOLOGICAL STUDY OF HODGKIN'S LYMPHOMA AND ITS ASSOCIATION WITH EPSTEIN-BARR VIRUS IN TURKISH PATIENTS

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SUMMARY

We evaluated 25 cases of Hodgkin's lymphoma for the presence of Epstein-Barr virus in Reed-Sternberg cells and Hodgkin cells. We also compared the epidemiological features of our patients with previous reports on Hodgkin's lymphoma in industrialized and developing nations. Among our cases, the most common subtype of Hodgkin's lymphoma was mixed cellularity (10 cases, 40%), followed by nodular sclerosis (9 cases, 36%). In 11 of 25 cases (44%), immunohistochemical studies demonstrated that Reed-Sternberg cells were positive for LMP-1 (four out of five in the lymphocyte-rich classic group, three of nine in the nodular sclerosis group and four out of 10 in the mixed cellularity group). The prevalence of Epstein-Barr virus and the high incidence of mixed cellularity in Turkey are similar to findings in developing countries. These findings further support the hypothesis that the prevalence of Epstein-Barr virus in Hodgkin's lymphoma and the epidemiological features of Hodgkin's lymphoma may be linked with socioeconomic conditions and geographic location.

Key Words: Hodgkin's Lymphoma, EBV, LMP-1

ÖZET

HODGKİN LENFOMA VE TÜRK HASTA POPÜLASYONUNDA EPSTEİN-BARR VİRÜSÜ İLE İLİŞKİNİ ARAŞTIRAN HİSTOPATOLOJİK BİR ÇALIŞMA

Bu çalışmada 25 Hodgkin lenfoma olgusunda Reed-Sternberg hücreleri ve Hodgkin hücrelerinde Epstein-Barr virüsü varlığını araştırdık. Ayrıca kendi hasta popülasyonumuzun epidemiyolojik özelliklerini daha önce gelişmiş ve gelişmekte olan ülkeler için bildirilmiş olan epidemiyolojik özelliklerle karşılaştırdık. Bu olgular arasında, 10 vaka (%40) ile en sık saptanan subtipin mikst sellüler suptip olduğu ve bunu 9 vaka (%36) ile nodüler sklerozan suptipin izlediği tesbit edilmiştir. Beş lenfositten zengin klasik subtipin 4'ünde, 9 nodüler sklerozan subtipin 3'ünde ve 10 mikst sellüler subtipin 4'ünde olacak şekilde toplam yirmibeş olgunun 11'inde (%44) immünohistokimyasal olarak Reed-Sternberg hücrelerinde LMP-1 ekspresyonu saptanmıştır. Türkiye'de Epstein-Barr virüs prevalansı ve mikst sellüler subtipin yüksek insidansı gelişmekte olan ülkeler için daha önce bildirilmiş olan oranlara benzer olarak saptanmıştır. Daha da ötesi, bu bulgular Hodgkin lenfoma olgularında Epstein-Barr virüs prevalansının ve Hodgkin lenfoma epidemiyolojik özelliklerinin sosyoekonomik özellikler ve jeografik lokalizasyonla ilişkili olabileceği hipotezini desteklemektedir.

Anahtar Sözcükler: Hodgkin Lenfoma, EBV, LMP-1

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The oncogenic potential of the Epstein-Barr virus (EBV), a lymphotropic herpesvirus, has been widely researched since the virus was first isolated by Epstein et al in 1964 (1). EBV has been detected in infectious mononucleosis (2), nasopharyngeal carcinoma (3) and certain types of non-Hodgkin's lymphomas (4-6). It has been detected in 95% of Endemic Burkitt's Lymphomas, 20% of Sporadic Burkitt's Lymphomas, 40% of lymphomas arising in patients with HIV infection and some subgroups of T-cell lymphomas (7). However, the association between EBV and Hodgkin's lymphoma (HL) remained speculative until Weiss et al showed monoclonal EBV-DNA in some HL tissues and the virus was localised in Reed-Sternberg (R-S) cells (8) using molecular genetic techniques (9). Data obtained from immunohistochemical studies also supports the association between HL and EBV by demonstrating LMP expression in R-S cells and variants (10,11), the neoplastic cell population of HL. LMP-1 is an EBV-gene product expressed from EBV-transformed B lymphocytes. The oncogenic potential of LMP-1 seems to act by preventing differentiation and immortalization of B lymphocytes (12).

HL has a bimodal age distribution in industrialized countries; however, in developing countries, it peaks in young adults (13-15). The association of HD and EBV seems to correlate with certain histological and geographic features. EBV is generally detected in R-S cells of mixed cellularity (MC) or lymphocyte depletion (LD) subtypes, in contrast to its rare detection in the nodular sclerosis (NS) subtype and still more rare detection in remaining subtypes (13). There are also some reports concerning the effects of geographic features on the prevalence of EBV. In developed countries, EBV is detected in 50% of HL cases, in contrast to developing countries, where EBV is detected in large numbers of HL cases together with a high incidence of MC subtype (14,15).

The aim of the present study is to investigate the association of EBV and HL in Turkish patients in terms of EBV prevalence among those subtypes

seen in children and adults and also to observe the age distribution of HL subtypes.

MATERIALS AND METHODS

Patient Population

This study included 25 cases of HL diagnosed at the Ankara University Medical School, Department of Pathology between 1989 and 1992.

Histopathologic Studies

Routinely fixed, paraffin embedded, 5- μ thick sections were stained with H&E, examined by a pathologist (AE) and subtyped according to WHO classification (16) as follows:

1. Nodular Lymphocyte Predominance (NLP)
2. Lymphocyte-rich classic (LRC)
3. Nodular Sclerosis (Grade I and Grade II) (NS)
4. Mixed Cellularity (MC)
5. Lymphocytic Depletion

Cases were evaluated according to histopathological subtype, sex and age distribution.

Immunohistochemical Studies

5- μ thick sections obtained from paraffin blocks were used to detect LMP-1 expression using the Streptavidin-Biotin Complex-Alkaline Phosphatase technique. Following antigen retrieval by microwave treatment using the monoclonal anti-LMP-1 antibody CS1-4, LMP-1 expression was evaluated (17, 18). The results were scored between (+) and (++++) according to the intensity of staining in R-S cells.

Statistical Analysis:

Patient groups were compared for LMP-1 expression in HL subtypes using the Chi-square test. A p value of less than 0.05 was considered significant.

RESULTS

Clinicopathological Findings:

Patient age ranged from 3.5 to 57 years, with a mean of 24.5 years. There were 17 males (68%) and eight females (32%), for an overall male to

female ratio of 2.1:1. However, the male to female ratio was found to be higher in children (5.5:1; 11 boys and two girls).

Overall, there were 10 cases (40%) of MC subtype, 9 cases (36%) of NS subtype, five cases (20%) of LRC subtype, and one case (4%) of NLP subtype. Thirteen of the cases (52%) were in the pediatric age group (17 years) and 12 of the cases (48%) were in the adult group. In the adult age group, 11 of the patients were between 18-49 years of age and one patient was over 50 years of age. Mean ages according to HL subtypes were as follows: MC: 25.7; NS: 25; LRC: 22.

Immunohistochemical Findings:

LMP-1 expression was detected in 11 (44%) of the cases overall. Immunohistochemical reaction was restricted to R-S cells, and no positivity was detected in the background small lymphocytes. LMP-1 positivity was slightly higher in the pediatric age group (46.1%) than in the adult age group (41.6%), although the difference was not statistically significant. When subtypes were evaluated, the LMP-1 expression rate was found to be 40% in the MC subtype, 30% in the NS subtype and 80% in the LRC subtype ($p>0.05$). Interestingly, vascular elements showed weak positivity in eight of 25 cases (32%), suggesting nonspecific staining.

DISCUSSION

Previous studies have shown significant geographic differences in both the prevalence of EBV infection and the epidemiological properties of HL. In industrialized countries, HL incidence shows bimodal distribution, with one age peak at 15-35 years of age and another at over 50 years of age (19,20). These peaks correlate with the prevalence of the NS subtype among adolescents and young adults and the MC subtype among adults over 50 years of age. However, in developing countries, the incidence of NS subtype is lower compared with MC subtype, which comprises the majority of HD cases (21-23). EBV prevalence in HL seems to correlate with the incidence of MC subtype in the pediatric age group (14).

In our study, the incidence of HL subtypes dif-

fered from that of industrialized countries, but was similar to that of developing countries (18). MC was the commonest subtype (40%) among all age groups, and NS and LRC subtypes were less frequently observed. There was no detection of any age-related distribution of subtypes. Overall, there were more patients in the pediatric age group (52%) than in the adult age group (48%). Gender distribution of the cases showed the NS subtype to be slightly higher in female patients (55%), while the MC and LRC subtypes were common among male patients. (80% and 100%, respectively). Our observation regarding children with a higher incidence of HL parallels the age distribution of HL in developing countries (18). Moreover, the distribution by gender of particular subtypes is in line with previously reported rates (24,25).

R-S cells are known to be the most common type of HL cells. When the results of LMP-1 expression were examined, expression was found to be restricted to R-S cells, with no reaction detected in the small lymphocytes in the background. These findings support the role of EBV in oncogenesis. In the present study, weak, non-specific vascular staining was also detected (32%) with LMP-1, which seems to be due to biotin cross-reacting with the vessel wall.

Previous studies have reported a correlation between MC subtype and EBV prevalence. Our study detected a 40% incidence of MC and LMP-1 expression in 11 of 25 cases, a rate of 44%. Overall, the age, gender, subtype and LMP-1 expression rates of HL seem to show similarities with previous reports in the literature (14,18-25). Despite this similarity and the reliability of immunohistochemical techniques in EBV detection, immunohistochemistry should not be used as the sole criteria to establish association. Other EBV-gene products that are also expressed by the EBV-transformed cells should be demonstrated by PCR and in-situ hybridization techniques, and this data should be combined with the immunohistochemical data. Our future goal is to combine immunohistochemistry with PCR and in-situ hybridization techniques in order to obtain more reliable data.

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