

EBER IN SITU HYBRIDIZATION EXPERIENCE IN HODGKIN LYMPHOMA

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ABSTRACT

Purpose: Several studies suggest that the relation of Epstein-Barr virus (EBV) and Hodgkin lymphoma (HL). EBV and HL associations are reported at rates ranging from 20% to 100% that varies nationally and regionally. In this study; in the case of Hodgkin's lymphoma, the presence of EBV encoded RNA (EBER) by in situ hybridization method and its distribution according to histopathological subtypes were investigated.

Methods: We investigated the presence of EBV in Hodgkin Lymphoma diagnosed 107 adult cases between 2013 and 2017, which chromogenic in situ hybridization with EBV RNA probe has been in routine use were evaluated retrospectively with pathology reports.

Results: There were 69 males and 38. Nodular Sclerosis Classical Hodgkin Lymphoma (NSCHL) was the most common (63 cases, 58,9%) followed by Mixed Cellularity Classical Hodgkin Lymphoma (MCCHL) (23 cases, 21,5%). EBV was expressed in 40 cases (45,4%). High EBV association was found with NSCHL (50%) and MCCHL (48%). None of NLPHL was positive with EBER.

Conclusion: In our series NSCHL is the most common histologic subtype and has higher percentages of EBV positivity as reported in developed countries. Our study has the largest series of patients investigating the relationship between HL and EBER in our country. For further comments more studies, particularly from Turkey, are needed to determine the actual epidemiological pattern of HL in the region and to define the role of EBV in HL.

Keywords: EBER, EBV, Hodgkin Lymphoma

INTRODUCTION

Hodgkin's Lymphoma (HL) is a monoclonal lymphoid neoplasm composed of Hodgkin/Reed-Sternberg cells in a background of inflammatory cells consisting of small lymphocytes, histiocytes, neutrophils, eosinophils, plasma cells, and fibroblasts (1). HL has unusually heterogeneous clinical, histologic, and epidemiologic characteristics (1-3). Epstein-Barr virus (EBV), a unique herpesvirus that has been associated with a number of lymphoid malignancies (2,4). Studies have proved a link between Epstein-Barr virüs (EBV) and Hodgkin's disease in adults and

children in past decade (1-2, 4-7). The proportion of the subtypes of HL and EBV positivity shows significant geographic variation worldwide (1-3,8). In this study; in the case of Hodgkin's lymphoma, the presence of EBV encoded RNA (EBER) by in situ hybridization method and its distribution according to histopathological subtypes were investigated.

METHODS

Review of cases

Hodgkin Lymphoma diagnosed adult cases between 2013 and 2017, which chromogenic in situ

Table 1: Distribution of histologic subtypes

	NSCHL (n:63)	MSCHL (n: 23)	LRCHL (n: 10)	CHL unclassifiable (n: 7)	NLPHL (n: 4)
Age	18-76	18-82	23-73	22-76	38-73
Sex					
Male	41	16	8	1	3
Female	22	7	2	6	1
EBER					
Positive	26	9	3	2	0
Negative	26	10	4	4	4
No data	11	4	3	1	0

NSCHL: Nodular Sclerosis Classical Hodgkin Lymphoma; MSCHL: Mixed Cellularity Classical Hodgkin Lymphoma; LRCHL: Lymphocyte-rich Classical Hodgkin Lymphoma; CHL: Classical Hodgkin Lymphoma; NLPHL: Nodular Lymphocyte Predominant Hodgkin Lymphoma

hybridization with EBV RNA probe has been in routine use, were evaluated retrospectively with pathology reports. Histologic subtypes according to the WHO classification, age, sex, localization, bone marrow involvement and in situ hybridization (ISH) for EBV encoded RNA (EBER) status were recorded for each patient.

In-situ hybridization (ISH)

EBV early RNAs (EBER) were investigated by chromogenic ISH method. 4-µm thick sections were taken from formalin fixed paraffin embedded tissue samples. Chromogenic ISH with the EBV RNA probe was performed using the standard procedure according to manufacturer's recommendations in full automatic immunohistochemistry and in situ hybridization staining device (Benchmark XT, Ventana Medical Systems, Tucson, USA). For this purpose, oligonucleotide EBV early RNA (EBER) Probe, (INFORM EBER Probe, Ventana Medical Systems, Tucson, USA), and ready-to use ISH kit suitable for probe (ISH iVIEW Blue Detection Kit, Ventana Medical Systems, Tucson, USA) was used.

RESULTS

Age, sex, localization and bone marrow involvement
 The range of patient age was 18 to 82 with an average of 44,2 years. There were 69/107 (64.5%) males and 38/107 (35,5%) females. The male: female ratio was 1,8:1. The age range for males was 18 to 82 with an average of 45,8 years and for females, the range was 18 to 76 with an average of 41,2 years.
 Data on site of lymph node was available in only 54/107 (50,4%) cases. The remaining 53 cases was consultation cases. The most common site was the cervical region, accounting for 16 (29,6%) cases, followed by mediastinal and supraclavicular lymph nodes, accounting for 13 (24,07%) cases of each. Data on bone marrow involvement was available in

96/107 (89,7 %) cases. Seven cases (7,3%) had bone marrow involvement and two cases was suspicious for bone marrow involvement.

Histopathologic classification

The distribution of histologic subtypes in the 107 cases reviewed is shown in Table 1. Nodular Sclerosis Classical Hodgkin Lymphoma (NSCHL) was the most common (63 cases, 58,9%) followed by Mixed Cellularity Classical Hodgkin Lymphoma (MCCHL) (23 cases,21,5%). There were 10 (9,3%) cases of Lymphocyte-rich Classical Hodgkin Lymphoma (LRCHL) and 4 (3,7%) Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL). 7 cases of Classical HL (6,5%) were unclassifiable subtype.

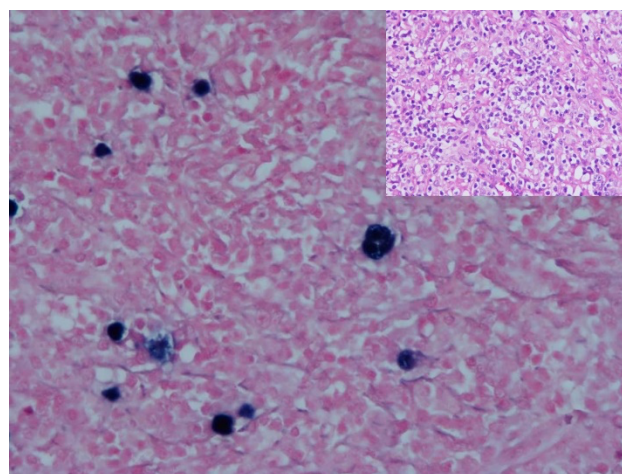


Figure 1. EBER positive HL cells

EBER expression

EBER results were available in 88 of 107 cases. Forty cases (45,4 %) were positive for EBER by ISH (Figure 1). The frequencies of EBV expression in the histological subtypes are summarized in Table 2. EBER was positive in 26/52 (50%) of NSCHL, 9/19 (48%) of MCCHL, but in only 3/7 (43%) of LRCHL.

None of NLPHL was positive with EBER. 19 cases had no data of EBER expression. The relation of EBV expression to patient age and gender is also shown in Table 2.

DISCUSSION

A role for EBV in HL pathogenesis has been further supported by the identification of EBV infection based on Southern blot DNA hybridization, assays for EBERs and immunohistochemical assays for the latency membrane protein-1 (LMP-1) (11). EBV and HL associations are reported at rates ranging from 20% to 100% that varies nationally and regionally (2,3,9-13). The average reported rate in the literature is 40-60% (2,3,10,13-14,).

Most studies described epidemiologic properties of EBV-associated HL, but these reports include tests with different sensitivities and small study samples (2). This may be the reason for the wide range of positivity rates in the literature. However, almost all studies, support that the EBV ratio differs according to age, ethnicity, socioeconomic level and histological subtypes (2-4,9-15). In economically less developed regions, the percentage of EBV positivity in all age groups is higher than in developed regions (2,3,14). Ratios between 30% and 50% in developed countries reach 70-100% in developing countries (3,11-13). EBV prevalence was uniformly higher in children than in young adults for all histologic subtypes (2).

In our study, 45,4% of cases were EBER positive, similar to developed country rates. But this rate is also lower than the some previous reports from Turkey (9-12,16). This may be due to the fact that we have evaluated a wider series of patients, selected

age range (only adult patients, EBV detection method or this could be a regional difference. Only two studies used in situ hybridization for detecting EBV (9,11).

In all age groups, NSCHL had relatively low percentages of EBV-positive cases, whereas MCCHL was associated with high levels (2) In our study NSCHL subtype has the higher percentages of EBV positivity as like the report of Ozdil et. all (11).

In conclusion, in our series NSCHL is the most common histologic subtype and has higher percentages of EBV positivity as reported in developed countries. Our study has the largest series of patients investigating the relationship between HL and EBER in our country. For further comments more studies, particularly from Turkey, are needed to determine the actual epidemiological pattern of HL in the region and to define the role of EBV in HL.

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Table 2: Distribution of EBV expression

	EBER (+)(n:40)	EBER (-)(n:48)
Age	18-71	20-76
Sex		
Male	26	31
Female	14	17
Subtype		
NSCHL	26	26
MCCHL	9	10
LRCHL	3	4
CHL	2	4
unclassifiable	0	4
NLPHL		

NSCHL: Nodular Sclerosis Classical Hodgkin Lymphoma

MSCHL: Mixed Cellularity Classical Hodgkin Lymphoma

LRCHL: Lymphocyte-rich Classical Hodgkin Lymphoma

CHL: Classical Hodgkin Lymphoma

NLPHL: Nodular Lymphocyte Predominant Hodgkin Lymphoma

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