Epstein-Barr Virus in Hodgkin's Disease: Correlation of Risk Factors and Disease Characteristics with Molecular Evidence of Viral Infection


Harvard Medical School [B. G. S., P. M. M., G. S. P., A. P. A.] and Harvard School of Public Health [I. T., N. M.], Boston, Massachusetts 02115; The Johns Hopkins School of Medicine [R. F. A., R. M.], Baltimore, Maryland 21231; and Beth Israel Deaconess Medical Center [M. E. K., B. S.], Boston, Massachusetts 02115

Abstract

Risk factors suggestive of relatively late exposure to EBV have been consistently associated with Hodgkin's disease (HD) in younger adults. In addition, evidence of EBV infection has been found in the Reed-Sternberg cells themselves in about one-third to one-half of all HD cases. However, no study yet published has correlated these childhood social environment risk factors with the presence of EBV in Hodgkin's tumor cells. We examined whether EBV-positive HD occurs in those patients whose childhood environment would predispose them to relatively late exposure to EBV. The study population consisted of 102 cases of mixed cellularity (MC; n = 25) or nodular sclerosing (n = 77) HD. Samples that tested positive for either EBV-encoded RNA or latent membrane protein or both were considered EBV-positive. Of the 102 cases, 83 completed a questionnaire regarding childhood social environment. The association with EBV-positivity was estimated by the odds ratio (OR) with 95% confidence intervals (CI). Twenty-two percent of the cases were EBV-positive. These cases were more likely to be MC (OR, 6.2; CI, 2.3-16.3) and male (OR, 3.4; CI, 1.3-9.0). History of infectious mononucleosis (IM) was not predictive of EBV-positivity, with only 3 of 14 such patients being EBV-positive (P = 0.82). Contrary to our hypothesis, no association between EBV and childhood environment risk factors was identified. The association of EBV with MC histology and male gender agree with previous reports. The most intriguing finding was the dissociation between IM history and EBV-positivity, in that almost all of the cases with a history of IM were EBV-negative.

Introduction

It has been proposed that an infectious agent plays a role in the development of HD ever since the disease was first described (1). In 1964, MacMahon put forth the "two-disease hypothesis" based on the bimodal age distribution of HD in developed countries. The incidence of HD peaks first at about age 25, decreases until the mid-50's, and then rises again in the elderly population. MacMahon noted the different risk factors among these two groups and suggested that cases in younger patients were caused by an infectious agent (2, 3).

EBV has been accepted as the likely candidate for several reasons. First, its association with Burkitt's lymphoma, nasopharyngeal carcinoma, and non-Hodgkin's lymphoma in immunocompromised patients is well defined (4). Recently, a working group of the IARC evaluated EBV as a carcinogenic risk to humans and concluded there is sufficient evidence that EBV is a causal factor in HD and other malignancies (5). In addition, the epidemiology of IM induced by EBV infection corresponds to that of HD in young adults. EBV is a widespread, relatively asymptomatic, early childhood infection (6). It is easily transmitted via oral secretions; therefore, a childhood environment allowing exposure to many other young children leads to higher rates of infection. In contrast, young adults with HD tend to have a more sheltered childhood environment. These cases are more likely to have fewer siblings, a higher level of maternal education, and lower density housing than controls (7). The same pattern of risk factors holds for patients diagnosed between 40 and 54 years (8). Therefore, the cases occurring within the first mode of incidence (the "young adult" peak) seem to be at risk of late infection with EBV, at which point it often manifests as IM (6). Several cohort studies have documented an approximate 3-fold increased risk for HD in young adults who have had IM (9-14). HD patients also show an altered serological pattern of antibody response to EBV both before and after diagnosis (15).

More recently, evidence of EBV infection has been found in the malignant cells of about one-third to one-half of HD cases (16). Molecular evidence includes detection of the epimammary viral genome, the abundant RNA transcripts expressed in latency called EBERs, and a restricted expression of the latent cycle proteins (17-20). Furthermore, these gene products are detected within the diagnostic malignant RS cells rather than in...
EBV and Hodgkin’s Disease

the infiltrating lymphocytes. Additional studies have demonstrated monoclonality of the Epstein-Barr virus (EBV) genome in tumor tissue, which indicated that infection occurred before or at the time of malignant transformation (21–23).

A paradox in our understanding of EBV and HD has recently emerged. In the recent analysis by Glaser et al. (24) of the demographic features of 1546 cases in relation to their tumor EBV status, the distribution of EBV-positive HD is the opposite of what the epidemiological data might predict. Specifically, children in underdeveloped countries, who have a high likelihood of early exposure to EBV but whose EBV serology does not predict risk of HD, had the highest levels of EBV-positive disease (40–80% in children 0–14 years old). In addition, 50–80% of adults 55 years and older, in whom socioeconomic factors do not correlate with risk for HD, were also EBV-positive. The young adult cases (15–54 years old) in which childhood social environment would predict the risk of HD as well as IM, in fact had the lowest rates (30–50%) of EBV-positive disease in Glaser’s series.

To our knowledge, no study has been published that evaluates childhood social environment risk factors for HD for correlation with EBV status. We wished to examine whether EBV-positive HD occurs in those patients whose childhood environment would predispose them to relatively late exposure to EBV. Thus, we undertook a retrospective case series analysis of HD in which we could correlate demographic data with the EBV status in biopsy tissue. We hypothesized that, within the group of young adults with HD, those subjects whose risk factors predicted the late onset of childhood illness such as IM would more likely be EBV-positive.

Methods

Using the HD database from the Joint Center for Radiation Therapy, we selected patients who were alive and met the following criteria: (a) age, 15–55 years old at diagnosis; (b) histological subtype NS or MC; (c) pathological stage IA–IIB; and (d) diagnosed 1980 or later. Cases were limited to early stages to evaluate whether there was an association between the site of presentation and EBV. Of 159 pathology specimens requested from regional hospitals including 11 from out-of-state facilities, 112 specimens were received. In the remaining cases, either tissue was unavailable or repeated attempts to obtain the material were unsuccessful.

Of the 112 pathology specimens, 2 were subsequently eliminated because they were stage III disease. All of the specimens were reviewed to confirm the diagnosis of HD. In six cases, the histological subtype did not agree with the original diagnosis. Reanalysis of the data using the reviewer’s histological diagnoses did not change the findings; therefore, the original diagnoses were used. In six additional cases, tissue was inadequate to confirm the subtype.

Tumor specimens were examined for EBER and LMP1 expression by in situ hybridization and immunohistochemistry, respectively. A digoxigenin riboprobe was used for EBER1 detection according to a standard protocol (25, 26). A cocktail of monoclonal antibodies (CS1–4) was used for LMP1 detection with microwave antigen retrieval (27). Positive controls consisted of known EBV-positive HD specimens and EBV cell lines fixed in formalin and embedded in paraffin. Molecular studies were inadequate in eight cases (six had nondiagnostic tissue, and in two, the tissue washed off the slide). Thus, results were available on 102 cases. Both studies were performed on all of the 102 cases except 1, in which no tumor was remaining for LMP1 testing after EBER was negative.

Information on age at diagnosis, gender, histology, stage, and sites of disease were obtained from the database. The patient’s socioeconomic status was estimated by median household income by ZIP code in the 1990 United States census. Income data were unavailable for eight ZIP codes, five at diagnosis and three current. Patients were contacted by mail, with follow-up telephone calls as needed, to obtain demographic information via a simple questionnaire. Data included parents’ and subject’s education, childhood housing at age 8, sibship size, birth order, and history of IM as diagnosed by a physician. Educational history was unavailable for one mother and one father. Patients gave written informed consent upon completing the questionnaire. A total of 83 questionnaires were received.

The study was designed as a case-series (28), comparing EBV-positive and -negative cases for etiological heterogeneity. Descriptive data (age, gender, histology, stage, and site of disease) on all of the 102 cases were examined as “exposures” for correlation with EBV status. For site of disease, neck involvement was defined as any cervical, submandibular, or supraclavicular nodes. Childhood social environment risk factors (education, income, housing, family size, and history of IM) were also correlated with EBV status for the 83 cases in which questionnaires were completed. The OR and its 95% CI were calculated as an estimate of the relative risk of EBV-positive disease for each exposure. When the 95% CI does not include unity, the association is statistically significant with P < 0.05. When appropriate, the lower limit of the CI was calculated using Cornfield’s method (29). The Ps given are by either χ² or Fisher’s exact test; t testing was used for continuous variables.

Results

The entire study population of 102 patients was diagnosed between 1980 and 1994 (median, 1988). The year of diagnosis did not differ significantly between the EBV-positive cases and EBV-negative ones. Table 1 shows the distribution of disease characteristics for all of the 102 subjects compared with the 83 cases who returned the questionnaire. The two groups were similar in disease characteristics and prevalence of EBV-positivity. Of the 102 cases, 22 were EBV-positive—3 by LMP1 only, 4 by EBER only, and 15 by both methods. Of the 83 cases that returned questionnaires, 16 were EBV-positive—3 by
Cancer Epidemiology, Biomarkers & Prevention

Table 2: The distribution and association of clinical and demographic factors by EBV status of tumor tissue

<table>
<thead>
<tr>
<th>Factor</th>
<th>EBV(+) (n = 22) N (%)</th>
<th>EBV(-) (n = 80) N (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>14 (64)</td>
<td>27 (34)</td>
<td>3.4 (1.3-9.0)</td>
<td></td>
</tr>
<tr>
<td>MC</td>
<td>12 (55)</td>
<td>13 (16)</td>
<td>6.2 (2.3-16.3)</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>12 (55)</td>
<td>50 (63)</td>
<td>0.72 (0.28-1.9)</td>
<td>0.53</td>
</tr>
<tr>
<td>Mean age</td>
<td>30 years</td>
<td>28 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neck</td>
<td>20 (91)</td>
<td>63 (79)</td>
<td>2.7 (0.60-12.2)</td>
<td></td>
</tr>
<tr>
<td>Stage I</td>
<td>10 (100)</td>
<td>15 (50)</td>
<td>Infinity (2.4-infinity)</td>
<td>0.006</td>
</tr>
</tbody>
</table>

a EBV(+), EBV-positive; EBV(-), EBV-negative.
b Cornfield's method used.
c Fisher's exact test.

Table 3: The distribution of cases by EBV status for childhood social environment risk factors and the association with EBV-positivity

<table>
<thead>
<tr>
<th>Variable</th>
<th>EBV(+) (n = 16)</th>
<th>EBV(-) (n = 67)</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Socioeconomic status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Education (mean years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient</td>
<td>16.5</td>
<td>15.5</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Mother</td>
<td>13.3</td>
<td>12.9</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Father</td>
<td>13.4</td>
<td>13.3</td>
<td>0.97</td>
<td></td>
</tr>
<tr>
<td>Income (mean)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At diagnosis</td>
<td>$44,910</td>
<td>$43,461</td>
<td>0.70</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>$43,770</td>
<td>$44,737</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>Childhood environment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single-family home</td>
<td>81%</td>
<td>72%</td>
<td>1.7 (0.44-6.7)</td>
<td></td>
</tr>
<tr>
<td>Family size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sibship size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>5 (31%)</td>
<td>24 (36%)</td>
<td>0.83 (0.22-3.1)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>5 (31%)</td>
<td>19 (28%)</td>
<td>1.1 (0.28-4.0)</td>
<td></td>
</tr>
<tr>
<td>4+</td>
<td>6 (38%)</td>
<td>24 (36%)</td>
<td>1.0^</td>
<td></td>
</tr>
<tr>
<td>Firstborn</td>
<td>44%</td>
<td>39%</td>
<td>1.2 (0.41-3.7)</td>
<td></td>
</tr>
<tr>
<td>History of IM</td>
<td>19%</td>
<td>16%</td>
<td>1.2 (0.28-4.9)</td>
<td></td>
</tr>
</tbody>
</table>

a EBV(+), EBV-positive; EBV(-), EBV-negative.
b Referent category.

Of the characteristics examined for a correlation with EBV status, three were statistically significant (Table 2). As expected, males were more likely to be EBV-positive, with an OR of 3.4 (95% CI, 1.3-9.0), as were MC cases, with an OR of 6.2 (2.3-16.3). Among the 40 stage I cases, disease involving neck nodes was much more likely to be EBV-positive than disease in other areas (OR, infinity; CI, 2.4-infinity; P = 0.006), with all of the 10 EBV-positive cases in this subset presenting in the neck. However, this association was not seen in stage II. Mean age was virtually the same for EBV-positive and EBV-negative cases (30 versus 28). For the 44 subjects ages 15-25, 23% were EBV-positive, compared with 16% of the 37 subjects 26-35 years old and with 29% of the 21 cases 36-55 years old (P for the trend = 0.76).

Table 3 displays the correlation of EBV with the childhood social environment risk factors for HD. No associations were evident; furthermore, the fact that 11 of 14 subjects with a history of IM were EBV-negative is remarkable. We examined the role of birth order, controlling for sibship size, using the method of Yule and Greenwood (30). This method uses the null expectation that for a given sibship size (e.g., three), a disease is equally likely to affect the first, second, and third child. For the entire case series, there was no association of birth order with HD itself or with EBV status.

Table 3 shows adjusted OR for each factor in the entire dataset and among only stage I cases. The increased risk of EBV in males is less evident when examining only stage I cases (OR, 2.3; CI, 0.52-9.7). Overall, MC histology was the strongest predictor of EBV-positivity.

Histological type did not correlate significantly with gender, age, or site of disease in the entire sample. However, patients with NS in this study were more likely to have stage II disease (P = 0.005). In addition, all of the cases with a history of IM were NS (P = 0.06). Neck involvement itself was not associated with any of the childhood social environment risk factors.

Because MC histology, male gender, and neck involvement (in stage I) were the only significant variables predicting EBV-positivity, these three variables were examined for mutual confounding. Table 4 shows adjusted OR for each factor in the entire dataset and among only stage I cases. The increased risk of EBV in males is less evident when examining only stage I cases (OR, 2.3; CI, 0.52-9.7). Overall, MC histology was the strongest predictor of EBV-positivity.

Discussion
This study examined both disease characteristics and childhood social environment risk factors for association with the presence of EBV in HD tumor cells. Surprisingly, only gender, histology, and stage I neck involvement were significantly correlated with EBV status, whereas the childhood environment factors were not predictive. Previous epidemiological studies have found that risk factors for HD in the 15-55-year-old range are small sibship size, low density childhood housing, and higher maternal education (7, 8). However, such associations...
were not evident in the present study population for EBV-positive HD.

EBV-positive cases were more likely to be male (OR, 3.4; CI, 1.3–9.0), a finding corroborated by Bosq et al. (31). They found that in early-stage disease, 67% of males were LMP1-positive compared with 42% of females (P < 0.001). In terms of histology, 48% of the MC cases were EBV-positive compared with only 13% of the NS cases. This finding is consistent with other reports (16). In general, EBV-positivity rates are highest in MC and somewhat lower in NS. Reported rates are low in lymphocyte-predominant HD. Data for lymphocyte-depleted cases are sparse and inconsistent.

Because the portal of entry of EBV is the oropharynx, HD presenting in neck nodes might be expected to be EBV-positive. For all of the stages combined, the OR (2.7; CI, 0.60–12.2) was not significant. However, all of the ORs of the EBV-positive stage I cases involved neck nodes (OR, infinity; CI, 2.4–infinity; P = 0.006). Our data agree with those of O’Grady et al. (32), who found that for stage I patients, LMP1 expression is correlated with presentation in the neck (OR, 17.7; CI, 2.0–156). They also found no association in stages II-IV, which is not surprising inasmuch as the site of origin becomes less clear as the disease spreads. When controlled for histology and gender, the association of neck involvement in our study disappeared. Of the 25 stage I neck cases, 8 of the 12 MC cases were EBV-positive compared with only 2 of the 13 NS cases. O’Grady’s cases showed a similar distribution. HD most often presents in the neck or mediastinum, with NS histology being strongly associated with mediastinal disease (33). Perhaps NS cases originate in the mediastinum, and MC cases originate in the neck in association with EBV infection.

The selection criteria for our patient population limit these findings. Only a limited age range was included; therefore, an age association may not be detected. We selected only early-stage, pathologically staged cases in an attempt to correlate EBV status with site of disease. Risk factors could only be analyzed in those patients who responded to the mailed questionnaire, but this should not introduce bias inasmuch as the rate of EBV-positivity was the same for the subset of 83 cases and the entire 102 cases (19% versus 22%). Our rate of EBV detection in tumor cells was 22%. Using either EBER or LMP1 in RS cells as evidence of EBV-positivity, Glaser et al. (24) found a similar rate of ~30% for age 25–30 (our mean age was 28). Because our study population by definition had an excellent prognosis, a lower rate may have been due to selection bias if EBV-positivity is associated with poor prognostic factors. In fact, a study of 560 HD patients by Bosq et al. (31) found that the 438 early-stage cases had a LMP1-positive rate of 35%, compared with 46% for the 122 advanced-stage cases. Thus, our EBV-positivity rate of 22% is in keeping with other reports of 30–35%.

The most intriguing finding is that 11 of 14 subjects with a history of IM had no evidence of EBV in their tumor tissue. This highlights the apparent paradox in the relationship between EBV infection and HD. EBV infection is ubiquitous but usually asymptomatic. Symptomatic EBV infection (IM) is associated with a distinctive immune response to viral infection characterized by lymphadenopathy and an atypical lymphocytosis consisting mainly of C8-positive T cells and natural killer cells. The history of IM is associated with an increased incidence of HD but seems not to be associated with the presence of EBV in the tumor cells. The first evidence linking IM with EBV in HD was a case report of an older adult with IM (34). However, the assumption that EBV-positive HD is usually preceded by IM is not supported by our findings, inasmuch as only 3 of the 16 EBV-positive cases had a history of IM.

These findings raise the possibility that EBV infection or the immune response that it evokes contributes to the pathogenesis of HD, even in cases in which the viral genome is not detected. In this regard, Frisan et al. (35) report detecting cytotoxic T cells specific for EBV latency antigens in HD tumor tissue in which EBV was not detected in the RS cells. In addition, Razzouk et al. (36) report the presence of fragments of the EBV genome in some cases of nonendemic Burkitt’s lymphoma. The evidence involving EBV in these cases would have been missed by the assays conventionally applied to HD, including those applied here. Thus, among the scenarios that might be considered is the possibility that EBV infection is a step on the pathway to malignancy but that, in some instances, all or most of the viral genome is lost, perhaps in the setting of a vigorous cytotoxic T-cell response to viral latency antigens. Additional studies of tumor tissue to detect viral fragments in cases that are EBV-negative by EBER in situ hybridization or LMP1 immunohistochemistry and studies of the cellular immune response to EBV in patients with EBV-positive and EBV-negative HD should shed light on these issues.

References


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