RESEARCH COMMUNICATION

Epstein-Barr Virus-Associated Nodal Malignant Lymphoma in Thailand

Winyou Mitarnun¹, Jintana Pradutkanchana¹, Takafumi Ishida²

Abstract

Specific subtypes of malignant lymphoma are highly associated with Epstein-Barr virus (EBV) infection. In the present study, the authors evaluated EBV-encoded RNA (EBER) expression by in situ hybridization in 300 cases of malignant lymphomas diagnosed by lymph node biopsy, with 100 cases of reactive lymphoid hyperplasia in lymph nodes as controls, for comparison. There were 100 consecutive cases of classical Hodgkin’s lymphoma (cHL), 100 consecutive cases of non-Hodgkin’s lymphoma, B cell (NHL-B), and 100 consecutive cases of non-Hodgkin’s lymphoma, T cell (NHL-T). EBER expression was detected in 46% of reactive lymphoid hyperplasia cases, but the positively stained cells in those cases constituted less than 5 percent of the total cell populations. When using the presence of EBER in 5 percent or more of the cell population and/or the presence of EBER in the Hodgkin’s Reed-Sternberg’s cells as indicators of positivity, 64% of cHL, 13% of NHL-B, and 51% of NHL-T were found to be positive. The study indicates a strong association of cHL and NHL-T with EBV infection, the link apparently being weaker for NHL-B except for the subtypes of Burkitt’s lymphoma and diffuse large B cell lymphoma.

Key Words: Epstein-Barr virus - malignant lymphoma - EBV-encoded RNA

Introduction

Epstein-Barr virus (EBV) is a lymphocryptovirus belonging to the subfamily of gammaherpesvirinae. It is an etiologic agent of infectious mononucleosis, which is a self-limiting disease, and a fatal form is an uncommon presentation of the primary infection characterized by an uncontrolled B cell proliferation due to a defect in T cell-mediated immune regulation (Straus et al, 1993, Okano et al, 1996). EBV infection has also been implicated in the development of a variety of malignancies, including nasopharyngeal carcinoma (Nideobitek, 2000), gastric carcinoma (Takada, 2000), smooth muscle tumors in immunocompromised patients (McClain et al, 1995), thymic lymphoepithelial carcinoma (Levraz et al, 1985), Hodgkin’s lymphoma (HL) (Flavell et al, 2000), and non-Hodgkin’s lymphomas of B cell and T cell origins (Graig et al, 1993, Okano et al, 2000, Mitarnun et al, 2002a, Mitarnun et al, 2002b).

EBV could either play a direct or indirect role in the pathogenesis of HL, and with the advent of cloned viral probes and Southern blot hybridization methods, it has been possible to detect EBV DNA in 20-25% of HL tumor specimens (Evans et al, 1984, Weiss et al, 1987). EBV-encoded RNA (EBER) expression in Hodgkin’s Reed-Sternberg cells appears to be less common in developed countries, at between 20-50%, in contrast to underdeveloped countries which have much higher rates (Weiss et al, 1991, Herbst et al, 1992, Chang et al, 1993, Leoncini et al, 1996, Weinreb et al, 1996). There is evidence that the association of EBV infections with classical Hodgkin’s lymphoma (cHL) is strongest for mixed cellularity Hodgkin’s lymphoma (>60%), and is weaker for nodular sclerosis Hodgkin’s lymphoma (approximately 35%) (Herbst et al, 1990, Chan et al, 1995, Glasser et al 1997).

The EBER positive rate was estimated to be 7% for one series of non-Hodgkin’s lymphoma, B cell (NHL-B) cases. A high percentage positivity is usually found in diffuse large B-cell lymphomas, Burkitt’s lymphomas, and posttransplantation lymphoepithelial diseases (Zur Hausen et al, 1970, Craig et al, 1993). In previous studies, non-Hodgkin’s lymphoma, T cells (NHL-T), especially

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angioimmunoblastic T-cell lymphomas from Eastern and Western countries, demonstrated frequent association (55% to 97%) with EBV infection (Anagostopoulos et al, 1992, Weiss et al, 1992, Noorali et al, 2003).

The aims of the present study were as follows: 1) to assess the frequencies of nodal cHL, NHL-B, and NHL-T in southern Thailand; and 2) to search for an association of nodal cHL, NHL-B, and NHL-T with EBV infection using lymph nodes with reactive lymphoid hyperplasia as a control for detection of positive cases.

**Materials and Methods**

**Patients Samples**

All of the cases were from the Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla province, Thailand. Retrospective consecutive cases from July 2003 back to January 1995 were obtained. There were 100 cases of reactive lymphoid hyperplasia (RH), 100 cases of classical Hodgkin’s lymphoma, 100 cases of non-Hodgkin’s lymphoma, B cell type, and 100 cases of non-Hodgkin’s lymphoma, T cell type. The histologic diagnosis of the lymphoid neoplasms in lymph nodes was made according to the WHO classification (Jaffe et al, 2001).

**In situ Hybridization**

An in situ hybridization (ISH) study for the Epstein-Barr virus (EBV) mRNA was performed on formalin-fixed, paraffin embedded, tissue using Epstein-Barr Virus Probe ISH Kit (Novocastra Laboratories, UK). The EBV probe hybridized to abundantly expressed Epstein-Barr virus-encoded RNA (EBER) transcripts which are concentrated in the nuclei of latently infected cells. The ISH procedure steps followed the manufacturer’s manual. Briefly, tissue sections of 5 microns were deparaffinized with xylene, rehydrated in 99% ethanol, 95% ethanol and graded water, respectively and digested with proteinase K (7 mg/mL in 50 mM Tris/HCl, pH 7.6) for 30 minutes at 37°C. After dehyrdration and air-drying, fluorescein-labelled oligonucleotide cocktail probes were applied to the sections for 2 hours at 37°C, then blocked with normal rabbit serum. Rabbit F(ab’) anti-FITC/AP was added for 30 minutes followed by overnight incubation of the enzyme substrate solution (BCIP/NBT/Levamisole). The slides were washed in running tap water and mounted with glycercol buffer. Appropriate positive and negative controls were run in every batch tested. The amount of positive cells were visually estimated in percent of the total cell population. Few positive cells were considered as less than 5% positive.

**Results**

During the two-year period 2001-2002, there were 192 cases of nodal malignant lymphomas diagnosed in the Department of Pathology, Faculty of Medicine, Prince of Songkla University. Of these, 31 cases (16.1%) were classical Hodgkin’s lymphoma (cHL), 119 cases (62.0%) were non-Hodgkin’s lymphoma, B cell (NHL-B), and 42 cases (21.9%) were non-Hodgkin’s lymphoma, T cell (NHL-T). The ratios of NHL-T to NHL-B was 1:2.8, and cHL to NHLs (-T, and -B) was 1:5.2.

EBERs were identified by ISH in 46 of the 100 consecutive cases (46%) of the nodal reactive lymphoid hyperplasia (Table 1). The degree of positivity was less than 5% of cells in all of the positive cases, and a majority showed few cells positive.

| Table 1. 100 Consecutive Cases of Reactive Lymphoid Hyperplasia in Lymph Nodes |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Total No.       | Males/Females   | Age (years)     | EBV-ISH study   |
|                 |                 | Mean | Range | Negative (cases) | Positive (percent of cells) | Positive <5% (percent) |
|                 |                 |  |       |                 | <5% | ≥ 5% | ≥ 5% |
| 100             | 48/52           | 35.1 | 1-75  | 54              | 46 | 0   | 46  |

| Table 2. 100 Consecutive Cases of Classical Hodgkin’s Lymphomas in Lymph Nodes |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Type of Hodgkin’s Lymphoma | Total No. | Male/Female | Age (years) | EBV-ISH study | Positive ≥ 5% |
| NSHL | 62 | 46/16 | 34.5 | 4-72 | 15 | 17 | 21  | 9 | 37 | 38 | 61.3 |
| MCHL | 20 | 15/5 | 32.3 | 7-54 | 1 | 3 | 14  | 2 | 17 | 14 | 95.0 |
| LRCHL | 14 | 6/8 | 42.2 | 7-70 | 6 | 4 | 2  | 2 | 4 | 7 | 35.7 |
| LDHL | 4 | 2/2 | 39.8 | 4-87 | 1 | 2 | 1  | 0 | 2 | 2 | 50.0 |
| Total | 100 | 69/31 | 35.4 | 4-87 | 23 | 26 | 38 | 13 | 60 | 61 | 64.0 |

NSHL = Nodular sclerosis Hodgkin’s lymphoma, MCHL = Mixed cellularity Hodgkin’s lymphoma
LRCHL = Lymphocyte rich classical Hodgkin’s lymphoma, LDHL = Lymphocyte depleted Hodgkin’s lymphoma
HRS-cell = Hodgkin’s Reed-Sternberg’s cell, BG-cell = Background cell

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positivity in the different cases, ranging from few positive cells to approximately 90% of cells. When using 5 percent or more than 5 percent of the EBER-positive cells as a cut-off value, the positivity was detected in 13% of NHL-B patients, and 51% of NHL-T patients.

Table 2 shows the results of the clinical data and ISH study for the EBER in 100 consecutive cases of the nodal cHL. The frequencies of the histological subtype were as follows: nodular sclerosis Hodgkin's lymphoma (NSHL) = 62%, mixed cellularity Hodgkin's lymphoma (MCHL) = 20%, lymphocyte rich classical Hodgkin's lymphoma (LRCHL) = 14%, and lymphocyte depleted Hodgkin's lymphoma = 4%. The mean age of all subtypes was 35.4 years (range 4-87 years), and the male to female ratio was approximately 2:1. The prevalence of EBER in Hodgkin's Reed-Sternberg (HRS) cells varied according to the subtype: 37 of 62 (59.7%) in NSHL, 17 of 20 (85%) in MCHL, 4 of 14 (28.6%) in LRCHL, 2 of 4 (50%) of LDHL, and the average prevalence was 60%. EBER positivity in HRS cells and/or 5 percent or more than 5 percent in the background cells was detected in 61.3% of NSHL, 95% of MCHL, 35.7% of LRCHL and 50% of LDHL.

Table 3 shows the results of the clinical data and ISH study for the EBER in 100 consecutive cases of NHL-B. The prevalence in each subtype was as follows: 75% of diffuse large B-cell lymphoma (DLBCL), 11% of follicular lymphoma (FL), 8% of mantle cell lymphoma (MCL), 5% of Burkitt's lymphoma (BL), and 1% of small lymphocytic lymphoma, B cell type (B-SLL). The mean age of NHL-B cases was 54.3 years (range 7-85 years), and the male to female ratio was about 2:1. The incidence rates of non-Hodgkin's lymphomas (NHLs) and classical Hodgin's lymphoma varied geographically (Stewart et al, 2003). They were commonly found in Western countries, Middle-Eastern countries and Australia. The incidence rates were low in Asia and some underdeveloped countries. In the past two decades, the incidence of NHLs (nodal and extranodal) in Songkhla province, Thailand has increased notably. Cancer registry reports from 1988 to 2000 showed a progressive increase in the age-standardized rate (ASR) per 100,000 population per year of both sexes from 1.1 to 4.5. The ASRs were as follows; 1.1 in 1988-1991 (Vatanasapt et al, 1993), 2.8 in 1992-1994 (Deerasamee et al, 1999), 4.4 in 1995-1997 (unpublished data), and 4.5 in 1988-2000 (unpublished data). The male to female ratio for the nodal and extranodal NHLs was approximately 1.6:1. The incidence of HL was

### Table 3. 100 Consecutive Cases of Peripheral B-cell Lymphomas in Lymph Nodes

<table>
<thead>
<tr>
<th>Type of lymphoma</th>
<th>Total No.</th>
<th>Male/Female</th>
<th>Age (years) Mean</th>
<th>EBV-ISH study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Negative (cases)</td>
</tr>
<tr>
<td>DLBCL</td>
<td>75</td>
<td>48/27</td>
<td>53.8</td>
<td>51</td>
</tr>
<tr>
<td>FL</td>
<td>11</td>
<td>6/5</td>
<td>62.6</td>
<td>8</td>
</tr>
<tr>
<td>MCL</td>
<td>8</td>
<td>5/3</td>
<td>60.4</td>
<td>8</td>
</tr>
<tr>
<td>BL</td>
<td>5</td>
<td>3/2</td>
<td>35.6</td>
<td>2</td>
</tr>
<tr>
<td>B-SLL</td>
<td>1</td>
<td>1/0</td>
<td>58.0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>63/37</td>
<td>54.3</td>
<td>69</td>
</tr>
</tbody>
</table>

DLBCL = Diffuse large B-cell lymphoma, FL = Follicular lymphoma, MCL = Mantle cell lymphoma, BL = Burkitt’s lymphoma, B-SLL = Small lymphocytic lymphoma, B-cell type

### Table 4. 100 Consecutive Cases of Peripheral T-cell Lymphoma in Lymph Nodes

<table>
<thead>
<tr>
<th>Type of lymphoma</th>
<th>Total No.</th>
<th>Male/Female</th>
<th>Age (years) Mean</th>
<th>EBV-ISH study</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Range</td>
<td>Negative (cases)</td>
</tr>
<tr>
<td>AILT</td>
<td>67</td>
<td>44/23</td>
<td>51.1</td>
<td>9</td>
</tr>
<tr>
<td>ALCL</td>
<td>9</td>
<td>7/2</td>
<td>44.2</td>
<td>2</td>
</tr>
<tr>
<td>PTCL-u</td>
<td>24</td>
<td>15/9</td>
<td>50.8</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td>66/34</td>
<td>50.4</td>
<td>18</td>
</tr>
</tbody>
</table>

AILT = Angioimmunoblastic T-cell lymphoma, ALCL = Anaplastic large cell lymphoma, PTCL-u = Peripheral T-cell lymphoma, unspecified
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References

hemophagocytic syndrome and fatal infectious mononucleosis. 


