Epstein-barr Nuclear Antigen-1 (EBNA-1) in Diffuse Large B-cell Lymphoma and Its Relationship to The bcl-2 Protein

Ibnu Purwanto*, Johan Kurnianda*, Susanna Hilda Hutajulu*, Kartika Widayati*, Mohammad Rizki*, Harijadi**

ABSTRACT

Aim: to determine EBNA-1 expression in the tissue of patients with diffuse large B-cell lymphoma and its relationship to bcl-2 expression.

Methods: paraffin-embedded tissue from 24 cases of diffuse large B-cell lymphoma were stained immunohistochemically with monoclonal antibody anti-EBNA-1 and anti-bcl-2 using Streptavidin Biotin Complex method. The bcl-2 expression was measured as negative (no staining), positive 1 (weak staining), and positive 2 (moderate to strong staining). The relationship between the immunohistochemistry results was examined.

Results: from the 24 samples, 12 (50%) were EBNA-1 positive and 14 (58.3%) showed expression of bcl-2. Eleven of the 14 samples (90%) showed expression of bcl-2 were EBNA-1 positive, while only 3 of these samples (10%) were EBNA-1 negative. The relationship between bcl-2 expression and EBNA-1 was statistically significant (Chi square-uncorrected 11.2571, p= 0.0036).

Conclusion: our results showed that 50% of diffuse large B-cell lymphoma were EBNA-1 positive. Furthermore, EBNA-1 seemed to be correlated with bcl-2 expression.

Key words: EBNA-1, diffuse large B-cell lymphoma, bcl-2.

INTRODUCTION

Epstein-Barr virus (EBV) is associated with several human lymphoid malignancies, including lymphomas. The biological activity of EBV that causally links it to lymphomagenesis is its capacity to growth-transform resting B cells to immortalized lymphoblastoid cells that proliferate indefinitely and harbor the virus in a latent state. Latent EBV infection is characterized by restricted expression of viral gene products, including six nuclear antigens (Epstein-Barr nuclear antigens [EBNAs]-1, -2, -3a, -3b, -3c, and LP) and two transmembrane proteins (latent membrane protein [LMP]-1 and -2) that function cooperatively in activating cellular genes that are involved in physiologically B-cell activation, proliferation, and survival. Expression of EBNA-1 has been proven to induce B cell neoplasia in transgenic mice. While EBNA-1 is the only EBV-protein that is thought to be expressed in all latently EBV-infected cells, only some of diffuse large B-cell lymphomas patients express EBNA-1.

The bcl-2 family of proto-oncogenes is a critical regulator of apoptosis, whose expression frequently becomes altered in human cancers, including lymphomas. In vitro studies have shown that expression of bcl-2 is important for the long survival of Epstein-Barr virus-positive cells and may be a first step in oncogenesis of lymphoma. Some reports have shown interactions among bcl-2 protein and EBV protein, whether EBNA-1 or other proteins.

The aim of this study is to determine the expression of EBNA-1 in the tissue of patients with diffuse large B-cell lymphoma. In addition, we also want to verify the correlation between EBNA-1 with other poor prognostic markers such as bcl-2 expression.

METHODS

This was a cross-sectional study conducted on 24 tissue samples of patients with diffuse large B-cell lymphoma of low, intermediate, and high grade histology. All patients had a biopsy either from a lymph node or extra-nodal site.

Paraffin embedded tissues of diffuse large B-cell lymphoma cases were cut 5μ on a poly-ilsined slide. The tissues were previously detected to be associated with EBV infection by EBER 1,2 RNA in situ hybridization (RISH). All slides were stained with monoclonal antibodies anti EBNA-1 (clone OTx) with dilution 1:100. All slides were also stained with anti bcl-2 (clone 124 DAKO); with dilution 1:50 using Streptavidin Biotin Complex (SABC) with DAB visualization. With
EBNA-1 staining, the presence of the nucleus of tumor cells indicated a positive result. With bcl-2 staining, cytoplasm of tumor cells of equal or more than 10% was considered positive, and less than 10% was considered negative. A bcl-2 staining showing more than 10% with low intensity was considered weak staining (positive 1), and those showing high intensity was considered as moderate to strong staining (positive 2).

Statistical analysis consisting of Chi-square test was performed using Epi-info program. A p value of < 0.05 was considered significant.

RESULTS

We studied 24 diffuse large B-cell lymphoma cases (13 men and 11 women) ranging in age from 30-69 years (mean age was 58±9.8 years). Biopsy specimens from 13 patients were derived from lymph nodes (mesenterial, colli, submandibular) and specimens from 11 patients were derived from extra nodal organs (tonsil, nasopharynx, gum, and other gastro intestinal tract organs).

From the 24 samples, 12 (50%) were EBNA-1 positive and 12 (50%) were EBNA-1 negative. Fourteen of these (58.3%) showed expression of bcl-2, while another 10 did not show any expression. Seven samples showed weak staining of bcl-2, while the other 7 showed moderate to strong staining.

Eleven of the 14 samples (90%) which showed expression of bcl-2 were EBNA-1 positive, while only 3 of these samples (10%) were EBNA-1 negative. Eleven of 12 samples that were EBNA-1 positive showed expression of bcl-2 (5 had weak staining, 6 had moderate to strong staining).

When the immunohistochemistry results were analyzed, there was a correlation between bcl-2 expression and EBNA-1. The relation was statistically significant (Chi square-uncorrected 11.257, p= 0.0036). When stratified based on nodal location, the relation showed a significant value (p= 0.0015), but not when stratified based on extra nodal location (p= 0.06).

Table 1 shows the expression of bcl-2 and EBNA-1.

<table>
<thead>
<tr>
<th>Variables</th>
<th>EBNA-1 positive</th>
<th>EBNA-1 negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bcl-2 negative</td>
<td>1 (8.3%)</td>
<td>9 (75%)</td>
<td>10</td>
</tr>
<tr>
<td>Bcl-2 positive</td>
<td>5 (41.7%)</td>
<td>2 (16.7%)</td>
<td>7</td>
</tr>
<tr>
<td>Bcl-2 positive</td>
<td>6 (50%)</td>
<td>1 (8.3%)</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>12</td>
<td>24 (100%)</td>
</tr>
</tbody>
</table>

DISCUSSION

Transformation of B-cells by EBV requires the expression of at least five latent viral genes (EBNA-1, EBNA-2, EBNA-3a, EBNA-3c, and LMP-1) and cannot be mediated by a single viral gene. The precise role of each of these latent gene products in transformation is not fully understood. All of them play a role in protecting EBV-infected cells from apoptosis. The presence of EBNA-1 in EBV-cell transformation will result in complete immortalization.3

The bcl-2 family of proto-oncogenes is a critical regulator of apoptosis, whose expression frequently becomes altered in human cancers, including lymphomas. Bcl-2 was the first member to be identified, by virtue of the involvement of t(14,18) in chromosomal translocation commonly found in B-cell non-Hodgkin’s lymphoma (NHL).11 The consequence of the translocation is the presence of high levels of bcl-2 protein. Bcl-2 is believed to contribute to oncogenesis by blocking programmed cell death, thereby by extending cell survival. Over-expression of bcl-2 protein also prevents cell death induced by nearly all cytotoxic drugs and gamma irradiation. More over, expression levels of bcl-2 family of protein changes as the tumor becomes more malignant or after treatment, suggesting that expression of these survival proteins is critical not only for tumor development, but also for tumor progression and resistance to therapy.12 Some studies showed that bcl-2 expression induction by EBV infection protects B infected cells from apoptosis.8,13

Bcl-2 protein expression has been found in 44-55% of aggressive lymphoma. It was associated with stage III-IV diseases and primary nodal diseases. The disease-free survival for patients with bcl-2-expressing tumors is significantly poorer and remains an independent prognostic factor. Survival is also lower in patients with bcl-2 expressing tumor.14

Another EBV-protein that is associated strongly to carcinogenesis of lymphomas is latent membrane protein-1 (LMP-1).9,15 However, EBNA-1 seemed to always be expressed in all kinds of lymphoid and epithelial EBV-associated disease.3

A study performed by Chen et al. used a method that detects viral transcripts for EBNA-1, EBNA-2, LMP-1 and LMP-2a, showed that EBV-positive cells among peripheral blood B-lymphocytes in type I Burkitt lymphoma cells expressed EBNA-1 m-RNA.16 A previous study by Roth showed that antisense oligonucleotide directed to EBNA-1 partially inhibited EBNA-1 expression and suppressed B cell proliferation.17 Drotar et al. reported that expression EBNA-1 by EBV may provide a selection pressure in addition to
translocation of the c-myc locus in the genesis of endemic Burkitt’s lymphoma. These data indicate that myc and EBNA-1 act cooperatively in lymphoma genesis.\textsuperscript{18}

In the study of rat monoclonal antibodies directed against EBNA-1 Reynolds et al demonstrated that all (8 cases) HIV associated cerebral B cell lymphoma expressed EBNA-1.\textsuperscript{19} These antibodies also examined the expression of EBNA-1 in 28 of 40 Hodgkin’s disease cases, 3 of 3 Burkitt’s lymphoma cases, 10 of 10 nasopharyngeal carcinoma cases, and 10 of 10 gastric carcinoma cases.

In their study with immunohistochemistry using a mixture of two EBNA-1 specific monoclonal antibodies (1H4-1 and 2B4-1), Oudejans et al detected EBNA-1 positive cells in all of 20 EBV associated B cell lymphomas. This study also detected EBNA-1 positive cells in 8 of 9 EBV-associated T cell lymphomas, and 11 of 27 EBV positive cases of Hodgkin’s disease.\textsuperscript{20}

Compared to other studies, there were some differences in detecting positive EBNA-1 cells. It could be derived from different EBNA-1 detection methods or from other unknown causes we still have not explored. EBNA-1 positive cases in our study was high enough (50%) even though was not so high as in previous studies of malignancies.\textsuperscript{19, 20}

Other EBV protein frequently reported to be expressed in lymphoma are LMP-1\textsuperscript{9,13,21,22} and EBNA-2.\textsuperscript{21,22} Other than B cell lymphomas, EBNA-1 expression was also reported on Hodgkin’s disease,\textsuperscript{19,23} T cell lymphoma,\textsuperscript{20} and nasopharyngeal cancer.\textsuperscript{19,20} These will be other interesting aspects we want to verify in our cancer patients.

Our study confirmed the expression of bcl-2 in 14 out of 24 DLBCL samples (58.3%). Komano et al.\textsuperscript{7} proved that bcl-2 expression on EBV infected cells increased apoptosis resistance compared to uninfected cells. Like our study, this result supported the association between bcl-2 expression with EBNA-1 in apoptosis resistance. Association of EBNA-1 with other proto-oncogen (bcl-xL) reported by Tsimbousuri et al.\textsuperscript{24} Finke et al.\textsuperscript{8} and Henderson et al.\textsuperscript{12} showed that bcl-2 was induced by an EBV latent gene which may be a part of independent viral survival strategy in the infected host cell.

As far as we know, there have not been any studies reporting correlation between EBNA-1 and bcl-2 based on tumor location (nodal or extra nodal). In our study, nodal location has an influence on that relationship. EBNA-1 expression, especially its relationship with bcl-2 proto-oncogen, is an interesting point for further investigation with a larger sample.

CONCLUSION

Our results showed that 50% of diffuse large B-cell lymphoma were EBNA-1 positive and EBNA-1 seemed to be correlated with bcl-2 expression. Nodal location (and not extra nodal) has an influence on the correlation. Since bcl-2 protein has been reported to play a critical role in oncogenesis as well as resistance to therapy of cancer,\textsuperscript{23} the clinical significance of EBNA-1 and bcl-2 expression in diffuse large B-cell lymphoma needs to be further investigated in a larger cohort of prospectively accrued patients.

REFERENCES


