RESEARCH COMMUNICATION

Epstein-Barr Virus-Associated Nodal Malignant Lymphoma in Thailand

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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

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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 (Leyvraz 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, Kumar 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 posttransplantion lymphoproliferative 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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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) <5%	Positive (percent of cells) ≥ 5%	Positive <5% (%)
100	48/52	35.1	1-75	54	46	0	46

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 dehydration 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 glycerol 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.

Tables 2, 3, and 4 show the results of the ISH study of the EBER in 100 consecutive cases of cHL, 100 consecutive cases of NHL-B, and 100 consecutive cases of NHL-T, respectively. There was a very wide range in the degree of

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%, or in HRS-cell (%)
			Mean	Range	Negative (cases)	Positive (percent of cells) <5%	Positive (percent of cells) 5-19%	Positive (percent of cells) 20-50%	Positive in (cases) HRS-cells	Positive in (cases) BG-cells	
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

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

Type of lymphoma	Total No.	Male/Female	Age (years) Mean	Age (years) Range	EBV-ISH study Negative (cases)	Positive (percent of cells) <5%	5-39%	40-90%	Positive ≥ 5% (%)
DLBCL	75	48/27	53.8	7-85	51	13	4	7	14.7
FL	11	6/5	62.6	39-82	8	3	0	0	0
MCL	8	5/3	60.4	34-79	8	0	0	0	0
BL	5	3/2	35.6	26-57	2	1	0	2	40.0
B-SLL	1	1/0	58.0	-	0	1	0	0	-
Total	100	63/37	54.3	7-85	69	18	4	9	13.0

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

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. Five percent or more than 5 percent of EBER-positive cells was detected in 14.7% of DLBCL, 40% of BL, and none in subtypes of FL, MCL and B-SLL.

The clinical findings and ISH study for EBER in 100 consecutive cases of NHL-T is shown in Table 4. The mean age of NHL-T cases was 50.4 years (range 3-82 years), with a male to female ratio of approximately 2:1. Angioimmunoblastic T-cell lymphoma (AILT) was 67%, anaplastic large cell lymphoma (ALCL) was 9%, and peripheral T-cell lymphoma, unspecified (PTCL-u), was 24%. Five percent or more than 5 percent of EBER-positive cells was detected in 49.3% of AILT, 55.6% of ALCL, and 54.2% of PTCL-u. In most cases of AILT, EBER was detected in both background lymphocytes and immunoblasts.

Discussion

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

Type of lymphoma	Total No.	Male/Female	Age (years) Mean	Age (years) Range	EBV-ISH study Negative (cases)	Positive (percent of cells) <5%	5-39%	40-90%	Positive ≥ 5% **(%)**
AILT	67	44/23	51.1	3-82	9	25	21	12	49.3
ALCL	9	7/2	44.2	13-69	2	2	2	3	55.6
PTCL-u	24	15/9	50.8	6-74	7	4	9	4	54.2
Total	100	66/34	50.4	3-82	18	31	32	19	51.0

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

apparently stable during those periods with an average ASR of 0.4, and male to female ratio of 2.8:1 (Vatanasapt et al, 1993, Deerasamee et al, 1999). An increase in incidence of NHLs was also reported elsewhere in Thailand, especially the high grade B-cell NHL (Sukpanichnant et al, 1988).

Classical Hodgkin's lymphoma (cHL) in this reported series accounted for 16% of all the nodal malignant lymphomas, which is a much lower figure than reports of Caucasians (25-40%), and was somewhat higher than other Oriental reports (5-10%) (Liang et al, 1989, Sukpanichnant et al, 1988). For the NHLs, the ratio of NHL-T to NHL-B was 1:2.8 similar to previous reports from Thailand and Pakistan (Sukpanichnant et al, 1988, Noorali et al, 2003), but in contrast to a study from Western countries in which the NHL-T to NHL-B ratio was 1:8-10 (The non-Hodgkin' s lymphoma classification project, 1997).

NSHL accounted for about two thirds of all cHLs, which was rather high when compared to other Asian reports (Sukpanichnant et al, 1988, Liang et al, 1989). For the NHL-B, DLBCL was more common than FL (75% versus 11%). The frequency of FL in Caucasians was a much higher percentage (22-40%), and the frequency of DLBCL was much lower (approximately 30%) (The non-Hodgkin's lymphoma classification project, 1997). AILT in Caucasians usually occurred in the middle-aged and elderly (median age = 64 years), with a slight male predominance (Siegert et al, 1995). In this reported series, AILT patients were much younger, with an average age of 51 years (range 3-82 years) and the male to female ratio of 2:1. The frequency of AILT in the Western countries was about 1-2% of all NHLs. It was much higher in this report: AILT accounted for about 9% of all nodal NHLs.

A small amount (less than 5 percent of cells) of EBER was detected in 46% of the nodal lymphoid hyperplasia cases. This concurred with a previous report in which EBV DNA in peripheral blood $CD3^-$ lymphocytes was detected in 50% of the healthy controls (Mitarnun et al, 2002b). These EBER positive cells were considered to be by-stander lymphocytes. In this study, ISH for EBER was interpreted as positive when the positively stained cells were ≥ 5 percent of the cell population, except for cHL which showed positivity in HRS cells.

The data presented here indicate a strong association of cHL and NHL-T with the EBV infection, and this association was less commonly found in NHL-B. Subtypes of malignant lymphoma showed different associations. The EBER was detected in 95% of MCHL cases, 40-60% of other cHL subtypes and all subtypes of NHL-T. For the NHL-B, only subtypes of DLBCL and Burkitt's lymphoma showed this association.

References

Anagnostopoulos I, Hummel M, Finn T, et al (1992). Heterogenous Epstein-Barr virus infection patterns in peripheral T-cell lymphoma of angioimmunoblastic lymphadenopathy type. *Blood*, **80**, 1804-12.

Chan JK, Yip TT, Tsang WY, et al (1995). Detection of Epstein-Barr virus in Hodgkin's disease occurring in an oriental population. *Hum Pathol*, **26**, 314-8.

Chang KL, Albujar PF, Chen YY, et al (1993). High prevalence of Epstein-Barr virus in the Reed-Sternberg cells of Hodgkin's disease occurring in Peru. *Blood*, **81**, 496-501.

Craig FE, Gully ML, Banks PM (1993). Posttransplantation lymphoproliferative disorders. *Am J Clin Pathol*, **99**, 265-71.

Deerasamee S, Martin N, Sontipang S, et al (1999). Cancer in Thailand Vol II, 1992-1994, International Agency for Research of Cancer technical No.34, Lyon, France, 130-1.

Evans AS, Gutensohn HM (1984). A population-based case control study of EBV and other viral antibodies among persons with Hodgkin's disease and their siblings. *Int J Cancer*, **34**, 149-57.

Flavell KJ, Murray PG (2000). Hodgkin's disease and the Epstein-Barr virus. *J Clin Pathol: Mol Pathol*, **53**, 262-9.

Glasser SL, Lin RJ, Stewart SL, et al (1997). Epstein-Barr virus-associated Hodgkin's disease: epidemiologic characteristics in international data. *Int J Cancer*, **70**, 375-82.

Graig FE, Gully ML, Banks PM (1993). Posttransplantation lymphoproliferative disorders. *Am J Clin Pathol*, **99**, 265-71.

Herbst H, Niedobitek G, Kneba M, et al (1990). High incidence of Epstein-Barr virus genomes in Hodgkin's disease. *Am J Pathol*, **137**, 13-8.

Herbst H, Steinbrecher E, Niedobitek G, et al (1992). Distribution and phenotype of Epstein-Barr virus harboring cell in Hodgkin's disease. *Blood*, **80**, 484-91.

Jaffe ES, Harris NE, Stein H, et al (2001). World Health Organization Classification of Tumours. Tumours of Haematopoietic and Lymphoid Tissues. International Agency for Research of Cancer Press, Lyon, France, 10-1.

Kumar S, Fend F, Quintanilla-Martinez L, et al (2000). Epstein-Barr virus-positive primary gastrointestinal Hodgkin's disease. Association with inflammatory bowel disease and immunosuppression. *Am J Surg Pathol*, **24**, 66-73.

Leoncini L, Spina D, Nyong A, et al (1996). Neoplastic cells of Hodgkin's disease show differences in EBV expression between Kenya and Italy. *Int J Cancer*, **65**, 781-4.

Leyvraz H, Henle W, Chahinian AP, et al (1985). Association of Epstein-Barr virus with thymic carcinoma. *N Engl J Med*, **312**, 1296-9.

Liang R, Choi P, Todd D, et al (1989). Hodgkin's disease in Hong Kong Chinese. *Hematol Oncol*, **7**, 395-403.

McClain KL, Leach CT, Jensen HB, et al (1995). Association of Epstein-Barr virus with leiomyosarcoma in young people with AIDS. *N Engl J Med*, **332**, 12-8.

Mitarnun W, Pradutkanchana J, Takao S, et al (2002). Epstein-Barr virus-associated non-Hodgkin's lymphoma of B-cell origin, Hodgkin's disease, acute leukemia, acute leukemia, systemic lupus erythematosus: a serologic and molecular analysis. *J Med Assoc Thai*, **85**, 552-9.

Mitarnun W, Suwiwat S, Pradutkanchana J, et al (2002). Epstein-Barr virus-associated peripheral T-cell and NK-cell proliferative disease/lymphoma: a clinicopathologic, serologic and molecular analysis. *Am J Hematol*, **70**, 31-8.

Nideobitek G (2000). Epstein-Barr virus infection in the pathogenesis of nasopharyngeal carcinoma. *J Clin Pathol: Mol Pathol*, **53**, 248-54.

Noorali S, Pervez S, Moatter T, et al (2003). Characterization of T-cell non-Hodgkin's lymphoma and its association with Epstein-Barr virus in Pakistani patients. *Leuk Lymphoma*, **44**, 807-13.

Okano M, Gross TG (1996). Epstein-Barr virus-associated

hemophagocytic syndrome and fatal infectious mononucleosis. *Am J Hematol*, **53**, 111-5.

Siegert W, Nerl C, Agthe A, et al (1995). Angioimmunoblastic lymphadenopathy (AILD)-type T-cell lymphoma: prognostic impact of clinical observations and laboratory findings at presentation. The Keil Lymphoma Study Group. *Ann Oncol*, **6**, 659-64.

Stewart BW, Kleihues P, eds. (2003). World Cancer Report International Agency for Research on Cancer. IARC Press, Lyon, France, 238-8.

Straus SE, Cohen JI, Tosato G, Meier J (1993). Epstein-Barr virus infection: biology, pathogenesis and management. *Ann Int Med*, **118**, 45-58.

Sukpanichnant S, Sonakul D, Piankijagum A, et al (1988). Malignant lymphoma in Thailand: change in the frequency of malignant lymphoma determined from a histologic and immunophenotypic analysis of 425 cases at Siriraj Hospital. *Cancer*, **83**, 1197-204.

Takada K (2000). Epstein-Barr virus and gastric carcinoma. *J Clin Pathol: Mol Pathol*, **53**, 225-61.

The Non-Hodgkin's lymphoma classification project (1997). A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. *Blood*, **89**, 3909-18.

Vatanasapt V, Martin N, Sriplung H, et al (1993). Cancer in Thailand 1988-1991. International Agency for Research of Cancer technical report No. 16, Lyon, France, 120-3.

Weinreb M, Day PJR, Niggli F, et al (1996). The role of Epstein-Barr virus in Hodgkin's disease from different geographical areas. *Arch Dis Child*, **74**, 27-31.

Weiss LM, Strickler JG, Warnke RA, et al (1987). Epstein-Barr viral DNA in tissues of Hodgkin's disease. *Am J Pathol*, **129**, 86-91.

Weiss LM, Chen YY, Liu XF, et al (1991). Epstein-Barr virus and Hodgkin's disease: a correlative in situ hybridization and polymerase chain reaction study. *Am J Pathol*, **139**, 1259-65.

Weiss LM, Jaffe ES, Liu XF, et al (1992). Detection and localization of Epstein-Barr viral genomes in angioimmunoblastic lymphadenopathy and angioimmunoblastic-like lymphoma. *Blood*, **79**, 1789-95.

Zur Hausen H, Schulte-Holthausen H, Klein G, et al (1970). EBV DNA in biopsies in Burkitt tumors and anaplastic carcinoma of the nasopharynx. *Nature*, **228**, 1056-8.