

RESEARCH COMMUNICATION

Epstein-Barr Virus-Associated Extranodal Non-Hodgkin's Lymphoma of the Sinonasal tract and Nasopharynx in Thailand

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Abstract

Epstein-Barr virus (EBV) infection is highly associated with specific subtypes of malignant lymphoma. In our previous report on nodal malignant lymphoma in Thailand, we found that 64% of classical Hodgkin's lymphoma (cHL), 51% of non-Hodgkin's lymphoma, T-cell (NHL-T), and 13% of non-Hodgkin's lymphoma, B-cell (NHL-B) were EBV-related. In the present research, we conducted a retrospective study of primary extranodal non-Hodgkin's lymphoma of the sinonasal tract (e-NHL-ST) and primary extranodal non-Hodgkin's lymphoma of the nasopharynx (e-NHL-NP) in Southern Thailand, between 1997 and 2004. EBV-encoded RNA (EBER) expression by in situ hybridization was performed in all cases and a T-cell receptor (TCR)- γ gene rearrangement study was performed in NHL-T cases. There were 18 cases of e-NHL-ST and 42 cases of e-NHL-NP detected by histologic and immunohistochemistry examinations. The percentages of e-NHL-ST and e-NHL-NP as compared to nodal malignant lymphoma were 3.7% and 6.8%, respectively. Sixteen cases (88.9%) of e-NHL-ST and 7 cases (16.7%) of e-NHL-NP were NHL-T, and the remainder were NHL-B. All of the NHL-T cases in both sites were EBER-positive. Two (5.4%) of the NHL-B cases in the nasopharynx showed EBER positive. Monoclonal bands of the TCR- γ gene were detected in 71.4% of the extranodal NK/T-cell lymphomas, nasal type, patients; 50.0% of peripheral T-cell lymphoma, unspecified, patients; and one case of angioimmunoblastic T-cell lymphoma. This study indicates a very strong association of NHL-T in the sinonasal tract or nasopharynx with EBV infection, the link apparently being weaker in NHL-B patients. The study also indicates that most cases of extranodal NK/T-cell lymphoma, nasal type, are not the germline configuration of the TCR genes.

Key-Words: Epstein-Barr virus - malignant lymphoma - sinonasal tract - nasopharynx - EBV-encoded RNA - T-cell receptor

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Introduction

About one-third of extranodal malignant lymphomas are localized in the head and neck region. The sequential frequencies of these are tonsil, salivary gland, thyroid gland, orbit, nasopharynx and sinonasal tract (Hart et al., 2004). About 15% of the total number of sinonasal malignancies are Non-Hodgkin's lymphoma (NHL) of the nasal cavity and paranasal sinuses (Goldenberg et al., 2001). Sinonasal NHLs are relatively common in Asia, comprising up to 7% of all NHLs with a marked preponderance demonstrating an extranodal NK/T-cell lymphoma, nasal type (NK/T-cell lymphoma), subtype (Chan et al., 1987, Ho et al., 1984). Other areas with relatively high frequency of NK/T-cell lymphomas have been reported from Central and South America (Arber et al., 1993, Navarro-Roman et al., 1994, Quintanilla-Martinez et al, 1999). In contrast, sinonasal NHLs are uncommon in Western countries, accounting for only about 1.5% of NHLs (Frierson et al., 1989). In the Asian

population, more than 90% of NHLs in the sinonasal tract are NHL, T-cell type (NHL-T) (Woo et al., 2004), and the majority of them demonstrate Epstein-Barr virus (EBV) genomes in their tumor cells (Huh et al, 1999., Kuo et al., 2004., Peh et al., 2003). In Western countries, the majority of NHLs in the sinonasal tract are NHL, B-cell type (NHL-B) (Cuadra-Garcia et al., 1999, Proulx et al., 2003, Quraishi et al., 2001).

In Western populations, nearly all cases of nasopharyngeal NHL are NHL-B, whereas NHL-B accounts for only about 50-60% in Asian populations (Chan et al., 2005). Diffuse large B-cell lymphoma (DLBCL) is the most commonly found NHL-B in the nasopharynx in adults, with Burkitt's lymphoma occurring more frequently in children and young adults (Chan et al., 2005). The commonly found subtype of nasopharyngeal NHL-T is NK/T-cell lymphoma (Garcia-Cosio M et al., 2003).

NK/T-cell lymphoma is a predominantly extranodal manifestation, the sites of predilection being the nasal cavity,

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nasopharynx, palate, skin, soft tissue, gastrointestinal tract and testis. This subtype of NHL-T is more prevalent in Asia, South and Central America, with germline configuration of the T-cell receptor (TCR) gene in a majority of the cases (Chan et al., 2001). In the United States only one of fourteen cases of sinonasal NK/T-cell lymphoma demonstrated a TCR- γ gene rearrangement (Gaal et al., 2000). In contrast, in Singapore showed a significantly higher proportion of cases demonstrate monoclonal TCR-g gene rearrangement (Ng et al., 2004).

The aims of this study were: (I) to describe the basic clinical data and histologic subtypes in patients with NHL in the sinonasal tract and nasopharynx; (II) to search for any association with EBV infection; and (III) to document clonality and TCR- γ gene rearrangement in patients with the various subtypes of NHL-T.

Materials and Methods

Study Samples

A retrospective study was made of patients diagnosed for primary sinonasal tract (nasal cavity and paranasal sinuses) and/or nasopharynx malignant lymphoma at the Department of Pathology, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand, between the years 1997 and 2004. There were 60 cases of extranodal non-Hodgkin's lymphomas; 18 cases were identified in the sinonasal tract, and 42 cases were identified in the nasopharynx. Hodgkin's lymphoma was not found at any site. The histologic diagnosis of malignant lymphoma was made according to the WHO classification (Jaffe et al., 2001).

In situ Hybridization

An in situ hybridization (ISH) study for the EBV mRNA was performed on formalin-fixed, paraffin embedded tissue, using the Epstein-Barr Virus Probe ISH Kit (Novocastra Laboratories, UK). The EBV probe hybridized to abundantly expressed EBV RNA (EBER) transcripts which are concentrated in the nuclei of latently EBV infected cells. The ISH procedure steps followed the manufacturer's manual. Appropriate positive and negative controls were run in every batch tested. Few positive cells or positive less than 5% of the total tumor cell population were considered to be negative (Mitarnun et al., 2004).

DNA Extraction from Tissues for Polymerase Chain Analysis

Paraffin blocks from the patients diagnosed of NHL-T

were made available for the TCR gene rearrangement study. DNA extraction was performed using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). The DNA extraction steps followed the tissue protocol outlined in the manufacturer's manual. The presence of DNA was confirmed by agarose-gel electrophoresis or by amplification of the β -globin gene.

Polymerase Chain Reaction (PCR) Analysis

DNA extracted from the paraffin-embedded NHL-T tissues was analyzed for the TCR gene rearrangement and clonality studies. For the TCR gene rearrangement and the T-cell clonality of the tumor cells, the TCR- γ chain gene was used. Amplification of the TCR- γ chain gene was carried out using the PCR technique as previously described (McCarthy et al., 1991). We used two separate reactions with the following oligonucleotide primer combinations: V γ I+V γ III/IV+J γ 1/2 (product sizes 70-95 bp) and V γ II+J γ 1/2 (product sizes 150-180 bp). The presence of a clonal T-cell population was determined by the presence of one or two clearly visible bands of the PCR product. The smear pattern was interpreted as polyclonal TCR gene rearrangement. DNA extracted from the reactive lymph node and from the cell line U8312 (a human T-cell lymphotropic virus type-1 positive) were used as a negative and a positive controls.

Results

During the two-year period 2001-2002, there were 161 cases of nodal NHL diagnosed in our institute. During the same period, 17 cases of extranodal NHL in the sinonasal tract and nasopharynx were identified. The percentages of extranodal NHL in the sinonasal tract and nasopharynx as compared to nodal NHL were 3.7% (6 cases) and 6.8% (11 cases), respectively.

Table 1 shows the basic clinical data and in situ hybridization (ISH) for the EBER of the 18 patients with extranodal NHL in the sinonasal tract. There were 14 males and 4 females, ranging in age from 27 to 80 years. All of the 11 cases of NK/T-cell lymphoma and all of the 5 cases of peripheral T-cell lymphoma, unspecified (PTCLu), were strongly EBER positive. The degree of positivity ranged from 50% to more than 90% of the tumor cell population. None of the 2 cases of diffuse large B-cell lymphoma (DLBCL) showed the EBV genome in the tumor cells.

Table 2 shows the results of clinical data and the ISH study of EBER in 42 patients with extranodal NHL in the

Table 1. Extranodal Non-Hodgkin's Lymphoma in the Sinonasal Tract

Type of Lymphoma	Total No.	Male/Female	Age (years)		EBV-ISH study		
			Mean	Range	Positive	Negative	%Positive
NK/T	11	10/1	47.7	27-80	11	0	100
PTCLu	5	3/2	45.0	31-56	5	0	100
DLBCL	2	1/1	55.0	42-68	0	2	0
Total	18	14/4	46.9	27-80	16	2	88.9

NK/T, extranodal NK/T-cell lymphoma, nasal type; PTCLu, peripheral T-cell lymphoma, unspecified; DLBCL, diffuse large B-cell lymphoma.

Table 2. Extranodal Non-Hodgkin's Lymphoma in the Nasopharynx

Type of Lymphoma	Total No.	Male/Female	Age (years)		EBV-ISH study		
			Mean	Range	Positive	Negative	%Positive
NK/T	3	3/0	34.3	20-53	3	0	100
PTCLu	3	3/0	51.7	34-66	3	0	100
AILT	1	1/0	30.0	-	1	0	100
DLBCL	35	17/18	60.5	29-90	2	33	5.7
Total	42	24/18	57.2	20-90	9	33	21.4

NK/T, extranodal NK/T-cell lymphoma, nasal type; PTCLu, peripheral T-cell lymphoma, unspecified; AILT, angioimmunoblastic T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma.

nasopharynx. There were 24 males and 18 females, ranging in age from 20 to 90 years. EBERs were identified in all cases of NK/T-cell lymphoma (n=3), PTCLu (n=3), and a case of angioimmunoblastic T-cell lymphoma (AILT). The degree of positivity ranged from 50% to more than 90% of the tumor cell population. Only 2 of 35 cases (5.7%) of DLBCL were EBER-positive.

The results of the TCR gene rearrangement and the clonality studies of NHL-T in the sinonasal tract and nasopharynx are shown in Table 3. Using two sets of oligonucleotide primers for the TCR- γ gene, TCR gene rearrangement and monoclonal bands were detected in 10 of the 14 cases (71.4%) of NK/T-cell lymphoma and 4 of 8 cases (50.0%) of PTCLu. A case of AILT in the nasopharynx also showed the monoclonal bands of TCR- γ from the clonality study.

Discussion

Primary sinonasal and nasopharyngeal NHL are more common in Asian populations, but also occur in European countries and the United States. We studied 18 and 42 cases of NHL with involvement of the sinonasal tract and nasopharynx, respectively. The male to female ratios were 3.4:1 in the sinonasal tract cases and 1.3:1 in the nasopharynx cases. The age of these patients ranged from 20 to 90 years, with the mean ages of 47 years in the sinonasal cases, and 57 years in the nasopharyngeal cases. Nearly all NHL cases in the sinonasal tract were NHL-T, and only 2 (11%) were NHL-B. There were twice as many NK/T-cell lymphoma subtypes as PTCLu subtypes. In contrast, the majority of NHL patients in the nasopharynx were NHL-B (83%). The histologic subtype of NHL-B in both sites was DLBCL. These findings concurred with previous reports on NHL of the sinonasal tract and nasopharynx (Chan et al., 1987, Chan

et al., 2005).

The EBV genome was detected in all of NHL-T cases regardless of subtype. The degree of positivity in the nuclei of neoplastic T- or NK/T-cells was very strong. Interestingly, there was no positive staining in the mucosal epithelia of either sinonasal tract or nasopharynx. The only subtype of NHL-B which was found in these regions were DLBCL. The EBV genome was detected in only 5% of DLBCL cases, which was some what lower than our previous report on nodal DLBCL (Mitarnun et al., 2004).

NK/T-cell lymphoma of the nasal cavity and nasopharynx has a very poor prognosis as compared to the other subtypes of NHL-T and NHL-B (median survival time is less than 5 months) (Cheung et al., 1998). Histopathologic patterns can not distinguish between NK/T-cell lymphoma and PTCLu. Only an immunophenotypic examination can differentiate between these two entities. It was previously found that NK/T-cell lymphoma almost always showed the germline configuration of the TCR genes (Chan et al., 2001, Gaal et al., 2002). Recently, a report from Singapore revealed that 27% of NK/T-cell lymphoma cases had a monoclonal TCR- γ gene rearrangement (Ng et al., 2004). In this reported series, more than two-thirds of the NK/T-cell lymphomas demonstrated a monoclonal TCR- γ gene rearrangement, which was significantly higher than those of the PTCLu subtype.

The data presented here indicate a strong association of NHL-T, regardless of subtypes, in the sinonasal tract and nasopharynx with the EBV infection. For the NHL-B, this association is insignificant. The etiology of NHL-T remains to be elucidated, although a constant association with EBV infection suggests that this virus is involved in the pathogenesis. Unlike the previous studies on NK/T-cell lymphoma, a significantly higher percentage of this subtype of NHL-T in our study showed a clonal TCR- γ gene

Table 3. The Monoclonal Bands of T-cell Receptor- γ of Non-Hodgkin's Lymphoma, T-cell

Type of Lymphoma	Sinonasal tract			Nasopharynx			Total %Positive
	No. Positive	No. Negative	%Positive	No. Positive	No. Negative	%Positive	
NK/T	9	2	81.8	1	2	33.3	71.4
PTCLu	3	2	60.0	1	2	33.3	50.0
AILT	-	-	-	1	0	100	100
Total	12	4	75.0	3	4	42.9	65.2

NK/T, extranodal NK/T-cell lymphoma, nasal type; PTCLu, peripheral T-cell lymphoma, unspecified; AILT, angioimmunoblastic T-cell lymphoma.

rearrangement. Extensive molecular study on this subset of lymphoma should be reconsidered.

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