Article is available online at http://www.webio.hu/por/2003/9/3/0159

ARTICLE

Epstein-Barr Virus Expression in Hodgkin's Lymphoma in Kuwait*

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The epidemiology of Hodgkin's lymphoma (HL) shows wide geographic variation in histological subtypes and in its association with the Epstein-Barr virus (EBV). The proportion of EBV positive HL is low in industrialized countries, high in non-industrialized countries and intermediate in early-industrialized countries. Reports from the Persian Gulf and Middle East are very limited. The aim of this study was to determine the epidemiology of HL in Kuwait, an early-industrialized country in the Persian Gulf, and to delineate the extent of its association with EBV. We reviewed 134 cases of HL for histological classification and demographic data. 107 cases were examined for the presence of EBV using immunohistochemistry (IHC) for the latent membrane protein I (LMPI) and in-situ hybridization (ISH) for EBV-encoded RNA (EBER). 70.4% of the patients were males and 29.6% were females. The male: female ratio was 2.4:1. The mean age was 30.6 years (range, 4-71 years). Mixed cellularity HL (MCHL) was the most common subtype (45.5%), followed by nodular sclerosis (37.3%), nodular lymphocyte predominant (6.7%), lymphocyte rich (3%) and lymphocyte depletion (3%). 4.5% of cases were unclassifiable. EBV expression was seen in 56%, was significantly higher in MCHL, in children, and in males. Our findings suggest that the frequency of EBV expression in HL in Kuwait is similar to other early-industrialized countries. Further research from other countries in the Persian Gulf and the Middle East should shed more light on the epidemiology of HL and its relation to EBV in this region. (Pathology Oncology Research Vol 9, No 3, 159–165)

Keywords: Hodgkin's lymphoma, Epstein Barr virus, in situ hybridization, Epstein Barr virus encoded RNA, immunohistochemistry, Epstein Barr virus latent membrane protein 1

Introduction

Hodgkin's lymphoma (HL), a neoplasm of germinal center related B cells in nearly all cases[18,31] occurs as two clearly delineated entities, nodular lymphocyte predominant HL (NLPHL) and classical HL (CHL).[31] The two differ in clinical presentation and behavior, morphology, phenotype and molecular features.[31] CHL is further classified into four subtypes: nodular sclerosis (NSHL), mixed cellularity (MCHL), lymphocyte rich (LRCHL), and lymphocyte depletion(LDHL). The proportion of the subtypes shows significant geographic variation worldwide.[7,9,10]

Initially implicated in the etiopathogenesis of the African type of Burkitt's lymphoma, the Epstein-Barr virus (EBV), an ubiquitous human herpes virus, is now associated with a growing number of human malignancies, which include nasopharyngeal carcinoma, gastric carcinoma and T-cell lymphoma.[12, 25,26] More importantly, there is also a growing consensus now on the relevance of EBV infection to HL in varying proportions of patients in different populations.[4,5,9,10,13,14,15,16,21,27,29]

Hodgkin's lymphoma (HL) is one of the five most common malignancies in Kuwait.[28] Yet little is known of the relative distribution of the various subtypes, or of the proportion of cases associated with EBV. Earlier reports on HL from the Persian Gulf region, of which Kuwait is a part, have been rather selective and limited.[11,20,21,23] The

Received: June 6, 2003; *accepted:* August 25, 2003

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*This work was supported by Grant 99/01 from Kuwait University Research Fund

Abbreviations: HL: Hodgkin's lymphoma; EBV: Epstein-Barr virus; MCHL: mixed cellularity Hodgkin's lymphoma; NSHL: nodular sclerosis Hodgkin's lymphoma; NLPHL: nodular lymphocyte predominant Hodgkin's lymphoma; LRCHL: lymphocyte rich classical Hodgkin's lymphoma; LDHL: lymphocyte depletion Hodgkin's lymphoma; CHL: classical Hodgkin's lymphoma; ISH: in situ hybridization; EBER: Epstein-Barr virus encoded RNA; IHC: immunohistochemistry; EBV-LMPI: Epstein-Barr virus latent membrane protein 1

purpose of the present study is therefore to determine the relative frequency of distribution of the various histological subtypes of HL in Kuwait, correlate the subtypes with EBV expression and compare our findings with similar studies from elsewhere. This is the first comprehensive study of HL and EBV from a Persian Gulf country.

Materials and Methods

Review of cases

Archival paraffin blocks of cases previously diagnosed as Hodgkin's disease or Hodgkin's lymphoma from 1983-2002 were obtained from the surgical pathology files of Mubarak Al-Kabeer Hospital, Kuwait Cancer Control Center, and peripheral hospitals. Hematoxylin and eosin (H&E) and immunohistochemical stained sections were reviewed to confirm the diagnosis and to classify the cases into histologic subtypes according to the WHO classification.[31] The age, sex, and nationality, where available, were recorded for each patient. For the detection of EBV, two methods were used: immunohistochemistry (IHC) for the EBV latent membrane protein I (EBV-LMPL) and in situ hybridization (ISH) for EBV encoded RNA (EBER).

Immunohistochemistry (IHC)

Immunohistochemical staining was performed using the following antibodies: CD20, CD3, CD30, EBV-LMPI (Dako, Carpenteria, CA, USA) and CD15 (Becton-Dickinson, Franklin Lakes, NJ, USA). All sections were cut at 5-micron thickness and mounted on aminopropyltriethoxysilane (APES) coated slides. After dewaxing with xylene and rehydrating with alcohol, slides were placed in a 0.01 M citrate buffer solution (pH=6.0) and pretreatment procedures to unmask the antigens were performed in a microwave oven for 10 minutes for CD20, CD3, CD30 and EBV-LMPI. For CD15, pretreatment consisted of pronase digestion and microwave pressure-cooking for 4 minutes in citrate buffer. Tonsils were used as positive controls for CD20, CD3, and CD30. For EBV-LMPL and CD15, a known positive case of HL was used. For negative controls, primary antibody was replaced with normal mouse serum.

In-situ hybridization (ISH)

In situ hybridization was performed using a specific oligonucleotide probe (Novocastra – kit number NCL-EBV-K, U.K.) which hybridizes to Epstein-Barr virus-encoded RNA (EBER) transcripts concentrated in the nuclei of latently infected cells. Five micron sections were mounted on APES coated slides, dewaxed and dehydrated. This was followed by 30 minutes digestion with Proteinase K at 37°C. Twenty microliters of probe hybridization solution were added. The slides were incubated at 37°C for two hours then washed with TBS containing 0.1% Triton x100. For detection, blocking solution (normal rabbit serum diluted 1:5 in TBS, 3%w/vBSA, 0.1% v/v Triton x100) was added for 10 minutes, followed by incubation with rabbit FITC/AP (alkaline phosphatase-conjugated antibody to fluorescein isothiocyanate) for thirty minutes. After washing in TBS and alkaline phosphatase substrate buffer, the slides were incubated overnight with the solution containing 1000 microliters of dilute enzyme substrate and alkaline phosphatase buffer to which was added 1 microliter of levamisole hydrochloride as an inhibitor. Following overnight incubation, slides were washed with water and counterstained with hematoxylin.

With each batch of cases studied, a positive and a negative control slide were also run. The positive control slide was a known case of EBV positive HL to which was added the specific EBER oligonucleotide probe. The negative control slide was another section of the same case of known EBV positive HL to which was added a random probe consisting of a fluorescein labeled oligonucleotide cocktail.

In addition, for each case studied two sections were used; the EBER oligonucleotide probe was added to one section, and the random probe was added to the other. Using this random probe as a negative background control alongside the EBV probe contributes to the validation of staining obtained by the EBV probe. If this negative control slide showed significant background staining in a particular case, the slide having the EBER probe was considered non-interpretable and the test was repeated for that particular case.

Interpretation of results

Two pathologists first reviewed slides independently. Where there was disagreement, the slides were reviewed and a consensus was reached. Using the H&E and immunohistochemical stains, cases were classified into histologic subtypes. Cases were considered unclassifiable if they had ambiguous features or had insufficient biopsy material.[30]

For EBV detection, slides stained by IHC and ISH were reviewed independently to prevent bias. For IHC, cases were labeled as positive for EBV-LMP1 expression if the neoplastic cells were reactive in a membrane, cytoplasmic and/or Golgi staining pattern (*Figures 1 and 2*). For ISH, cells with a black precipitate in the nucleus were identified as positive for the presence of EBER when the negative control slide showed a clean background (*Figures 3 and 4*). EBV expression was considered positive if the neoplastic cells were positive by either method.

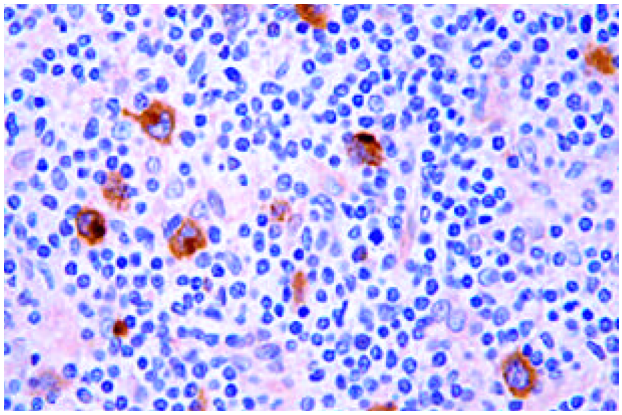

Figure 1. *EBV-LMPI positivity in a Golgi and cytoplasmic pattern in Hodgkin's cells (x400)*

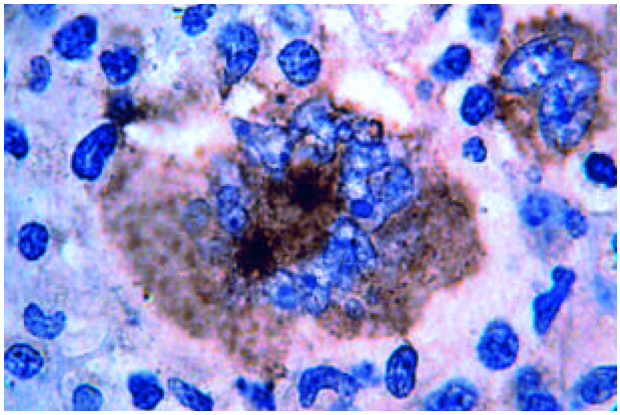

Figure 2. *EBV-LMPI positivity in a multinucleated Hodgkin's cell (xl000)*

Statistical analysis

Statistical analysis of data was performed using Statistical Package for Social Sciences (SPSS) with Chi-square tests, and z-test of proportions. Histologic subtypes were compared with patient age, sex, and nationality (Kuwaiti vs. non-Kuwaiti). EBV expression was compared to the histologic subtypes, as well as to the available demographic data. The two methods used to study EBV expression were also compared. Level of statistical significance was defined as a p-value corresponding to <0.05.

Results

Age, sex, and nationality

Data on age was available for 118/134 patients (88%) and sex for 125/134 patients (93%). The range of patient age was 4 to 71 with an average of 30.6 years. There were 88/125 (70.4%) males and 37/125 (29.6%) females. The male: female ratio was 2.4:1.

Among the 125 patients for whom gender data was found, data on age was available for 83/88 (94%) males and for 35/37 (94.6%) females. The age range for males was 4 to 71 with an average of 30.9 years (±16.9) and for females, the range was 5 to 64 with an average of 30 years (±17.5). Twenty-five (21%) of the 118 cases were children aged 14 years and younger. These included 18 males (72%) and 7 females (28%). The male: female ratio for children was therefore 2.6:1.

Data on nationality was available for 78/134 (58%) cases. Among these there were 30 (38.5%) Kuwaitis and 48 (61.5%) non-Kuwaitis, reflecting the distribution of the general population of Kuwait. Among the Kuwaitis, there were 23 (76.7%) males and 7 (23.3%) females, giving a male:female ratio of 3.3:1. Among the 28 of 30 Kuwaitis for whom age data was available, age range was 4 to 71 years, with a mean of 32 (+ 20.6). Among the non-Kuwaitis, there were 29 males (60.4%) and 19 females (39.6%) giving a male: female ratio of 1.5:1. The age range for the 44 cases where age data was available was 5 to 59 years with a mean of 29.7 (± 12.6). The number of Kuwaiti children was 8 of 30 (28.6%) whereas the number of non-Kuwaiti children was 6 of 44 (13.6%). This difference was not statistically significant.

Data on site of lymph node was available in only 47/134 (35%) cases. The most common site was the cervical region, accounting for 28 (59.6%) cases, followed by inguinal lymph nodes, accounting for 5 (10.6%) cases.

Histopathologic classification

The distribution of histologic subtypes in the 134 cases reviewed is shown in ***Table 1.*** MCHL was the most common (61 cases, 45.5%) followed by NSHL (50 cases, 37.3%). There were 4 (3%) cases of LRCHL, 4 (3%) LDHL, and 9 .(6.7%) NLPHL. 6 cases (4.5%) were unclassifiable.

Histopathologic classification in relation to age, sex and nationality

MCHL was the most common subtype in males, accounting for 44/88 (50%) followed by NSHL (24/88, 27.3%). However, among females, NSHL was the most common (22/37, 54.1%) followed by MCHL (14/37, 37.8%). Using the z-test, the proportion of MCHL was significantly higher in males while the proportion of NSHL was significantly higher in females (p=0.035).

The proportion of MCHL in children was 14/25 (56%) and in adults was 41/93 (44%) whereas NSHL occurred in 6/25 (24%) children and in 35/93 (37.6%) adults. These differences in proportions were not statistically significant.

Twelve of 30 Kuwaitis (40%) had MCHL and 11 (36.7%) NSHL, while of the 48 non-Kuwaitis 21 (44%) had MCHL and 16 (33%) NSHL.

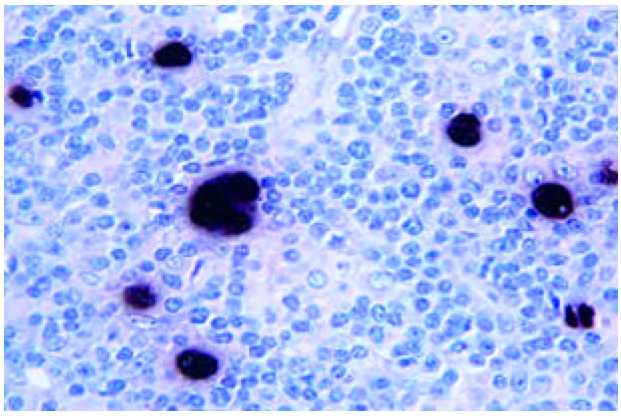

Figure 3. *EBER-1 positivity localizes to the nucleus of Hodgkin's cells (x400)*

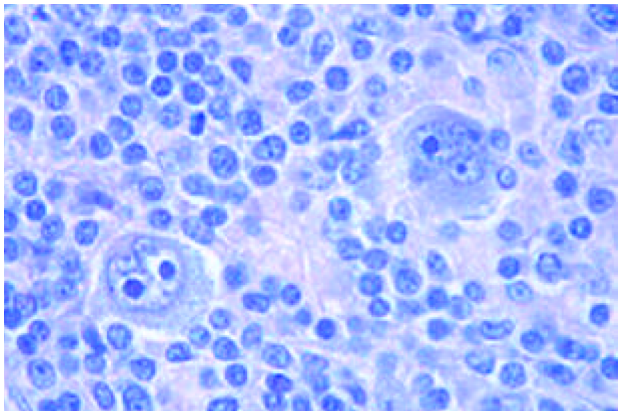

Figure 4. *In situ hybridization using a random oligonucleotide probe for negative control. There is no background staining. (x400)*

EBV expression

EBV expression was studied in 107 cases where sufficient tissue was available and fixation was adequate. Sixty (56.1 %) were positive for EBER by ISH and 56 (52.3%) were positive for EBV-LMP I by IHC. This difference was not statistically significant. None of the cases that were positive by IHC were negative by ISH, whereas 4 cases that were positive by ISH were negative by IHC.

The frequencies of EBV expression in the histological subtypes are summarized in ***Table 1***. EBV was expressed in 38/48 (79%) of MCHL, but in only 13/38 (34%) of NSHL, a statistically significant difference ($p< 0.001$).

The relation of EBV expression to patient age is shown in ***Table 2***. The rate of EBV positivity was significantly higher in children ($p<0.05$). However, the mean age of the EBV negative tumors (34 years) was similar to that of the EBV positive tumors (33 years).

Table 3 shows the relation of EBV expression with gender. There was a higher rate in males than in females, but this was not statistically significant for either MCHL or NSHL. However, in children (***Table 4***), EBV expression was significantly greater in males than females ($p<0.025$)

EBV expression was seen in 12/21 (57%) of HL in Kuwaitis, and in 18/36 (50%) in non-Kuwaitis. Among the children in each group, 4/5 (80%) were EBV positive.

Discussion

Three main epidemiological patterns (I, II, and III) have been described in HL.[7,10,22] In the Type I pattern, there is a relatively high incidence in male children, a low incidence in the third decade and a second peak of high incidence in older

Table 1. **EBV Expression in subtypes of HL in Kuwait**

Histological subtype	*Total cases (% of total)*	*Cases EBV studied*	*EBV positive (%)*
MCHL	61 (45.5)	48	38(79)
NSHL	50 (37.3)	38	13 (34)
LRCHL	4 (3)	4	2 (50)
LDHL	4 (3)	3	3 (100)
NLPHL	9 (6.7)	8	2 (25)
UNUnclass	6 (4.5)	6	2 (33)
Total	134	107	60 (56.1)

MCHL: mixed cellularity Hodgkin's lymphoma; NSHL: nodular sclerosis Hodgkin's lymphomas; LRCHL: lymphocyte rich Hodgkin's lymphoma; LDHL: lymphocyte depletion Hodgkin's lymphoma; NLPHL: nodular lymphocyte predominance Hodgkin's lymphoma; Unclass: unclassifiable

Table 2. **Age Distribution in EBV associated Hodgkin's lymphoma**

Age	*Total cases*	*Cases EBV studied*	*EBV positive (%)*
0-14	25	19	15 (79%)*
15-39	57	43	18 (42%)
40-54	22	19	10 (53%)
> 55	14	11	9 (82%)
Total	118	92	52 (56.5%)

*EBV expression in children (0–14) is significantly higher than in adults (p: <0.05)

Table 3. **Sex Distribution of EBV – Associated Hodgkin's lymphoma**

Sex	*No of patients*	*EBV positive (%)*
Males	70	44 (63)
Females	28	13 (46)
Total	98	57 (58.2)

Table 4. Proportion of EBV Positive Hodgkin's lymphoma in children

Sex	*Total*	*EBV positve (%)*
Males	14	11 (78.6%)*
Females	5	1 (20%)
Total	19	11 (61%)

*EBV expression is significantly higher in male than female children (p<0.025)

age groups. In this pattern, which is prevalent in developing countries, the histological subtypes are those with a less favorable prognosis, usually either MCHL or LDHL.

Type III is the opposite of the type I pattern. It is characterized by a low rate in children and a pronounced initial peak in young adults. This is usually seen in developed countries where the most common histological subtype is NSHL, which has the most favorable prognosis. It is exemplified by the bimodal distribution reported from the USA where the first peak is seen between 15 and 34 years and the second after 50 years.[7,17]

The third pattern known as type II is intermediate and reflects a transition between types I and III. In this pattern there is both a childhood and a second decade peak.[2] This type II pattern is described in many Asian countries, such as Japan,[14] and Taiwan,[16] and Malaysia.[29]

A review of 82 cases of Saudi nationals resident in the Kingdom of Saudi Arabia showed an epidemiological pattern suggestive of type III with two distinct peaks at 18 and 48 years.[23] In contrast, the pattern of age distribution in the present study is most suggestive of a type II transitional epidemiological pattern. No bimodal pattern or peak in any specific age group was seen. Initially, this discrepancy of epidemiological patterns for two adjacent countries with similar ethnic backgrounds was thought to be due to a difference in the populations studied. However, analysis of the Kuwaiti vs. non-Kuwaiti populations separately showed similar age distribution type II patterns in both groups.

Earlier studies on HL in the Middle East from Lebanon, Oman, Egypt, and Kuwait suggested that a significant proportion of HL occurs in children, with a male: female ratio of approximately 2:1.[2,3,8,20] A review of pediatric HL in Kuwait from 1968 to 1981 found that MCHL was the most common subtype (49% of children), followed by NSHL (32%).[20] This distribution is similar to that of the present study where 56% of the children had MCHL, and 24% had NSHL. Although it has been suggested that the distribu-

Table 5. **Correlation of EBV in Hodgkin's lymphoma by geographic location**

Country	*MCHL Pos/total (%)*	*NSHL Pos/total (%)*	*LDHL Pos/total (%)*	*LPHL Pos/total (%)*	*Unclass Pos/total (%)*	*Total totalpos/ total (%)*
Arabian Gulf and Middle East Region						
Kuwait (present study)	38/48 (79)	13/38 (34)	3/3 (100)	2/8 (25)	2/6 (33)	60/107* (56)
Saudi Arabia (Kandil, 2001)	24/48 (50)					
Saudi Arabia/Canada (Mourad, 1998)	8/9 (88)	21/45 (47)		0/8 (0)		29/62 (47)
Southern Israel (Benharroch, 1997)	15/33 (45)	14/64 (22)				29/97 (30)
Type II Epidemiologic pattern: Asia						
Malaysia (Peh, 1996)	27/31 (92)	12/33 (33)	1/2 (50)	0/14 (0)	1/1 (100)	41/8] (51)
Japan (Kusuda, 1998)	12/18 (67)	5/14 (36)	5/7 (71)	4/6 (67)	0	26/45 (58)
Taiwan (Liu, 1998)	18/26 (69)	23/36 (64)	2/2 (100)	0/1 (0)	0	43/65 (66)
Type III Epidemiologic pattern: North America, Western Europe						
USA (Weiss, 1991)	12/13 (92)	17/30 (57)	1/1 (100)	0/1 (0)	0/1 (0)	30/46 (65)
UK (Khan, 1993)	15/22 (68)	9/38 (24)	1/7 (14)	0/10 (0)	0	25/77 (32)
Sweden (Enblad, 1999)	8/23 (38)	20/87 (23)	3/3 (100)	1/5 (20)	0	32/118 (27)
Type I Epidemiologic pattern: Africa and South America						
Peru (Chang, 1993)	20/20 (100)	6/7 (86)	2/2 (100)	1/1 (100)	0	29/30 (96)
Kenya (Kusuda, 1998)	28/33 (85)	4/7 (57)	4/5 (80)	2/3 (67)	0	38/48 (79)
Cumulative total	201/276 (72.8)	168/447 (37.6)	22/32 (68.8)	10/57 (17.5)	3/8 (37.5)	382/776 (49)

MCHL: mixed cellularity Hodgkin's lymphoma; NSHL: nodular sclerosis Hodgkin's lymphomas; LRCHL: lymphocyte rich Hodgkin's lymphoma; LDHL: lymphocyte depletion Hodgkin's lymphoma; NLPHL: nodular lymphocyte predominance; Hodgkin's lymphoma; uncclass: unclassifiable. *Total includes 4 cases of LRCHL

tion of HL subtypes has changed post-1985 in the US and Canada,[10] the pattern of HL in Kuwait does not appear to have changed significantly over the last 30 years, at least in children.

The few reports from the Middle East, with two exceptions, show that MCHL is the most common subtype of HL[1,3,8,20,23] The two exceptions in which NSHL was the most common were one study which combined cases from Saudi Arabia and Canada[21] and another study from Southern Israel.[4] Further studies are needed to delineate the epidemiological pattern of HL in the Persian Gulf region.

In addition to the wide geographic variation in epidemiological patterns and subtypes of HL, there are also large epidemiological differences in the frequency and pattern of EBV expression in HL. The most comprehensive report of this association was a meta-analysis of more than 1500 cases from several countries.[9] This included data from Asia, Europe, North and South America. The study showed that the prevalence of EBV-positive HL ranged widely from a maximum of 87.5% in Saudi Arabia (n=8) to a minimum of 30.8% in the United Kingdom (n=394). Other and subsequent studies have reported frequencies of EBV expression in HL ranging from 27% in Sweden[6] to 94% in Peru.[5]

The frequency of EBV expression appears to parallel the epidemiological pattern of HL in these different geographic areas (*Table 5*). For example, in Sweden, which has a type III pattern, the proportion of EBV positive cases is quite low at only 27%.[6] On the other hand, in Peru[5] and in Kenya,[14,15] that have type I epidemiologic patterns, the frequency of EBV expression in HL is more than 90%. In Asian countries with a type II pattern, EBV is expressed in 52% to 62.9% of cases.[14,16,29]

However, the limited reports from the Middle East are unusual in that the observed epidemiologic patterns did not match the frequency of EBV expression. For example, in Israel, where the reported age distribution is intermediate between the bimodal pattern of Western countries and the pediatric peak seen in developing countries, EBV expression was only 30%.[4] Interestingly, the small number of Bedouin patients in Israel showed a significantly higher rate of EBV expression at 66.7%.[4] In Saudi Arabia, on the other hand, where the bimodal age distribution is suggestive of a type III epidemiologic pattern[23], EBV expression was seen in as many as 50% of NSHL,[11] a frequency close to the 56.1 % obtained in this study and to figures reported from regions with a type II pattern.[14,16,21] (Please see *Table 5*).

In conclusion, the epidemiology of HL in Kuwait is suggestive of a type II epidemiologic pattern in terms of age distribution, histopathologic subtypes and frequency of EBV expression. This pattern is seen in both the native Kuwaiti population and the large non-Kuwaiti resident expatriate population. A significant proportion of cases are EBV-related and the frequency of EBV expression is significantly higher in MCHL, in children, and in males. It is important to assess all cases of HL for EBV status, since its presence may have a significant impact on prognosis and response to therapy.[19,24] Further studies, particularly from the Persian Gulf and the Middle East, are needed to determine the actual epidemiological pattern of HL in the region and to define the role of EBV in HL. These future studies should also recognize the possibility of different disease patterns between the native Kuwaiti people and expatriate settlers and laborers.

Acknowledgement

Mr. Edison – Dept. of Community Medicine and Biostatistics for assistance with statistical analysis.

References

1. *A1-Idrissi HY, Ibrahim EM:* Hodgkin's disease in adults in Saudi Arabia. Clinical features, prognostic factors and an analysis of therapy. Outcome of combination chemotherapy only for both localized and advanced disease. Int J Cancer 47: 822-826, 1991.
2. *Azzam SA:* High Incidence of Hodgkin's disease in children in Lebanon. Cancer Research 26: 1202-1203, 1966.
3. *Bamanikar S, Thunold S, Devi KR, Bamanikar A:* The pattern of malignant lymphoma in Oman. J Trop Med Hyg 98: 351-354, 1995.
4. *Benharroch D, Brousset P, Goldstein J, et al:* Association of the Epstein-Barr virus with Hodgkin's disease in Southern Israel. Int J Cancer 71: 138-141, 1997.
5. *Chang KL, Albujar PF, Chen YY, Johnson RM, Weiss LM:* High prevalence of Epstein-Barr virus in the Reed-Sternberg cells of Hodgkin's disease occurring in Peru. Blood 81: 496-501, 1993.
6. *Enblad G, Sandvej K, Sundstrom C, et al:* Epstein-Barr virus distribution in Hodgkin's disease in an unselected Swedish population. Acta Oncologica 38: 425-429, 1999.
7. *Flavell KJ, Murray PG.* Hodgkin's disease and the Epstein-Barr virus: J Clin Pathol: Mol Pathol 53: 262-269, 2000.
8. *Gad-El-Mawla N, El-Deeb BB, Abu-Gabal A, et al:* Pediatric Hodgkin's Disease in Egypt. Cancer 52: 1129-1131, 1983.
9. *Glaser SL, Lin RJ, Stewart SL, et al:* Epstein-Barr virus-associated Hodgkin's disease: epidemiologic characteristics in international data. Int J of Cancer 70: 375-382, 1997
10. *Harris NL:* The many faces of Hodgkin's disease around the world: what have we learned from its pathology. Annals of Oncology 9(suppl5): S45-S56, 1998.
11. *Kandil A, Bazarbashi S, Mourad W:* The correlation of Epstein-Barr virus expression and lymphocyte subsets with the clinical presentation of nodular sclerosing Hodgkin disease. Cancer 91: 1957-1963, 2001.
12. *Kawa K:* Epstein-Barr virus – associated diseases in humans. Int J Hematol 71: 108-117, 2000.
13. *Khan G, Norton AJ, Slavin G:* Epstein-Barr virus in Hodgkin Disease. Relation to age and subtype. Cancer 71: 3124-3129, 1993.
14. *Kusuda M, Toriyama K, Kamidigo NO, et al:* A Comparison of epidemiologic, histologic and virologic studies on Hodgkin's disease in Western Kenya and Nagasaki, Japan. Am J Trop Med Hyg 59: 801-807, 1998.

15. *Leoncini L, Spina D, Nyong A, et al:* Neoplastic cells of Hodgkin's disease show differences in EBV expression between Kenya and Italy. Int J Cancer 65: 781-784, 1996.
16. *Liu SM, Chow KC, Chiu CF, et al:* Expression of Epstein-Barr virus in patients with Hodgkin's disease in Taiwan. Cancer 83: 367-71, 1998.
17. *MacMahon B:* Epidemiology of Hodgkin's disease. Cancer Research 26: 1189-1200, 1966.
18. *Marafioti T, Hummel M, Foss HD, et al:* Hodgkin and Reed-Sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. Blood 95: 1443-1450, 2000.
19. *Morente MM, Piris MA, Abraira V, et al:* Adverse clincial outcome in Hodgkin's Disease is associated with loss of retinoblastoma protein expression, high Ki67 proliferation index, and absence of Epstein-Barr virus latent membrane protein 1 expression. Blood 90:2429-2436, 1997.
20. *Motawy MS, Omar YT:* Hodgkin's disease in children in Kuwait. Cancer 57:2255-2259, 1986.
21. *Mourad W, Alsohaibani MO, Saddik M, et al:* Epsztein-Barr Virus Expression in Hodgkin's disease: correlation with histologic subtypes and T and B lymphocyte distribution. Ann Saudi Med 18: 296-300, 1998.
22. *Mueller NE, Grufferman S:* The Epidemiology of Hodgkin's disease. In: Mauch PM, Armitage JD, Diehl V et al. Hodgkin's Diseases, pp. 61-77. Philadelphia, USA: Lippincott Williams & Wilkins, 1999.
23. *Mughal TI, Robinson WA, Padmos MA:* Adult Hodgkin's disease in Saudi Arabia. Eur J Surg Oncol 11:41-45, 1985.
24. *Murray PG, Billingham LJ, Hassan HT, et al:* Effect of Epstein-Barr virus Infection on Response to Chemotherapy and Survival in Hodgkin's disease. Blood 94: 442-447, 1999.
25. *Niedobitek G, Meru N, Delecluse HJ:* Epstein-Barr virus infection and human malignancies. Int J Exp Pathol 82: 149-170, 2001.
26. *Okano M:* Epstein-Barr virus infection and its role in the expanding spectrum of human diseases. Acta Paediatr 87: 11-18, 1998.
27. *Oudejans JJ, Jiwa NM, Meijer CJ. Editorial:* Epstein-Barr virus in Hodgkin's disease: more than just an innocent bystander. J Pathol 181: 353-356, 1997.
28. *Parkin DM, Whelan SL, Ferlay J, et al.* Cancer Incidence in Five Continents Vol VII. International Agency for Research on Cancer (IARC) Press, Lyon, 1997.
29. *Peh SC, Looi LM, Pallesen G:* Epstein-Barr virus (EBV) and Hodgkin's disease in a multi-ethnic population in Malaysia. Histopathology 30: 227-233, 1997
30. *Pileri SA, Ascani S, Leoncini L, et al.* Hodgkin's lymphoma: the pathologist's viewpoint: J Clin Pathol 55: 162-176, 2002.
31. *Stein H, Delsol G, Pileri S, et al:* Hodgkin Lymphoma. In: Jaffe ES, Harris NL, Stain H, Vardiman JW. Pathology and genetics of tumors of haematopoietic and lymphoid tissues. International Agency for Research on Cancer (IARC) Press, Lyon, 2001. pp. 23 7-253