

## RESEARCH COMMUNICATION

# Hodgkin Lymphoma in Pakistan: An Analysis of Subtypes and their Correlation with Epstein Barr Virus

Samia Fatima\*, Rashida Ahmed, Arsalan Ahmed

### Abstract

The epidemiology of Hodgkin lymphoma (HL) shows a wide geographic variation with regard to age, gender, histological subtypes and their association with Epstein-Barr virus. The proportion of EBV positive cases appears higher in developing than in developed countries. EBV is a common infection in Pakistan due to poor socioeconomic conditions, but reports regarding HL subtypes have been rather selective. Our aims were to establish the relative frequencies of the five subtypes of Hodgkin lymphoma, to determine their associations with Epstein-Barr virus, and finally to establish whether such association follows patterns seen in developing or developed countries. Among 100 cases, the male: female ratio was 4.5:1, with an age range of 4-82 years and an average of 26.6 years. Similar to the subtype distribution in developing countries, mixed cellularity was the commonest 57%, followed by nodular sclerosis 35%, lymphocyte rich 6% and nodular lymphocyte predominant 2%. EBV-LMP1 staining was demonstrated in 41/57 (71%) of the mixed cellularity and the 19/35 (54.2%) of nodular sclerosis subtypes. All 6 cases of lymphocyte rich and 2 cases of nodular lymphocyte predominant were negative for EBV-LMP1. Speculation about prognostic effects of EBV infection on the course of HL are tempting. Thus the EBV-positive HL could in the future prove to be an excellent candidate for targeted cellular immunotherapy.

**Keywords:** Hodgkin lymphoma - Epstein-Barr virus - latent membrane protein-1 (LMP-1)

*Asian Pacific J Cancer Prev*, 12, 1385-1388

### Introduction

Hodgkin lymphoma (HL), encompasses a group of lymphoid neoplasms characterized morphologically by the presence of distinctive cells called Reed Sternberg (RS) cells and/or its variants that induce the accumulation of reactive lymphocytes, histiocytes (macrophages) and granulocytes (Kumar et al., 2004; Makar et al., 2003; Rosai et al., 2004). Epstein - Barr virus (EBV) a ubiquitous human herpes virus is now associated with a growing number of human malignancies including Hodgkin's lymphoma (Makar et al., 2003; Zhou et al., 2001). This association of EBV with HL may be causal (Jarrett et al., 2003).

The epidemiology of HL shows a wide geographic variation with regard to age, gender, histological subtypes and their association with EBV (Jarrett et al., 2003). In the West the most frequent subtype of HL is nodular sclerosis (NS) (60-75%), followed by mixed cellularity (MC) (20-25%), lymphocyte rich (LR) (rare), lymphocyte dominant (LD) (5%) and nodular lymphocyte predominant (NLP) (5%). (Akram et al., 2001). EBV can be detected in 40-50% of the cases of HL and this association is stronger in cases of MC as compared to NS (Zhou et al., 2001).

The proportion of EBV positive HL is high in developing countries as compared to developed countries.

EBV is a common infection in Pakistan due to poor socioeconomic conditions (Chang et al., 2005). However, reports regarding its subtypes have been rather selective, that is only clinical features and distribution among children have been studied (Akram et al., 2001; Khan et al., 2005).

EBV has been implicated in the pathogenesis of HL in international studies. (Khan et al., 2005) Local literature regarding the epidemiology of HL is very limited. Hence in our study we aim to establish the distribution of subtypes of HL and their association with EBV. Our aims were to find out the relative frequencies of the five subtypes of Hodgkin's lymphoma, to determine their association with Epstein-Barr virus, and finally to establish whether such association follows patterns seen in developing or developed countries. The demographics of these patients in terms of age, gender, and site of presentation will also be elucidated.

### Materials and Methods

A total of 100 consecutive cases of HL from 2007-2008 were retrieved and reviewed from the pathology files at the Section of Histopathology, Aga Khan University Hospital. Specimens not received in 10% buffered formalin, cases with incomplete clinical data and cases on which

*Section of Histopathology, Department of Pathology and Microbiology, The Aga Khan University Hospital, Karachi, Pakistan \*For correspondence: samia\_fatima90@yahoo.com*

the histological subtype could not be determined were excluded from the study.

For each case one representative section was selected and immunohistochemical analysis was performed using a panel of antibodies for CD 30, CD15, CD20, LCA, EMA, CD3, ALK protein and EBV LMP1 (DAKO, Denmark). Membranous and cytoplasmic staining was regarded as positive for all of these immunohistochemical stains [Figure 1 (b)]. Diagnosis of Classic Hodgkin lymphoma (CHL) was given for those cases in which typical RS cells were present in a mixed background comprising of lymphocytes, eosinophils and plasma cells [Figure 1 (a)]. These cases demonstrated a positivity of CD15 and CD30 in the RS cells. A diagnosis of NLPHL was given in cases which showed a nodular architecture, L& H cells which demonstrated positivity for LCA and CD20.

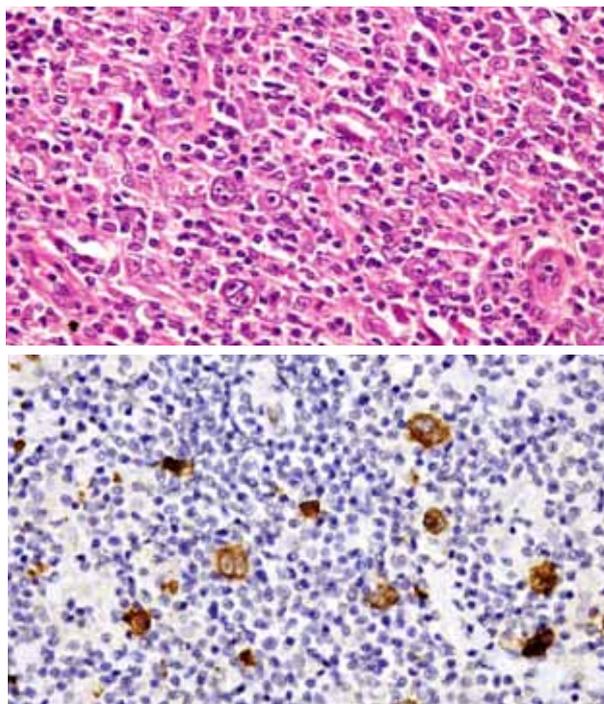
This immunohistochemical staining was performed on deparaffinized tissue sections of formalin-fixed material after microwave-enhanced epitope retrieval. Detection was done with streptavidin-biotinylated peroxidase-conjugated reagents, with 3- amino-9-ethyl carbazole as the chromogen and hematoxylin for counterstain.

Data was analyzed using SPSS version 17. Mean and standard deviation were computed for qualitative variables like age. Frequencies and percentages were calculated for categorical variables like gender, site, and subtypes of Hodgkin's lymphoma. Chi-square test was used to see the association of histological subtype of HL with respect gender and age. P value of 0.05 was considered level of significance.

## Results

### Age and gender

The ages of patients ranged from 4 to 82 years with an average age of 26.6 years. There were 82 males and



**Figure 1. a) HL with Reed-Sternberg Cells (H&E 20X); b) Reed-Sternberg Cells Positive for EBV-LMP1 (40X)**

18 females. The male to female ratio was 4.5:1. The age range for males was 4-82 years with an average age of 25.62 years and standard deviation of  $\pm 18$ . For females the age range was 10 to 70 years with an average age of 33.83 years and standard deviation of  $\pm 17$ . Of the 100 cases 31 were children under 15 years and younger. These included 29 males (93.3%) and 2 females (6.7%). The male to female ratio for children was 4.6:1.

### Site

Data on the site of lymph node involved was also available for all 100 cases. The most common site was the cervical region (73/100), followed by axillary (12/100), inguinal (11/100), mesenteric (2/100) and mediastinal (2/100).

### Histopathological classification

MC was the most common (57), followed by NS (35), LR (6) and NLP (2). There were no cases of LD. MC was the most common subtype in males, accounting for 66/82 (80%), followed by NS 27/82 (32%), LR 6/82 (7.3%) and NLP 1/82 (1.23%). Among the females MC was again the most common 9/18 (50%), followed by NS 8/18 (44.4%) and NLP 1/18 (5.5%). This difference in proportion was not significant, p value 0.32. The proportion of MC in children was 19/31 (61.2%) and in adults was 39/69 (56.5%) whereas NS occurred in 9/31 (29%) children and in 27/69 (39.1%) in adults. These differences in proportion were not significant by using chi square test, p value was 0.13.

### EBV expression

60 cases showed positivity for EBV-LMP1. The strongest association was with MCHL followed by NSHL. EBV-LMP1 staining was demonstrated in 41/57 (71%) of MCHL and 19/35 (54.2%) of NSHL. All 6 cases of NLPHL were negative for EBV-LMP1. In children, 29/31 (87.1%) of the cases showed EBV positivity. In adults 34/69 (49.3%) were EBV positive.

## Discussion

EBV infects the epithelial cells of the oropharynx and B lymphocytes. It gains entry into the B cells via the CD21 molecule which is present on all B cells. Within the B cell the linear genome of EBV circularizes to form an episome in the cell nucleus. The infection of B cells is latent. Several viral genes dysregulate the normal proliferative and survival signals in latently infected cells. The latent membrane protein-1 (LMP-1) binds to and activates a signaling molecule that is normally activated by the CD 40 receptors in the B cells. This receptor is the key recipient of the helper T-cell signals, which are normally responsible for full B cell responses. Mimicking CD40, LMP1 activates the NFkB and JAK/STAT signaling pathways and promotes B cell survival and proliferation, all of which are helper T cell induced responses that occur in the absence of T cells in EBV infected B cells. Thus the virus efficiently co-opts a normal pathway of B cell activation in order to increase the number of B cells it can infect and inhabit. The EBV encoded EBNA-2 gene

transactivates several host genes, including CYCLIN D and members of the SRC family promoting the transition of resting B cells from G0 to G1. EBNA-2 also activates the transcription of LMP-1 and is a key regulator of viral gene expression. Thus, several viral genes collaborate to render B cells immortal (Kumar et al., 2004)

In latent EBV infection, 11 genes are expressed, encoding two small nonpolyadenylated RNAs (EBER-1, EBER-2), six nuclear proteins (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP) and three integral latent membrane proteins (LMP-1, LMP-2A, LMP-2B). In contrast to transplant associated lymphomas where the whole spectrum of these genes is expressed (latency type III) and the restricted expression pattern observed in Burkitt's lymphoma (latency type I: EBNA-1, EBER-1, EBER-2), an intermediate latency pattern is seen in HL. Here, LMPs, EBERs and EBNA-1 are detectable (Claviez et al., 2005).

It has been known for some time that there is diversity in the incidence, age, and sex distribution and morphology of HL in different populations (Jaffe et al., 2001). A definite bimodal age peak, absent in most lymphomas, is present in the incidence of HL (Rosai et al., 2004). In developing countries it is reported to occur in children, with a male preponderance and a higher proportion of MCHL subtype whereas in developed countries it occurs in young adults; the second peak occurs in late adulthood in both developed and developing countries. NSHL, the predominant histological subtype in the United States and Western Europe is generally much less common in developing countries (Khan et al., 1993; Matteo et al., 2003)

Our study demonstrates a male predominance with MCHL being the commonest subtype. Hence it indicates that Pakistan, being a developing country shows similar epidemiological features as other developing countries however these features are in contrast to the developed nations.

Several studies using different methods have confirmed EBV association with certain subtypes of HL. The results of such studies have indicated that the frequency of EBV association with HL is related to the subtype of HL, the age of the patients, and the geographic location i.e. developed versus developing countries. MCHL has the highest rate of EBV association, whereas the NPHL has the lowest rate of EBV association. As for age, most studies have found higher rates of EBV presence among childhood HL as opposed to adult onset HL (Lukes et al., 1996). While in the developing countries with low socioeconomic status, 70-100% of HL is EBV associated; only 30-50% of HL in the developed countries shows such an association. In the oriental population from China and Hong Kong, Taiwan and Japan the association is intermediate (60 to 65%) (Naresh et al., 2000; Dinand et al., 2006).

Analogous to the previous observations, in the current study EBV association was noted among 71% of MCHL and 54% of NSHL of classic HL. There was no association with any of the 6 cases of LRHL subtype and neither with the 2 cases of LPHL. The overall EBV association with classic HL was 60%. Children of 15 years and below

showed an overall positivity of 87% while in adults it was 49.27%. A similar study from a South Indian hospital also demonstrated a 96% positivity of EBV-LMP1 in children (Karnik et al., 2003).

The stronger association of EBV with HL in children might be related to environmental factors and the patients' lowered immune status (Zhou et al., 2001).

In developing countries EBV might be acquired at an earlier age, and this compounded by the lower socioeconomic status might play a role in altering the immune status of the EBV infected children. Thus age, ethnicity and physiological effects of poverty may function as biological modifiers that could account for EBV association in HL (Naresh et al., 2000).

Although the association of HL and EBV is now established, a potentially more important question is whether the presence of EBV within the RS cells influences outcome for patients with HL (Murray et al., 2005)

Studies investigating the impact of latent EBV infection on outcome of patients with HL have demonstrated conflicting results. While EBV infection was reported to be a favorable prognostic parameter in nine studies, two studies observed the opposite effect, and in nine studies no significant statistical difference was observed. In the majority of the studies the expression of LMP-1 was studied for the demonstration of latent EBV infection. (Claviez et al., 2005). In the study by Claviez et al, EBV did not emerge as an independent risk factor for failure-free survival. LMP1 positivity, however, was associated with a poorer overall survival in a subgroup of patients with higher risks for treatment failure.

On theoretical grounds, there are two opposing biological effects that could impact on prognosis. Firstly, by virtue of its well documented effects promoting cell growth in vitro, the expression of LMP 1 in the malignant cells of HL might be expected to exert a negative effect on survival for EBV positive patients. On the other hand, cytotoxic T lymphocytes (CTL) responses to LMP1 and LMP2, proteins that are consistently expressed by EBV-positive RS cells, have been demonstrated in the blood of EBV-seropositive individuals. Furthermore, LMP2-specific CTLs have been identified and expanded from the peripheral blood of HL patients, suggesting that the expression of LMP2 by the RS cells might stimulate an antineoplastic CTL response (Matteo et al., 2003)

Morente et al., (1997), reported a significant association between the detection of LMP 1 in RS cells and longer survival in a series of 140 HL patients.

The finding that EBV-positive patients were more likely to achieve a complete response to chemotherapy than were EBV-negative patients, suggest that the malignant cells of EBV-positive HL are likely to be more sensitive to the chemotherapy agents or that residual EBV-positive cells after cytoreduction might be targets for immune cytotoxicity. (Murray et al., 1999)

EBV is likely to confer higher proliferating cell nuclear antigen (PCNA) expression and also contribute towards maintaining the RS cells of classical HL in cell cycle. Hence, RS cells in EBV associated HL would be more responsive to chemotherapy and radiotherapy

associated DNA damage. Thus, EBV-association provides survival advantage to HL patients treated with standard chemotherapy and radiotherapy protocols. (Naresh et al., 2000)

Since there are controversial studies regarding the prognostic effect of EBV on chemotherapy and radiotherapy, it is paramount that EBV positive and EBV negative patients be investigated for response to therapy to establish the importance of EBV association in HL in our population.

Speculations about a prognostic effect of EBV infection on the course of HL are tempting. To prove either a possible adverse effect by the oncogenic capacity of LMP-1 or a putative protective effect due to elucidation of a specific immune response of EBV infection, large series of patients with comparable clinical criteria and standardized therapeutic regimens need to be looked at in our population. Thus EBV-positive HL could, in the future prove to be an excellent candidate for targeted cellular immunotherapy.

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