ORIGINAL ARTICLE

# FREQUENCY OF EPSTEIN-BARR VIRUS IN CLASSICAL HODGKIN LYMPHOMA

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**Background:** Epstein-Barr virus plays an important role in pathogenesis of Hodgkin lymphoma. The first patient with Epstein-Barr positive Reed Sternberg cells was described in 1985. Since then association between Epstein-Barr virus and Hodgkin lymphoma has been shown in many parts of the world and its occurrence shows significant variation from continent to continent and from country to country. **Method:** The study was carried out at department of histopathology, Armed Forces Institute of Pathology from 27th April 2013 to 10th March 2014. A total of 55 cases of classical Hodgkin lymphoma were included in the study. **Results:** Out of 55 patients, 38 (69%) were male and 17 (31%) were female. The age of the patients ranged between 4–67 years with an average age of 29.4±21.72 years. Out of these, 44 cases (80%) were positive for latent membrane protein-1. Among positive cases 32 (72.72%) were male and 12 (27.28%) were female. Based upon histological subtypes MCHL was the commonest as a whole accounting for 87.3% as well as among both genders. Out of total 55 cases, 79.16% (38/48) of mixed cellularity Hodgkin lymphoma cases showed positivity for latent membrane protein-1 while 83.33% (5/6) cases of nodular sclerosis Hodgkin lymphoma and 100% (1/1) cases of lymphocyte depleted Hodgkin lymphoma showed positivity. No case of lymphocyte predominant classical Hodgkin lymphoma was diagnosed during the study. 80% of our classical Hodgkin lymphoma cases showed association with EBV expression. A total of 79.16% cases of mixed cellularity Hodgkin lymphoma showed LMP1 expression while 100% of lymphocyte depleted Hodgkin lymphoma showed LMP1 expression. **Conclusion:** The highest expression seen in lymphocyte depleted Hodgkin lymphoma subtype in contrast to mixed cellularity requires to be confirmed by a larger scale study comprising of substantial number of patients of lymphocyte depleted Hodgkin lymphoma and lymphocyte rich classical Hodgkin lymphoma.

**Keywords:** Hodgkin lymphoma, EBV expression, LMP1.

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## INTRODUCTION

Hodgkin lymphoma, previously known as Hodgkin's disease, comprises of a group of malignant lymphoid neoplasms of B-cell lineage that differ from Non-Hodgkin lymphoma in several aspects. It is characterized by scattered large malignant cells known as Reed-Sternberg cells (typical and variants) admixed with reactive infiltrate comprising of variable proportions of lymphocytes, histiocytes, eosinophils and plasma cells. Thomas Hodgkin was the first to mention the anatomic description to Hodgkin lymphoma in his article "On some morbid appearances of absorbent glands and spleen" in 1832. Later, Sternberg in 1898 and Reed in 1902 described multinucleated giant cells, a pathognomonic finding of this disease, which are now known as Reed-Sternberg cells. The Reed-Sternberg cells are large often binucleate cells with prominent nucleoli and an unusual CD45-, CD30+ and CD15± immunophenotype.[1]

Epstein-Barr virus is a widespread tumorigenic virus of herpes family. It is named after Michael Epstein and Yvonne Barr, who discovered the virus in 1964.[2] Epstein-Barr virus is associated with a number of lymphoproliferative and other disorders including nasopharyngeal carcinoma, Burkitt lymphoma, post-transplantation lymphoproliferative disease (PTLD), Angioimmunoblastic T-cell lymphomas (AITL), natural killer (NK) cell lymphoma, diffuse large B-cell lymphomas and Hodgkin lymphoma.[3]

While the exact role of EBV in pathogenesis of each type of lymphoma still needs to be elucidated, epidemiologic studies have shown strong associations between Epstein-Barr virus and Hodgkin lymphoma. Following natural infection by Epstein-Barr virus, numerous virus proteins are produced which include Epstein-Barr nuclear antigen-1 (EBNA-1), EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-leader protein (EBNA-LP), latent membrane protein (LMP) -1, LMP-2A, LMP-2B and Epstein-Barr encoded RNAs (EBERs). LMP-1 is one of the proteins that plays a role in transformation mechanism that allows RS-cells to survive from apoptosis. EBNA-1 protein is essential for maintenance of viral genome.[4]

The expression of Epstein-Barr virus in Hodgkin lymphoma can be identified by immune-histochemical detection of Epstein-Barr virus latent

membrane protein (LMP).[5,6] The first patient with Epstein-Barr positive RS cells was described in 1985.[7] Since then association between Epstein-Barr virus and Hodgkin lymphoma has been shown in many parts of the world and its occurrence shows significant variation from continent to continent and from country to country.[5] In a study by Katebi *et al* in Iran 93% of the cases of Hodgkin lymphoma studied were EBV-positive.[8] It ranges from 30% in Swedish patients to 100% in patients from Kenya.[5]

Not much studies have been conducted to establish the frequency of Epstein-Barr virus in patients of classical Hodgkin lymphoma in Pakistani population. The significance of this correlation between Epstein-Barr virus and Hodgkin lymphoma has been highlighted in various studies proving that the presence of Epstein-Barr virus has poor disease specific survival[9] and worse prognosis.[10] Since the tumour cells of Hodgkin lymphoma do not express immunoglobulin gene and those B-cells that lack functional immunoglobulins are destined to die through apoptosis. When there is EBV infection, Latent membrane protein (LMP) provides a tonic signal that mimics with immunoglobulin expression thus preventing apoptosis of these cells. The role of this viral protein in preventing apoptosis in tumour cells suggests the possibility that residual EBV-positive tumour cells after cytoreduction by chemotherapy could be target for immune cytolysis. The rationale of our study is to ascertain the frequency of Epstein-Barr virus in patients of classical Hodgkin lymphoma in local population. This has implications for the potential development of specific immunotherapy thus rendering improved patient care.

## MATERIAL AND METHODS

A total 55 patients of classical Hodgkin lymphoma were included through purposive sampling, which were diagnosed from 27th April 2013 to 10th March 2014. All newly diagnosed cases of classical Hodgkin lymphoma by histological examination and immunohistochemistry at Armed Forces Institute of Pathology, Rawalpindi were included in this study. Cases of Hodgkin lymphoma having received treatment were excluded. All the samples were received in 10% formal saline. For each case one representative section was selected and immune-histochemical analysis was performed using a panel of antibodies for LCA, CD3, CD20, CD15, CD30 and EBV LMP1. Membranous and cytoplasmic staining was taken as positive for all these immune-histochemical markers. Diagnosis of Classic Hodgkin lymphoma (CHL) was based on the presence of typical RS cells lying in a mixed background comprising of lymphocytes, plasma cells and eosinophils. This immune-histochemical 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 haematoxylin for counterstain.

Statistical analysis was done using SPSS version 19.0. Descriptive statistics were calculated for both quantitative and qualitative variables. Mean and SD were calculated for quantitative data like patient's age. EBV frequencies and percentages were calculated for qualitative variables like gender.

## RESULTS

During the study a total of 55 cases of classical Hodgkin lymphoma were included from 27th April 2013 to 10th March 2014.

The ages of patients ranged from 4 to 67 years with mean age of 29.4 years and standard deviation of ±21.72. There were 38 males and 17 females. The male to female ratio was 2.2:1. The age range for males was 4 to 67 years with a mean age of 27.4 years and standard deviation of ±21.70. For females the age range was 6–65 years with a mean age of 31.3 years and standard deviation of ±22.35. Of the 55 cases 23 were children under 15 years of age. These included 18 males (78.26%) and 5 females (21.74%). The male to female ratio for children was 3.6:1. In our study two peaks were observed, first in the first decade of life (31%, n=17/55) and second in late adulthood during sixth decade (18%, n=10/55).

Data on the site of lymph node involved was also available for 48 cases. The most common site was the cervical region 65.5% (36/55), followed by inguinal 9.1% (5/55), axillary 7.3% (4/55), and 1 case each of epitrochlear, mediastinal and para-aortic lymph nodes. MCHL was the most common type comprising of 87.3% (48/55), followed by NSHL 10.9% (6/55), LDHL 1.8% (1/55). There was no case of LRHL. MCHL was the most common subtype in males, accounting for 86.84% (33/38), followed by NSHL 10.52% (4/38), LDHL 2.63% (1/38). Among the females MCHL was again the most common 88.24% (15/17) followed by NSHL 11.76% (2/17). There was no case of LDHL or LPHL type among the females.

Forty-four (80%) out of 55 cases were positive for latent membrane protein-1. Among positive cases 32 (72.72%) were male and 12 (27.28%) were female. That means out of 38 males included in the study 32 (84.21%) were EBV-LMP1 positive and out of 17 females included in the study 12 (70.58%) were positive for EBV-LMP1. Out of total 55 cases, 79.16% (38/48) of mixed cellularity Hodgkin lymphoma cases showed positivity for latent membrane protein-1 while 83.33% (5/6) cases of

nodular sclerosis Hodgkin lymphoma and 100% (1/1) cases of lymphocyte depleted Hodgkin lymphoma showed positivity. No case of lymphocyte predominant classical Hodgkin lymphoma was diagnosed during the study period. In children, 91.30% (21/23) of the cases showed EBV-LMP1 positivity. In adults 71.87% (23/32) of the cases were EBV-LMP1 positive.

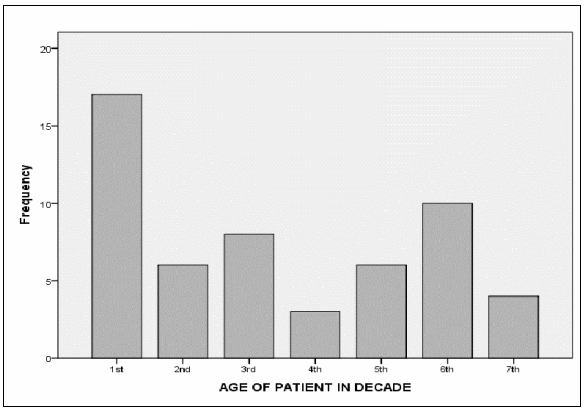


**Figure-1: Distribution of Hodgkin Lymphoma cases according to age groups**

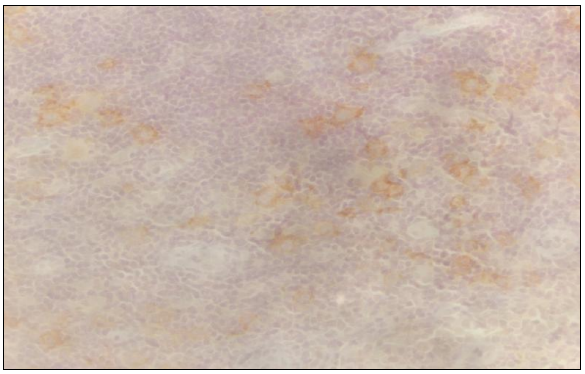

**Figure-2: Photomicrograph showing LMP1 reactivity in RS cells**

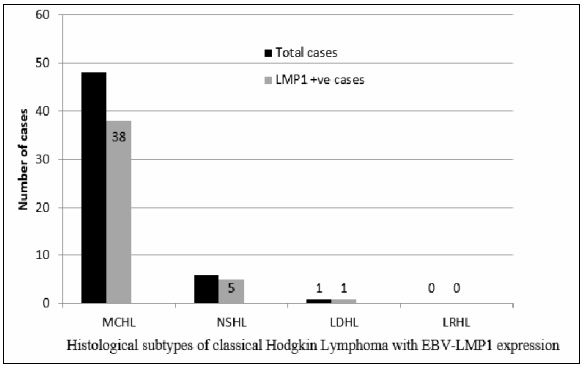


**Figure-3: MCHL=Mixed cellularity Hodgkin Lymphoma, NSHL=Nodular sclerosis Hodgkin lymphoma, LDHL=Lymphocyte depleted Hodgkin Lymphoma, LRHL=Lymphocyte rich Hodgkin lymphoma**

## DISCUSSION

EBV infects epithelial cells of oropharynx and B lymphocytes. It enters into the B cells with the help of CD21 which is present on all B cells. Within B cells linear genome of EBV circularizes to form episome within the cell nucleus. The infection of B cells is latent. Viral genes cause dysregulation of normal proliferative and survival signals in latently infected cells. The latent membrane protein-1 triggers a signalling molecule that is usually activated by the CD 40 receptor in B cells. This receptor plays a key role in helper T-cell signals, which are responsible for B cell responses. LMP1, like CD40, activates the NF-kB and JAK/STAT signalling pathways thus promoting the survival and proliferation of B cell.

In latent EBV infection, 11 genes are expressed, which encode two RNAs (EBER- 1, EBER-2), six nuclear proteins (EBNA-1, EBNA-2, EBNA-3A, EBNA-3B, EBNA-3C, EBNA-LP) and three integral proteins (LMP-1, LMP-2A, LMP-2B). In transplant associated lymphomas the whole range of these genes is expressed (latency type III) while the controlled expression is seen in Burkitt's lymphoma (latency type I: EBNA-1, EBER-1, EBER-2). An intermediate latency pattern is seen in Hodgkin's lymphoma where LMPs, EBERs and EBNA-1 are detected.[11]

There is diversity in the incidence, age, and sex distribution of HL in different populations.[12] A definite bimodal age peak is present in HL. In developing countries first peak occurs in children with male dominance and MCHL is the main subtype whereas in developed countries it occurs in young adults; the second peak follows in late adulthood. The major histological subtype in the United States and Western Europe is NSHL while it is less common in developing countries.[13,14]

Our study demonstrated that there is male predominance and MCHL is the commonest subtype. Similar results are found in a study conducted in Iran.[8] In our study two peaks were observed, first in children of 1–10 years and second in late adulthood between 51 to 60 years. In west male predominance persists but the commonest subtype is nodular sclerosis.[15] Predominance of MC subtype and first bimodal peak in children indicates that Pakistan, being a developing country displays 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. Comparing our study with other studies revealed that in our study the mean age (29.4 years) was 1.4 years younger and 4.4 years older than the study

conducted by Huang *et al* (mean age 28 years)[16] and Katebi *et al* (mean age 25 years)[8] respectively. However the expression of bimodal peak was consistent with the study conducted by Huang *et al*. In Western population first peak occurs at relatively later age (3rd decade). The second peak in our study occurred during 6th decade which was in concordance with Chinese population[16] and Western population[17,18].

Our study showed overall male predominance in Hodgkin lymphoma cases with male to female ratio of 2.2:1, which was in concordance with study by Katebi *et al*[8] and Huang *et al*[16] in which male to female ratio was 2:1 and 1.8:1 respectively.

The predominant site of involvement in our study was cervical lymph nodes constituting 65.5% of cases which was in concordance with the study conducted by Fatima *et al*[19] in which 73% cases showed cervical lymph nodes involvement.

On histopathological subtyping, in our study MCHL constituted highest percentage accounting for 87.3% of cases (n=48/55) followed by NSHL and LDHL constituting 10.9% (n=6/55) and 1.8% (n=1/55) respectively. These percentages were in concordance with study by Fatima *et al*[19] and Katebi *et al*[8] in which MCHL constituted 57% (n=57/100) and 63.3% (n=19/30) respectively. However in study by Huang *et al* highest percentage was seen in NSHL 61% (n=78/157) followed by MCHL 36% (n=46/157).[16]

EBV-LMP1 status in our study showed 80% expression which was similar to that seen in study by Katebi *et al*[8] but was 41% and 18.78% more than that seen in study by Huang *et al*[16] and Fatima *et al*[19] respectively.

Based on EBV-LMP1 status and its association with various subtypes of classical Hodgkin lymphoma, in our study highest expression was seen in LDHL (100%) which was in contrast to studies by Fatima *et al*[19], Katebi *et al*[8] and Huang *et al*[16] in which MCHL showed highest expression. This difference seems to arise from very less number of cases of LDHL. So this preposition needs to be validated through further studies comprising of substantial number of patients of LDHL.

## CONCLUSION

80% of our classical Hodgkin lymphoma cases showed association with EBV expression. 79.16% cases of mixed cellularity Hodgkin lymphoma showed LMP1 expression while 100% of lymphocyte depleted Hodgkin lymphoma showed LMP1 expression. This highest expression seen in lymphocyte depleted Hodgkin lymphoma subtype in contrast to mixed cellularity requires to be confirmed by a larger scale study comprising of substantial number of patients of lymphocyte depleted Hodgkin lymphoma and lymphocyte rich classical Hodgkin lymphoma.

## AUTHOR'S CONTRIBUTION

MA, HUD, FA: Research concept and design. MA, HUD: Collection and/or assembly of data, Data analysis and interpretation, Statistical analysis, writing the article. MA, HUD, IM, SH, FA: Critical revision of the article, Final approval of article

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