

**Brief Article**

## Analysis of the association between Epstein Barr virus infection and Hodgkin lymphoma

Jasmine Akhter Jahan<sup>1</sup>, S M Rashed Ul Islam<sup>2</sup>, Md. Shafikul Alam Tanim<sup>3</sup>, Tasnim Binte Ahmed<sup>4</sup>, Md. Rabiul Alam<sup>5</sup>, Ferdousy Begum<sup>3</sup>

<sup>1</sup>Department of Pathology, Mugda Medical College, Dhaka, Bangladesh.

<sup>2</sup>Department of Virology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

<sup>3</sup>Department of Pathology, Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh.

<sup>4</sup>Department of Pathology, US Bangla Medical College and Hospital, Narayanganj, Bangladesh.

<sup>5</sup>Department of Nephrology, National Institute of Kidney Diseases and Urology, Dhaka, Bangladesh.

Correspondence to: Dr. Jasmine Akhter Jahan, Email: drjasmine30@gmail.com

### Abstract

Epstein Barr virus plays an important role in the pathogenesis of Hodgkin lymphoma. However, the frequency of its association in Bangladeshi people has not been widely studied. The aim of this study was to determine the association of Epstein Barr virus association in Hodgkin Lymphoma through detection of Latent Membrane Protein 1 by immunohistochemistry and serum Epstein Barr virus viral capsid antigen IgG antibody titer by serology. This was a cross-sectional study in purposively selected 45 histologically diagnosed cases of Hodgkin lymphoma at Bangabandhu Sheikh Mujib Medical University, Dhaka, during the period of March 2017 to December 2018. Latent membrane protein 1 was positive in 71.1% of cases of Hodgkin lymphoma. Among these positive cases, 96% of cases had significantly raised titer of serum Epstein Barr virus viral capsid antigen IgG antibody ( $P < 0.0001$  obtained by Fisher's Exact test), which had a male predominance. Mixed cellularity classical Hodgkin lymphoma showed the highest positivity.

**Keywords:** Epstein Barr virus, Hodgkin Lymphoma, Latent membrane protein 1, Viral capsid antigen IgG.

### INTRODUCTION

Hodgkin lymphoma (HL) is one of the most common malignant neoplasms of the lymphoid system. In the South East Asia region, the prevalence of Hodgkin lymphoma is 1.61%. In Bangladesh, it is in the 28th position according to the incidence of malignant tumors and about 548 new cases of HL are diagnosed each year.<sup>1</sup>

According to the World Health Organization (WHO), HL is broadly classified into classical Hodgkin lymphoma (CHL) and nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL). CHL is further subdivided into nodular sclerosis classical Hodgkin lymphoma (NSCHL), mixed cellularity classical Hodgkin lymphoma (MCCHL), lymphocyte rich classical Hodgkin lymphoma (LRCHL) and

lymphocyte depleted classical Hodgkin lymphoma (LDCHL).<sup>2</sup> Though the pathogenesis of HL are unknown, EBV plays an important role.<sup>3</sup> The frequency of EBV positivity in HL varies with each histological subtype, sex, age and geographical distribution.<sup>3,4</sup>

After primary infection, many EBV proteins are produced.<sup>5</sup> Latent Membrane Protein 1 (LMP1) is a protein that plays a role in the transformation mechanism that allows EBV-infected Reed-Sternberg (RS) cells in HL to survive apoptosis which is detected by immunohistochemistry.<sup>6</sup> In EBV-induced CHL cases, MCCHL is more common in developing countries and the RS cells show LMP1 positivity.<sup>2</sup> The EBV antigens classically used for serological testing is viral capsid antigen (VCA). High anti-EBV-VCA IgG titers are considered to be markers of EBV past infection and

### Highlights

1. Data on the relationship of Epstein Barr virus, as measured by latent membrane protein 1 immunohistochemistry and IgG against Epstein Barr virus viral capsid antigen, with Hodgkin lymphoma in Bangladeshi people are not available.
2. This study reports a positive association of the latent membrane protein 1, and viral capsid antigen.

reactivation.<sup>7</sup> However, serological methods are less used; serum EBV-VCA IgG antibody titer can be analyzed.

The significance of association between EBV and HL has been highlighted in various studies. Many studies state that about 30-50% of cases of HL in developing countries are associated with EBV infection.<sup>8</sup> Therefore, this study is aimed to evaluate the frequency by expression of LMP1 by immunohistochemistry and serum EBV-VCA IgG antibody titer to detect EBV association in HL.

## METHODS

### Patients

This was a cross-sectional study carried from March, 2017 to December, 2018 at the Department of Pathology with co-operation of Department of Virology, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka. The sample size was calculated according to the EBV positivity in HL.<sup>8</sup> The study was conducted on paraffin blocks of lymph node biopsies and blood samples of purposively selected 45 histologically diagnosed cases of HL patients who gave informed consent for the serological test. Recurrent and treated cases of HL were excluded. Relevant demographic information and clinical findings were retrieved from the departmental registry during the study period.

### Laboratory diagnosis

Hematoxylin and eosin-stained slides of HL were already available in the laboratory of Department of Pathology. We have re-examined all these slides and selected representative tissue sections from each case for immunohistochemical analysis. Thin sections (5.0

µm) were taken on poly-L-lysine coated slides from the selected paraffin blocks, air-dried and incubated for 16 hours at 37°C in an incubator. Dewaxing of slides was done by xylene for 5 min. The slides were dehydrated in decreasing the strength of alcohol for 10 min in each. For antigen retrieval, the slides were treated with Dako Target Retrieval Solution (Code no S2023). The retrieval solution was taken in Coplin jar and pre-heated in the water bath at 65°C. Then slides were kept in this solution and heated in the water bath at 95-99°C for 30-40 minutes. After 20 minutes, each slide was washed with deionized water for 5 min. Then the sections were stained successively with LMP1 antibody following the DAKO standard procedure. Each case was run with a control slide (diagnosed positive case of HL). Cytoplasmic membrane and Golgi body staining of LMP1 in at least 10% of RS cells were interpreted as positive.<sup>4</sup>

Blood samples of the same patients of HL were collected in the Department of Virology, BSMMU to evaluate serum EBV VCA IgG antibody titer by chemiluminescence immunoassay (CLIA) technology. The analyzer automatically calculates VCA IgG antibody concentrations expressed as U/mL and grades the results. The assay range is 10 to 750 U/mL. The cut-off value discriminating between the presence and the absence of VCA IgG is 20 U/mL. Sample results were interpreted as samples with VCA IgG concentrations below 20 U/mL graded as negative and equal to or above 20 U/mL were graded as positive.

### Statistical analysis

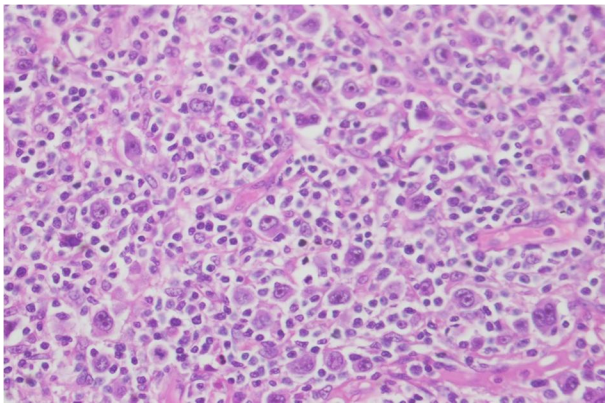
The results were obtained by using Statistical Packages for Social Sciences (SPSS 20). Proportions were compared using Fisher's Exact test.  $P < 0.05$  was considered statistically significant.

### Ethical consideration

Informed written consent were collected from patients or the legally authorized representatives of the minors in the preformed data collection form before the procedure. The study procedure also conformed to the ethical guidelines of the 1975 declaration of Helsinki and it was duly approved by the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University.

## RESULTS

Among the 45 cases of Hodgkin lymphoma 71.1% cases were LMP1 positive (Figures 1 and 2). EBV VCA IgG antibody titer was raised in 96% cases of LMP1 positive HL. The male-to-female ratio was 3:1. The highest proportion of LMP1 positivity (100%) was observed in older patients ( $\geq 51$  years). The histological analysis revealed that 77.1% of cases were mixed cellularity CHL. In immunohistochemical analysis, the highest LMP1 positivity was found in mixed cellularity CHL (84.3%). Among 32 cases of LMP1 positive HL, EBV-VCA IgG antibody titer was found to be raised in 81.2% of cases which shows a significant association between LMP1 positive HL and EBV-VCA IgG antibody titer. The detailed findings of the study are given in Table 1.



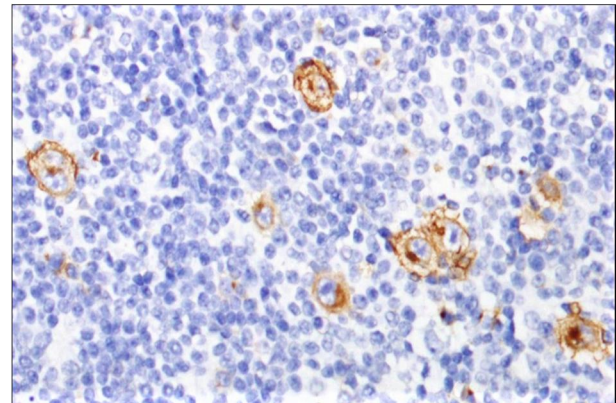
**FIGURE 1** Photomicrograph of mixed cellularity classical Hodgkin lymphoma showing different types of RS cells in a polymorphous background (H & E stain x200 magnification)

## DISCUSSION

This study shows 71.1% LMP1 positivity and 96% raised EBV-VCA IgG antibody titer in LMP1 positive Hodgkin lymphoma which is statistically significant. Among these cases 75% is male and the male to female ratio is 3:1. Similar observation regarding male predominance was also found in Honduras, the United States and in Bangladesh.<sup>9,10</sup> In this study, 51.1% patients were in 16-50 years; 40% patients were in  $\leq 15$  years and 8.9% patients were in  $\geq 51$  years age group. But LMP1 immunomarker is observed to be 100% positive in the older ( $\geq 51$  years) age group and 83.3% in the younger ( $\leq 15$  years) age group. In developing countries, HL shows bimodal age distribution and in developed countries, the peak incidence is in adulthood.<sup>3,11</sup> Adult case is more in this study but LMP1 positivity is highest in older cases although no

statistically significant difference was found between the age groups.

Nodular sclerosis CHL is the most encountered type in the USA and South Africa.<sup>12</sup> In this study, the most common subtype is mixed cellularity CHL with 84% LMP1 positivity. Several studies in Pakistan also reported mixed cellularity CHL as the commonest subtype in their study.<sup>4,13</sup> In this study, the majority (86.7%) of cases presented with unilateral lymphadenopathy, and the most involved lymph node was in the cervical region (73.3%) followed by inguinal (20%), abdominal (13.3%), and axillary region (6.7%). Hepatosplenomegaly was present in 15.6% of cases and 6.7% of cases had mediastinal involvement. In this study population, B-symptoms were present in 62.2% of



**FIGURE 2** Photomicrograph of LMP1 immunomarker showing brown cytoplasmic membrane and Golgi pattern staining of Reed-Sternberg cells (x400 magnification)

cases and 40% cases had increased serum LDH level. Most of the patients of LMP1 positive HL were presented with stage I disease. The percentage of LMP1 positivity in RS cells of HL has a variation in studies of different countries. LMP1 positivity was observed in Jordan (43%), Italy (40%), South Africa (68%) and Peru (83%).<sup>3,5,12,14</sup> In Pakistan, some studies found 68.1% and 80% LMP1 positivity.<sup>4,13</sup> This study shows 71.1% positivity in our population which is close to above mentioned studies.

Serological studies in the 1970s indicated that EBV infection was associated with Hodgkin lymphoma.<sup>15</sup> Studies in France and America reported significantly higher IgG titers in patients of HL.<sup>7,16</sup> The results obtained from the present study revealed that 96% of cases of LMP1-positive HL had significantly raised EBV-VCA IgG titer. Statistically, this association was highly

**TABLE 1** Clinico-pathological presentation of Hodgkin Lymphoma patients as per Latent membrane protein 1 positivity (n= 45)

| Subjects                    | Total | LMP 1 positive n (%) | LMP 1 negative n (%) | P*    |
|-----------------------------|-------|----------------------|----------------------|-------|
| Gender                      |       |                      |                      |       |
| Male                        | 35    | 24 (68.6)            | 11 (31.4)            | 0.48  |
| Female                      | 10    | 08 (80)              | 02 (20)              |       |
| Age group                   |       |                      |                      |       |
| ≤ 15 years                  | 18    | 15 (83.3)            | 3 (16.6)             | 0.041 |
| 16-50 years                 | 23    | 13 (56.5)            | 10 (43.5)            |       |
| ≥ 51 years                  | 04    | 04 (100)             | 0 (0)                |       |
| Lymph node involvement      |       |                      |                      |       |
| Cervical                    | 33    | 25 (75.8)            | 08 (24.3)            | 0.25  |
| Axillary                    | 03    | 2 (66.7)             | 01 (33.3)            |       |
| Inguinal                    | 09    | 5 (55.5)             | 04 (44.5)            |       |
| Nodal involvement           |       |                      |                      |       |
| Unilateral                  | 39    | 28 (71.8)            | 11 (28.2)            | 0.79  |
| Bilateral                   | 06    | 04 (66.7)            | 02 (33.3)            |       |
| Stage                       |       |                      |                      |       |
| I                           | 41    | 28 (68.3)            | 13 (31.7)            | 0.64  |
| II & III                    | 04    | 04 (100)             | 0 (0)                |       |
| Mediastinal mass            | 03    | 01 (33.3)            | 02 (66.7)            | 0.13  |
| Hepatosplenomegaly          | 07    | 05 (71.4)            | 02 (28.6)            | 0.98  |
| Presence of B-symptoms      | 28    | 21 (75)              | 07 (25)              | 0.46  |
| Increased LDH level         | 18    | 15 (83.3)            | 03 (16.7)            | 0.14  |
| Histological subtype        |       |                      |                      |       |
| Nodular sclerosis CHL       | 04    | 02 (50)              | 02 (50)              | 0.27  |
| Mixed cellularity CHL       | 35    | 27 (77.1)            | 08 (22.9)            |       |
| Lymphocyte rich CHL         | 05    | 03 (60)              | 02 (40)              |       |
| Lymphocyte depleted CHL     | 00    | 0 (0)                | 0 (0)                |       |
| NLPHL                       | 01    | 0 (0)                | 01 (100)             |       |
| EBV- VCA IgG antibody titer |       |                      |                      |       |
| Raised                      | 27    | 26 (96.2)            | 01 (3.8)             | <0.00 |
| Not raised                  | 18    | 06 (33.3)            | 12 (66.7)            | 01    |

CHL: Classical Hodgkin lymphoma; LDH: Lactate dehydrogenase; LMP1: Latent membrane protein 1; NLPHL: Nodular lymphocyte predominant Hodgkin lymphoma; EBV-VCA IgG: Epstein Barr virus viral capsid antigen IgG; \*Fisher's Exact test

significant. An elevated titer of IgG antibody against EBV-VCA was also found in patients with CHL in studies of Iraq and America.<sup>17,18</sup>

There are evidence that EBV-associated HL has poor survival rate especially in older patients.<sup>19</sup> The emerging understanding of the cellular immune response of the association of EBV infection suggests the possibility to reduce the incidence of Hodgkin lymphoma by interventions to prevent or modulate primary viral infection. Immunotherapy with T- cells targeting EBV antigens are being investigated. Therefore, the presence of EBV latent antigens in Hodgkin lymphoma represents an attractive opportunity for targeted immunotherapy in future.<sup>5</sup> In spite of this promising finding, this study has

limitations too. No validation analysis could be done for the titer of IgG titers. Moreover, recent primary infection by EBV in the cases could not be excluded. The participants were selected from a tertiary care hospital and the sample size was small. Therefore, the result may have uncertainty.

### Conclusion

Seventy one percent of the study sample of Hodgkin lymphoma expressed LMP1 positivity. Among 96% showed raised EBV-VCA IgG antibody titer. There was a significant association of EBV infection with Hodgkin lymphoma. Mixed cellularity classical Hodgkin lymphoma subtype was the most common.

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### Author contributions

- Conception and design: JAJ
- Acquisition, analysis, and interpretation of data: JAJ, MSAT, FB
- Manuscript drafting and revising it critically: JAJ, SMRI, TBA, MRA
- Approval of the final version of manuscript: All
- Guarantor accuracy and integrity of the work: JAJ

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### Conflict of interest

Authors declare no conflict of interest.

### Ethics approval

Ethical approval was given by the Institutional Review Board of BSMMU: Memo No. BSMMU/2017/9012 dated 29 August 2017.

### ORCID iDs:

Jasmine Akhter Jahan, <https://orcid.org/0000-0002-3470-9822>  
SM Rashed UI Islam <https://orcid.org/0000-0002-8164-5905>

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