Epstein-Barr virus latent membrane protein-1 expression in Hodgkin lymphoma

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Article Info

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Abstract

Expression of latent membrane protein-1 of Epstein-Barr virus is documented in Hodgkin lymphoma indicating its relationship in disease process. A total of 50 cases of Hodgkin lymphoma were analyzed for latent membrane protein-1 expression by immunohistochemistry. The mean age was 28.7 years with male predominance. Mixed cellularity classical Hodgkin lymphoma was the commonest subtype (50% cases). Out of 50 cases, 48% cases were found positive for latent membrane protein. Of the five histologic subtypes, all cases of lymphocyte depleted classical Hodgkin lymphoma showed positivity which was followed by 60% positivity of mixed cellularity classical Hodgkin lymphoma. Association of latent membrane protein-1 expression in relation to different age group, site of involvement and subtypes of Hodgkin lymphoma were found statistically insignificant while latent membrane protein-1 expression was significantly higher in male than female.

Introduction

Hodgkin lymphoma is an unusual malignancy because only a small number of cells constitute the tumor mass. This small fraction of cells are admixed with abundant heterogeneous population of non-neoplastic inflammatory and accessory cells. As a result, the study on these cells is challenging. Regarding pathogenesis of the Hodgkin lymphoma, it is found that Epstein -Barr virus is associated with some fractions which are causal.[1]

Several studies were performed to see the association of Hodgkin lymphoma with Epstein -Barr virus infection using immunohistochemical method in neoplastic cells of Hodgkin lymphoma[2] The detection of Epstein-Barr virus nucleic acids and proteins in neoplastic cells of Hodgkin lymphoma defines Epstein-Barr virus positive Hodgkin lymphoma. Viral RNAs referred to as EBERs detection by *in situ* hybridization technique and immunohistochemical detection for the latent membrane protein-1 are widely used in the diagnostic laboratories. Its association is dependent on various factors such as geographic location, histological subtypes, sex, ethnicity and age. Epstein-Barr virus infection is associated with various malignancy such as nasopharyngeal carcinoma, peripheral T cell lymphoma, nasal natural killer (NK) and T-cell lymphoma.[3-5] As the histological features of Hodgkin lymphoma sometimes overlap with anaplastic large cell lymphoma and reactive lymphoid hyperplasia, Epstein-Barr virus detection tests also help to arrive accurate diagnosis. Latent membrane protein-1 is expressed in the cytoplasm and surface membrane of Reed-Sternberg/Hodgkin cells which can be detected by immunohistochemical stain.[4] This study is planned to explore the latent membrane protein-1 detection to see its overall positivity in Hodgkin lymphoma, its pattern of positivity in different subtypes of Hodgkin lymphoma along with their correlation with demographics of 50 cases of Hodgkin lymphoma in terms of age and gender.

Materials and Methods

A total of 50 cases of Hodgkin lymphoma were obtained from the Pathology Department from January 2013 to December 2016. The clinical parameters like age and gender were recorded from the patient's requisition form.

All hematoxillin and eosin stained slides were reexamined and classified according to WHO Classification 2008 into to five subtypes viz. nodular sclerosis classical Hodgkin lymphoma, mixed cellularity classical Hodgkin lymphoma, lymphocyte rich classical Hodgkin lymphoma, lymphocyte depleted classical Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma.

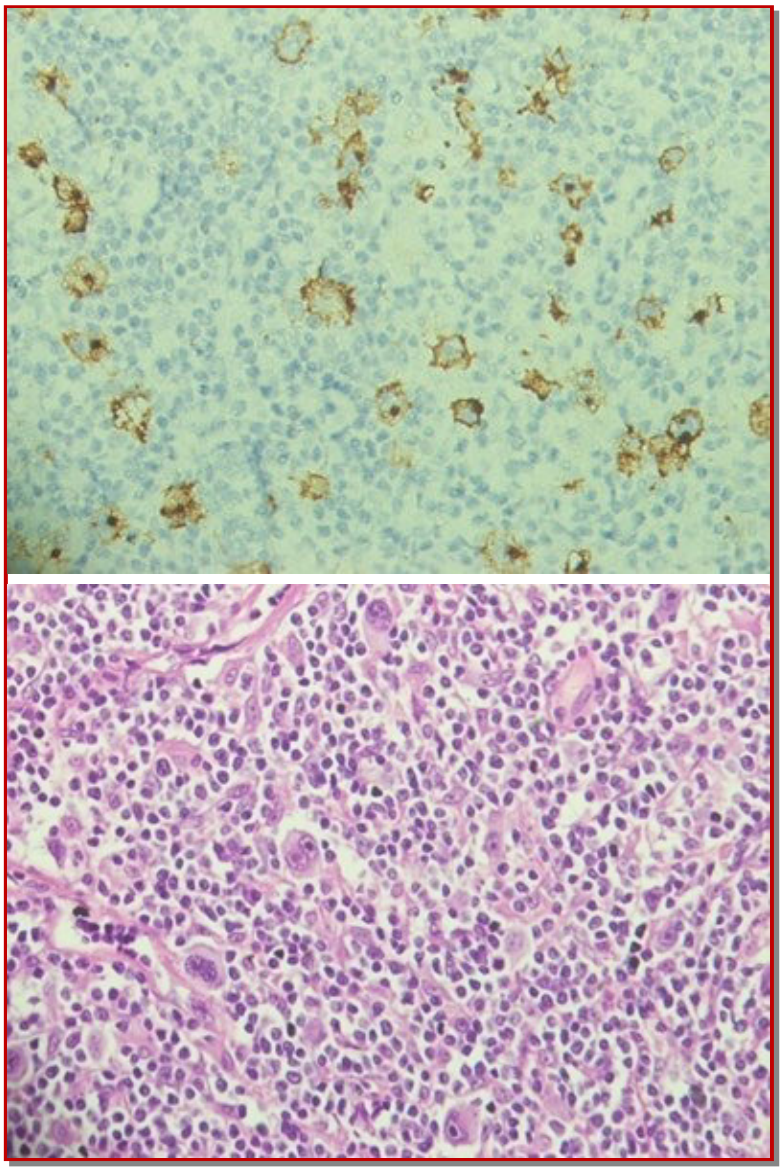

Figure 1: Latent membrane protein-1 expression in mixed celluarity classical Hodgkin's lymphoma Reed-Sternberg cells showing positive membrane and Golgi type staining (Immunohistochemical stain x400) (upper image)

Mixed cellularity classical Hodgkin lymphoma (Hematoxillin and eosin stain x400) (lower image)

All cases were examined for latent membrane protein-1 detection by immunohistochemical stain. Thin sections (4.0 µm) were taken on poly-L-lysin coated slides from the selected paraffin blocks. The slides were air-dried and incubated for 16 hours at 37°C in an incubator. Dewaxing of slides was done by subsequent two changes in xylene for 5 min duration each. The slides were dehydrated in decreasing strength of isopropyl alcohol (100, 90, 80, 70%) for 10 min in each. Then the slides were treated with Dako target retrieval solution (Code No. S1700). Retrieval solution was taken in coplin jar and preheated in the water bath at 65°C. the slides were kept in this solution and heated in the water bath at 95-99°C for 30-40 min. After 20 min, each slide was washed with deionized water for 5 min. Peroxidase–blocking solution DakoReal™ (Code No S2023) applied for 10 min followed by wash in Tris buffer saline for 5 min. Monoclonal mouse anti-Epstein-Barr virus, LMP clones CS, 1-4 (Code No M0897) was used as the primary antibody with a dilution of 1:200 and incubated for 30 min. This was followed by Tris buffer saline wash 5 min twice. The incubation in Envision™ Detection System, HRP, rabbit/mouse, (code K5007) was done for 30 min followed by wash in Tris buffer saline for 5 min twice. Application of 3,3-diaminobenzidine substrate (DAB) was used as chromogen and kept for 10 min. This step was followed by deionized water wash. Counter-staining of nucleus was performed by Mayer's Hematoxillin for 2 min. Dehydration was done by increasing strength of isopropyl alcohol (70, 80, 90 and 100%), each step for 10 min. Clearing was done by subsequent two changes in xylene for 5 min duration each. The slide was mounted with DPX. Immunostaining procedure was followed according to Dako, Denmark product Envision™ Detection System, HRP, rabbit/mouse, (code K5007). Each case was run with control slide (diagnosed positive case of Hodgkin lymphoma). Cytoplasmic and membrane staining of the latent membrane protein-1 were interpreted as positive. Hematoxillin was used as counter stain (Figure 1).

Statistical analysis

Statistical analysis was done using SPSS version 20. Chi-square test of independence was used to find out the association of latent membrane protein-1 expression between/among different age group, gender and subtypes of Hodgkin lymphoma along with latent membrane protein-1 positivity.

Results

Among the 50 studied cases of Hodgkin lymphoma, 48% cases were positive for Epstein-Barr virus latent membrane protein-1 by immunohistochemistry (Table I).

The age of studied cases ranged from 4 to 75 years with the mean age of 28.7 years (SD ± 16.95). Male cases outnumbered the female (76/24%).

Among the subtypes of Hodgkin lymphoma, latent membrane protein-1 was found 100% positivity in lymphocyte depleted classical Hodgkin lymphoma followed by 60% in mixed cellularity classical Hodgkin lymphoma, 41.7% in classical Hodgkin lymphoma, unclassifiable, 30% in nodular sclerosis classical Hodgkin lymphoma and 0% each for lymphocyte rich classical Hodgkin lymphoma and nodular lymphocyte predominant Hodgkin lymphoma (Table I).

Table I

Characteristics of patients and latent membrane protein-1 expression in subtypes of Hodgkin lymphoma (n= 50)

	LMP1 positive		p value
	n	(%)	
Age group			
<15	7/15	46.7	0.663
15 to 50	12/27	44.4	
>50	5/8	62.5	
Gender			0.047
Male	21/38	55.3	
Female		25.0	
Diagnosis			0.332
Classical Hodgkin lymphoma	24/49	49.0	
NLPHL	0/1	0.0	
Nodular lymphocyte predominant Hodgkin lymphoma	0/1	0.0	0.320
Nodular sclerosis classical Hodgkin lymphoma	3/10	30.0	
Mixed cellularity classical Hodgkin lymphoma	15/25	60.0	
Lymphocyte depleted classical Hodgkin lymphoma	1/1	100.0	
Lymphocyte rich classical Hodgkin lymphoma	0/1	0.0	
Classical Hodgkin lymphoma, unclassifiable	5/12	41.7	

LMP1: Latent membrane protein-1; NLPHL: Nodular lymphocyte predominant Hodgkin lymphoma

The association of latent membrane protein-1 expression in relation to different age group and subtypes of Hodgkin lymphoma was found statistically insignificant. However, the mentioned association was found statistically significant in respect to gender (Table I).

Discussion

This study shows 48% positivity in Hodgkin lymphoma. Only the positivity of latent membrane protein-1 in male patients (55.3%) were significantly higher than the females, however the association of latent membrane protein-1 expression in relation to different age group and subtypes of Hodgkin lymphoma was found statistically insignificant. The most common subtype was mixed cellularity classical Hodgkin lymphoma with 60% latent membrane protein-1 positivity, however lymphocyte depleted classical Hodgkin lymphoma showed 100% positivity (Table I). In addition, each case of nodular lymphocyte predominance Hodgkin lymphoma and lymphocyte rich classical lymphoma showed negativity to the latent membrane protein-1.

Glasser et al. (1997) reported 50% positivity of latent membrane protein-1 in Hodgkin lymphoma with significant male predominance in their international data of epidemiologic characteristics of Epstein-Barr virus-associated Hodgkin disease cases.[6] Male predominance has also been reported by Ambinder et al. (1993) in Honduras and in the United States and Gully et al. (1994) in Hispanic American ethnicity.[7, 8]

Percentage of latent membrane protein-1 positivity in Reed Sternberg cells of Hodgkin lymphoma has wide range of variation noticed in studied cases of different countries. Chang et al. (1993) found 83% positivity in Peru whereas cases from USA, Italy and Greece studied by Herling et al. (2003) found 21%.[9, 10] In Pakistan, Hashmi et al. (2007) and Fatima et al. (2011) found 68.1 and 60% respectively.[11, 12] Latent membrane protein-1 positivity in India described by Radha et al. (1997) in Chennai as 31%, Karnik et al. (2003) as 82% and Rajalakshmi et al. (2006) as 55%.[13-15] Irshaid et al. (2013) reported 60% latent membrane protein-1 positive Hodgkin lymphoma cases.[16] Cickusic et al. (2007) found 33.3% positive cases in their studied cases of Hodgkin lymphoma in Bosnia and Herzegovina.[17] Variation of percentage of latent membrane protein-1 positivity in different countries is reflected by socioeconomic condition, geographical location and ethnicity of people. This study showed 48% positivity which is more or less close to the findings of Pakistan and some area of India.

Mixed cellularity classical Hodgkin lymphoma subtype has been described as most predominant form by others which is similar to the present study whereas a large study performed by Campos et al. found nodular sclerosis classical Hodgkin lymphoma to be most common subtype.[9, 11, 16, 18, 19]

Hodgkin lymphoma is presented with bimodal age incidence. In developing countries, the first age incidence peak occurs in childhood and the second peak occurs in elderly patients. However in developed countries the first peak delayed until young adulthood.[20] Dinand et al. found statistically significant association between Epstein-Barr virus–latent membrane protein-1 detection in younger age group of Indian children. This study also described statistically significant association between Epstein-Barr virus-latent membrane protein-1 detection and middle-low socioeconomic status as compared with the higher socioeconomic status.[21] There is no statistically significant differences found between <15 years age group, 15-50 years age group and >50 years age group in our series.

The mean age of Hodgkin lymphoma cases of the present study is similar to the study performed by others[12, 22] but lower than Cickusic et al. (2007).[17] However, a retrospective analysis of diagnosed hematological malignancies in Bangladesh over

5,000 cases has reported median age of Hodgkin lymphoma to be 36 years.[23] The present study showed that male patients are more affected by the Hodgkin lymphoma than the females. This finding is similar to the earlier studies.[18, 17, 23]

Histological features like background lymphocyte population, presence of fibrosis, relative percentage of Reed Sternberg cells and immunophenotype determine the histologic subtypes of Hodgkin lymphoma. Nodular sclerosis Hodgkin lymphoma is the most commonly encountered type in USA.[1] People of higher socioeconomic status have predominant nodular sclerosis Hodgkin lymphoma subtype and lower socioeconomic condition have mixed cellularity Hodgkin lymphoma subtype described in a study performed.[19] Like the variation of these two histologic subtypes of Hodgkin lymphoma percentages of latent membrane protein -1 expression also varies. Regarding the latent membrane protein-1 expression in mixed cellularity Hodgkin lymphoma subtypes Ambinder et al (1993) found 100% positive cases of latent membrane protein-1 in Hondurus and 85.7% cases in USA, Hashmi et al. (2007) found 73.9% positive cases in Pakistan, Armstrong et al. (1993) 100% positive cases in Saudi Arabia, Sughayer et al. (2014) found 80.7% positive cases in Jordan.[07, 11, 20, 24] The present study showed 60% positive cases which is lower in comparison to others data. One explanation could be due to inclusion of 24% cases of unclassifiable classical Hodgkin lymphoma in our series.

In this study, Epstein-Barr virus latent membrane protein-1 positive staining was seen in 100% cases of lymphocyte depleted classical Hodgkin lymphoma subtype. A study carried out in Iran found 100% positive cases of latent membrane protein-1 in lymphocyte depleted classical Hodgkin lymphoma subtype while in Pakistan Hashmi et al. (2007) found 66.6% positive cases in this subtype.[16, 11]

Regarding pathogenesis of Hodgkin lymphoma, several molecular studies on Reed-Sternberg cells and it's variants have shown V(D)J recombination and somatic hypermutation. This established that origin of the malignant cells is from germinal center or postgerminal center B cells. Despite presence of genetic signature of B cells, most B cell specific genes including Ig genes are not expressed in Reed-Sternberg cells. This may be due to epigenetic changes of unknown etiology.[25] The concept of causal association of Hodgkin lymphoma and infectious agent was proposed by MacMahon in 1966.[26] Initially raised antibody titers to Epstein-Barr virus antigen was found in patients with Hodgkin lymphoma.[27] Epstein-Barr virus could play role in the pathogenesis of Hodgkin lymphoma either by direct infection or indirectly. Moreover, direct or acquired depression of immunoregulation might be responsible for reactivation of Epstein-Barr virus or causation of malignancy.[2] Epithelial cells of oropharynx and B lymphocytes are infected by Epstein-Barr virus. The virus enters into the B lymphocytes with the help of CD21 which is present on all B lymphocytes. Within the B cell, genome of Epstein-Barr virus transform to episomal form within the nucleus. This is supported by study performed by Deacon et al.[3] In latently infected cells the viral genes cause desregulation of normal proliferation and survival signals. A signaling molecule that is usually stimulated by CD40 receptor in B cells is triggered by latent membrane protein-1. CD40 receptor plays a key role in helper T cell signals which control B cell responses. Latent membrane protein-1 like CD40 receptor activates NF-kB and JAT/STAT signaling pathways that leads to the sustained survival and proliferation of B cells. NF-kB transcription factor activation is a common event that occur by several mechanism. This may be activated by Epstein-Barr virus infection or other mechanism.[25] During natural infection of B cells by Epstein-Barr virus six nuclear proteins (called either EBNA1-6, or EBNA1, 2, 3a, 3b, 3c and LP), three membrane proteins (LMP1 and LMP2A and 2B protein) and two small non-polyadenylated RNA transcripts ($EBER_{S}$) are expressed. Hodgkin and Reed Sternberg cells express EBNA1, LMP1 and LMP2A and 2B protein and EBERRNAs, the so called latency II pattern of transcription. Among the latent membrane proteins, latent membrane protein-1 involves in the activation of NF-kB transcription factor. So, its detection may be related to causal.[28-30.] The potential importance of LMP1 in the pathogenesis of Hodgkin lymphoma is being reflected by present study which showed 48% positivity of LMP1 in Hodgkin lymphoma cases by immunohistochemistry.

A population based study on Epstein-Barr virus status of Hodgkin lymphoma showed that Epstein-Barr virus negative cases have better disease specific survival rate.[31] Moreover, it has already been established that vaccine and antiviral drugs can modulate the clinical course of infectious mononucleosis virus which is detected in some Hodgkin lymphoma. Their impact on Hodgkin lymphoma is not known. T-cell function may have important role in Hodgkin lymphoma because higher CD4 counts are associated with an increased incidence of Epstein-Barr virus positive Hodgkin lymphoma in HIV patients. Treatment related to adoptive immunotherapy with T cells targeting Epstein-Barr virus antigens has been investigated. Recent advances in targeted therapy to have shown more effective therapeutic impact on Hodgkin lymphoma. So, the detection of Epstein-Barr virus association in biopsy samples either by identification of latent membrane protein-1 by immunohistochemistry or Epstein-Barr virus encoded RNA by *in situ* hybridization will be utilized for targeted therapy in future.[32]

Conclusion

Forty eight percentage cases of the present study series of Hodgkin lymphoma expressed latent membrane protein-1 positivity. Among other parameters male patients showed significant positivity (55.3% cases) of latent membrane protein-1 than the female. Though the mixed cellularity classical Hodgkin lymphoma was found to the most common subtype (50% cases), their association with latent membrane protein-1 showed 60% positivity while 100% cases of lymphocyte depleted classical Hodgkin lymphoma showed positivity for latent membrane protein-1.

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