The Frequency of Epstein - Barr Virus (EBV) expression in various histological subtypes of Hodgkin's Lymphoma

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Abstract

Introduction

The incidence of Hodgkin's Lymphoma (HL) shows marked heterogeneity according to age, gender, race, geographical state, socioeconomic position and histological subtypes. Recently, multiple studies in different countries have been applied by newer technologies such as Immunohistochemistry (IHC) and polymerase chain reaction (PCR), being more familiar with the pathogenesis of this neoplasm. The Epstein-Barr virus (EBV) is detected with high incidence in HL cases, approximately 40-50% in developed countries and much more (up to 95% of cases) in developing countries. There is evidences that mixed cellularity (MC) Hodgkin's Lymphoma is more likely to be EBV-associated which is against association of nodular sclerosis (NS) subtype. With regard to the geographical location of Iran and absence of similar documented research in our knowledge, it is need to perform studies like this.

Materials and Methods

This study was done by IHC method with antibody against latent membrane protein-1 (LMP-1) antigen of EBV for assessment of relationship between EBV infection and parameters such as age, gender and histological subtype. We collected 30 paraffin section samples of classic HL and positive cytoplasmic reactivity of Reed-Steinberg (RS) cells was evaluated.

Results

From 30 cases surveyed in this study only 2 cases were not immunoreactive for EBV marker of which both were NS subtype in two adult males. We confirm frequency of 93% EBV associated HL in our cases, and also confirm the above histological subtype distribution, and that childhood cases are more likely to be EBV-associated than adult cases. There is possible female predominance of EBV associated HL. This survey as a pilot study needs further studies with more cases for distinct confirmation.

Conclusion

It seems that EBV is a strong etiologic factor especially in developing countries like Iran and in childhood cases.

Keywords: Epstein - Barr Virus, Hodgkin's Lymphoma, EBV associated HL, Immunohistochemistry.

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Introduction

Despite multiple epidemiologic studies, the causes of Hodgkin's Lymphoma remain unknown (1, 2, 3). As early as 1966 MacMahon proposed that Hodgkin's Lymphoma might be caused by an infectious agent (4). Recent studies on the possible etiology of Hodgkin's Lymphoma have focused on the Epstein - Barr virus (EBV) (1-6).

In developing countries EBV can be demonstrated in RS cells in up to 95% of HL cases, and in industrialized countries only about 50% of HL cases are associated with EBV.(7,8) EBV genomes have been identified within the RS cells of Hodgkin's Lymphoma in up to 40 to 50 percent of cases (9,10). Patients with a history of infectious mononucleosis have a two to four times higher Lymphoma incidence than those without such a history (11). In addition, more patients with Hodgkin's Lymphoma than expected have higher levels of antibody against EBV capsid antigen (12). In EBV-positive cases, Hodgkin cells express EBV-LMP1, a protein that has transforming potential in transfection assays (13). EBV genome commonly detected by either LMP-1 immunostaining or EBV-encoded RNA (EBER) in situ hybridization (ISH) in RS cells (14, 15, 16). By combining Southern blot analysis and ISH, it has been shown that, in EBV-positive HL, there are clonal EBV copies in RS cells. (17, 18) EBV-positive RS cells might be rescued from apoptotic death by activation of the nuclear factor B through the LMP-1-mediated induction of A20 expression. (19) LMP-1 itself is known to exhibit an oncogenic potential in B cells, because LMP-1-transgenic mice develop B-cell lymphomas (20).

EBV is most commonly found in the mixed cellularity subtype and in patients under 15 and over 50 years (1, 2, 3, 12, 13, 21). The clear role of EBV in Hodgkin's Lymphoma has not been documented yet, but its presence in a high percentage of cases suggests an important role in pathogenesis of HL (1). Recently most scientists speculated that RS cells are of clonal germinal center B-cell origin, (1, 22) but except for nodular lymphocyte predominance subtype do not express a B cell phenotype (1, 2). Thus, EBV infection might have an important role in the rescue of these cells from apoptosis and in the development of the malignancy.

EBV might escape detection by standard screening methods such as IHC due to deletions in the viral genome or absence of LMP-1 antigen expression. It can better detect by methods like PCR which investigates fragments of the viral genome of virus (16).

Materials and methods

This retrospective study was performed by IHC method. Paraffin sections of formalin fixed lymph node biopsies from 30 known cases of classic HL were included in this study.

The cases had been referred to Ghaem Hospital of Mashhad University of Medical Sciences. 4µ thick sections prepared for staining with Hematoxylin and Eosin (H&E) and IHC procedure. First, H&E slides and their previous IHC stained slides with markers as CD15, CD30, CD45 (LCA), and FASCIN reviewed by two pathologists for conforming diagnosis.

After deparaffinization and hydration of slides, antigenic retrieval was done by incubation with Molar citrate Buffer %1 (PH=6) in microwave oven for 12 minutes.

For immunostaining, immunoperoxidase streptoavidin–biotin procedure was performed by incubation in room temperature respectively as: 1- with 3% hydrogen peroxide for 10 minutes, 2- then for 60 minutes with antibody against EBV LMP-1 prepared from Serotec Company, England [IgG1 mouse anti Epstein Barr Virus LMP

cocktail of 4 antibodies recognizing different epitopes of EBV-LMP-1 (MCA1874), clone (CS1, CS2, CS3, and CS4)] 3-with, biotinylated link anti mouse and anti rabbit immunoglobulin, then streptoavidin- peroxides (DAKOLSAB 25 system, peroxidase kit, Denmark) and finally with chromogen (Diamino Benzidin hydrochloride-DAB), 10 minutes for each one. Counter staining was done with Hematoxylin Mayer and mounted in canada balsam. For further evaluation cytoplasmic immunoreactivity of only RS cells (and not reactive cells) was evaluated.

Results

Between 30 classic HL cases with different subtypes, that we studied, there were 19 cases, mixed cellularity (MC) (63.3%), 8 cases, nodular sclerosis (NS) (26.7%), 2 cases, lymphocyte reach (LR) (6.7%) and one case, lymphocyte depletion (LD) (3.3%) subtypes (Chart 1).

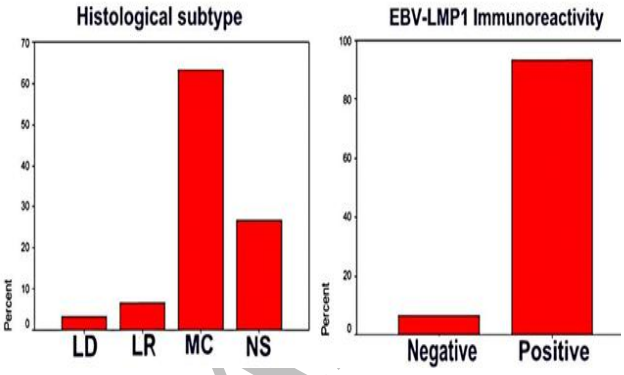

Chart 1: Percent of histological subtype and EBV immunoreactivity for Hodgkin's Lymphoma cases in this study.

Age range was between 6 to 76 years (mean=31.6, mode=21, SD=17.79) and the most frequent age group was young adult (14-40 years old) equal to 59.9 % (less than 14 years =13.3%, more than 40 years were 26.4%). There were 20 males (66.7%) with average age equal to 29.75 (min=12, max=60) and 10 females (33.3%) with average age equal to 35.30 (min=6, max=76) years (Chart 2). Except 2 cases, all of immunostained slides were reactive for EBV marker (Figure 1).

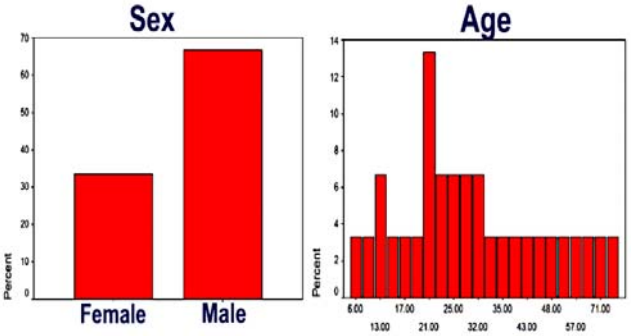

Chart 2: Percent of sex and age distribution of Hodgkin's Lymphoma cases in this study

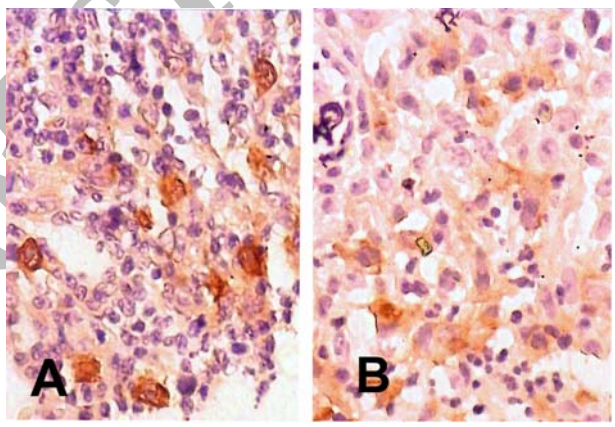

Figure 1: Positive EBV- LMP-1 immunoreactivity in Hodgkin's cells (A=Mixed Cellularity, B=Nodular Sclerosis subtype), Immunohistochemistry staining ×400.

Both of EBV-Negative cases were NS subtype and were adult male (47and 37 years old) (Table-1). There was a frequency of 93% for EBV-Positive HL in our cases.

Table 1: Numbers of EBV-LMP-1 immunoreactive and Non-immunoreactive male and female cases for each histological subtype.

Reactivity	SEX	TYPE	Number
Negative	Male	NS	2 cases
positive	Female	MC	8
		NS	2
	Male	MC	11
		NS	4
		LR	2
		LD	1

Neither of females nor childhood cases was negative for EBV detection and we didn't show any correlation between EBV immunoreactivity and histological types with age and gender. We confirmed previous results about subtype and age predominance of positivity of EBV detection in HL studies as was explained above and there is possible female predominance of EBV associated HL.

Discussion

EBV is a human herpes virus that infects B lymphocytes as an asymptomatic lifelong infection. EBV is associated with endemic Burkitt lymphoma, nasopharyngeal carcinoma, post transplantation lymphoproliferative disease (PTLD), some T-cell and natural killer (NK) cell lymphomas, Hodgkin Lymphoma , gastric carcinoma ,breast carcinoma and etc (4, 5, 6, 8, 11, 14, 15, 16, 17, 21, 23, 24, 25, 26).

In 1997, EBV was classified by the World Health Organization–International Agency for Research on Cancer (WHO-IARC) as a group I human carcinogen. Epidemiologic studies have shown strong associations between infectious mononucleosis and EBV positive Hodgkin Lymphoma (5).

Three distinct forms of HL have been identified by epidemiologic studies: a childhood form (0 to 14 years) common in developing countries, a young adult form (14 to 40 years) common in developed countries and an older adult form (55 to 74 years), occurring in both groups. In our study the most common age groups were young adult forms with 59.9% frequency. Disease incidence varies among different countries, with a relatively high incidence in the Netherlands, Denmark, and Israel, and a low incidence in Japan and Australia, particularly in the younger ages. In the United States there has been a decrease in the incidence of childhood HL and increasing in incidence in young adults (26).

In a considerable proportion of HL cases, RS cells harbor clonal EBV genomes. It has been suggested that EBV contributes to the malignant transformation of RS cells in these cases. On standard screening methods, like IHC, detection of EBV in RS cells is mainly based on the detection of the gene LMP-1 or EBER (EBV Encoded RNA). Our study was based on immunohistochemistry method of EBV-LMP-1 antigen detection (Figure-1). The detection of EBV DNA, is the most sensitive method to detect the evidence of EBV in the lymph node sections (27). According to an extensive cohort study linked to the Danish and Swedish cancer registries to identify cases of cancer following infectious mononucleosis diagnosis, published in year 2000, the relative risk of Hodgkin's Lymphoma remained increased for up to 20 years after the diagnosis of infectious mononucleosis (28).

In regard to this survey and other studies in developing countries it seems that EBV infection might have a strong relationship to etiology of HL, especially in childhood cases although it needs further studies with more cases for distinct confirmation.

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