Epstein-Barr Virus in Hodgkin's Disease Patients in Northeast Anatolia

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ABSTRACT

Epstein-Barr virus (EBV) is implicated as an etiologic factor in Hodgkin's disease (HD). The proportion of HD patients with EBV is high in developing countries but low in developed countries. In the present study, the EBV association with HD in Northeast Anatolia was investigated. Thirty-six formalin-fixed paraffin-embedded cases of HD were analysed for the presence of EBV and, for the latent membrane protein (LPM-1) by immunohistochemistry. There were 26 males and 10 females; age distribution ranged from 12 to 73 years (mean ± standard deviation, 34.1 ± 15.2 years). Overall, LPM-1 was detected in 27 of the 36 cases (75%). LPM-1 expression varied according to the histological subtype of HD (9/55 cases of lymphocyte predominance subtype, 3/4 cases of nodular sclerosis, 18/18 cases of mixed cellularity subtype, and 1/5 cases of lymphocyte depletion subtype). In this study, the EBV-positivity ratio in HD was found extremely high in Northeast Anatolia.

Key Words: Epstein-Barr virus, Hodgkin's disease, Northeast Anatolia.

Turk J Haematol 2000;17(2):61-65.

INTRODUCTION

Since 1970, serologic studies have linked Epstein-Barr virus (EBV) to Hodgkin's disease (HD). Patients with a history of EBV-associated infectious mononucleosis have been shown to have an increased risk of HD over populations and patients with HD have been shown to have abnormally high levels of antibodies against EBV antigens. EBV genomic DNA was first reported in HD in 1987 [1].

Epidemiologic studies also indicated a casual association between EBV and HD[1,2]. The association of EBV with HD is intimately related to socio-economic status. The EBV positivity ratio in HD is high in developing countries but low in developed countries[1,6].

In this study, the association of EBV with HD in Northeast Anatolia, having low socioeconomical status in Turkey, was estimated according to age, sex, and histologic subtype and the results were compared with those reported from other industrialised and developing countries.

MATERIALS and METHODS

Thirty-six formalin-fixed paraffin-embedded cases of HD were analysed. The cases were derived from the files of the Department of Pathology, Medical School of Atatürk University, Erzurum, Turkey. The morphologic criteria for the diagnosis and classification of HD were previously established [1]. Histologic sections, cut at 3 μm, were stained with monoclonal antibody EBV, which recognises anti EBV latent membrane protein (LMP-1) which was available as a pool of four anti-LMP monoclonal antibodies (CS 1-4) (Dakopatts, Copenhagen; Denmark) by the alkaline phosphatase-anti-alkaline phosphatase (APAAP) method[7]. Paraffin sections were treated with microwaves for 5 minutes in 0.01% citrate buffer before incubation with the antibody. EBV LPM-1 positive lymph node of HD and a normal tonsil were used as positive and negative controls, respectively.

The cases were interpreted as EBV-positive when any Reed-Stenberg (R-S) cell or variant was positive for LPM-1, and as EBV-negative when the R-S cells and variants were negative.

RESULTS

There were 26 males and 10 females, with a 2.6:1 ratio. The age distribution ranged from 12 to 73 years (mean ± standard deviation, 34.1 ± 15.2 years). Both male and female patients had the highest incidence in the third decade[8].

Histologic subtypes included lymphocyte predominance (LP) in 9 (25%), nodular sclerosis (NS) in 4 (11.1%), mixed cellularity (MC) in 18 cases (50%), and lymphocyte depletion (LD) in 5 (13.8%), according to the Rye Classification[8].

Overall, LPM-1 was detected in 27 of the 36 cases (75%). The following histologic subtypes were associated with EBV: LP in 5 of 9 cases, NS in 3 of 4 cases, MC in 18 of 18 cases, and LD in 1 of 5 cases (Table 1). LPM-1 positive patients were 22 male and 5 female. The age range for LPM-1 positive patients was 12-73 years (mean ± standard deviation; 36.3 ± 16.4 years), that for LPM-1 negative patients was 14-59 years (34.7 ± 15.1 years).

DISCUSSION

Hodgkin's disease is one of the most common cancers, but little is known about its etiology. Association between HD and EBV has been demonstrated in epidemiologic, serologic, and molecular biologic studies[3]. Especially the high EBV association with HD in the developing countries has been discussed in relation to high incidence ratio of HD in these areas[2,5].

In most populations, primary EBV infection is asymptomatic. However, in economically advantaged populations, in which viral exposure is delayed until early adulthood, primary infection is frequently associated with the syndrome of infectious mononucleous[6,9]. Healthy Turkish children developed anti-EBV viral capsid antigen (VCA) antibodies at about 1 year of age (46.3%), reflecting early exposure to EBV[10]. In the healthy children, ages under 15, anti-EBC (VCA) antibodies was reported as 75% and 77.7%[11,12].

The percentage of cases of HD with evidence of EBV has varied among different studies. The EBV association in Northeast Anatolia HD, such as other developing countries, was predominantly young adulthood (69% in patients younger than 40 years of age). Epidemiologic studies have shown a peak of incidence in

Table 1. Epstein-Barr virus (EBV) positivity according to histologic subtype, sex, and age

Subtype	Total of cases	Age distribution of EBV-positive cases	EBV-positive cases M	EBV-positive cases F	Positivity ratio to LPM-1
Lymphocyte predominance	9	16-55 (34)	1	4	5/9
Nodular sclerosis	4	30-60 (48)	1	2	3/4
Mixed cellularity	18	12-73 (37)	3	15	18/18
Lymphocyte depletion	5	43 (43)	-	1	1/5
Total	36	12-73 (34)	5	22	27/36

young adulthood among economically disadvantaged populations, while in economically disadvantaged populations no such peak is seen. Also, children who develop HD tend to be from lower social classes. The findings have led to the hypothesis that HD disease may arise as a rare consequence of infection by a common virus and that the risk is markedly increased when infection occurs after childhood, similar to what is observed with infections by polio virus[2,13].

Although EBV-positivity could be detected in nearly 100% of patients with HD in developing countries as well as the Orientals, it was only detected in approximately 45% of HD cases in developed countries[2]. However, the frequency of EBV in HD was reported as different both in developing and developed countries. LPM-1 were detected in 54.3% cases of Taiwan HD, in 60% cases of Costa Rica HD, in 49% cases of Denmark HD and in 26.7% cases of South Africa HD by using immunohistochemistry method[3,14,16].

The frequency of EBV infection in the HD patients in Turkey defined by serology was significantly high reported, e.g. the anti-EBC (VCA) antibodies were shown 100% and 94%[11,12]. Also, EBV-DNA was shown in 88% of Turkish HD patients who had been studied by polymerase chain reaction technique[17]. There is limited information with respect to the association of HD with EBV-related LPM-1 in Turkey. EBV-related LPM-1 positivity has been shown in 75% of HD patients in this study. Similarly, Çavdar et al. reported that LPM-1 was found 73.6% in the Turkish children with HD[12]. On the other hand, Yamaç et al. reported that LPM-1 was found 42% in HD patients[18]. This difference may be related with the socioeconomic status.

The most common histologic subtype of HD is NS in developed countries but MC in many developing countries. The reason for this discrepancy is not known, but it may be related to racial differences, endemic viral infections, or socio-economic conditions[1,3]. The MC subtype of HD has been reported by several developing countries. The most common histologic subtype in this study is MC subtype (50%) like many developing countries, such as Peru, Korea, Egypt, and Turkey[1,19,22].

Several studies including largely adult cases have shown an excess of EBV-positive cases within MC subtype compared to other subtypes. LPM-1 was detected in 84% cases of MC subtype, in 44% cases in other subtype in Japan; in 86% cases of MC subtype, in 20% cases of NS subtype in Costa Rica; in 96% cases of MS subtype, in 10% cases of NS subtype, in 32% cases of LP subtype in Denmark and in 96% cases of NS subtype in Honduras[2,6,14,15].

In this study, as shown in Table 1, the current results revealed that EBV association in this area HD was possibly correlated with the histologic subtypes. The association was found most frequently with MC subtype. Although it was also found at the high ratio in the NS subtype but the number of cases was not enough for a statistical evaluation.

EBV association in male patients with HD was higher than female patients in the most other studies. Liu SM et al. reported 67.9% for male and 47.1% for female[3]. Tomita et al. reported 76% for male and 33% for female[2]. Gulley et al. reported 54% for male and 36% for female[23]. In our study, the male and female ratio was 2.6:1 and the detection rate of female patients (50%) was lower than that of male patients (84.6%). These results were in agreement with the suggestion by Glaser et al. that hormonal factor may be one of the causes in the susceptibility of patients to EBV infection and subsequent disease manifestation[24].

Familial clustering of HD and a three-to-sevenfold increased risk of the disease among the sibling of affected patients suggest that both genetic and environmental factors may be important in the pathogenesis of the disease. Detailed HLA studies may help to elucidate the complex variations between populations in the risk of HD and its principal subtypes[5]. However, genetic susceptibility does not exclude a role for environmental factors in the pathogenesis of HD[23]. To determine this, environmental and genetic studies should be combined.

There is no definitive evidence that EBV plays a causal role in HD, but the available data suggest that it is likely to play a role in the pathogenesis of HD, particularly in MC subtype, and raises the possibility that there are important socio-economic status and geographic factors in the EBV association with HD.

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