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Seroprevalence of Human Herpes Simplex, Hepatitis B and Epstein-Barr Viruses in Children with Acute Lymphoblastic Leukemia in Southern Iran

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Abstract To investigate the seroprevalence of Herpes Simplex Viruses (HSV1 and HSV2), Ebstein-Barr Virus (EBV) and Hepatitis B Virus (HBV) in children with acute lymphoblastic leukemia (ALL) in southern Iran. 90 patients with ALL and 90 age-sex matched healthy participants were enrolled in this study. Antibodies (IgG) against HBsAg, HSV1, HSV2, EBV different antigens including Epstein-Barr nuclear antigen-1 (EBNA-1), viral capsid antigen (VCA) and early antigen (EA) were measured by enzyme-linked immunosorbent assay (ELISA). There were 54 (60%) male and 36 (40%) female in both study groups with mean age of 8.47±3.61 and 8.61±2.84 years in case and control group respectively ($P= 0.812$). The prevalence of antibodies against HBsAg ($P= 0.002$), HSV1 ($P< 0.0001$), VCA ($P= 0.021$) and EA ($P< 0.0001$) antigens of EBV were significantly higher in ALL patients. With the results of this study, we could not exclude a connection between these viral infections and later leukemogenesis in childhood ALL, although nor latent infection nor congenital infection cannot be excluded by this method.

Keywords Acute Lymphoblastic Leukemia (ALL) · Epstein-Barr Virus (EBV) · Hepatitis B Virus (HBV) · Human Herpes Simplex Virus (HSV) · Iran

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children [1]. Many studies have focused on the natural history of this disease but have received little advantage. Epidemiologic studies [2, 3] suggest that infectious agents play role in at least one step in the process of ALL [4]. Defective or inhibited repair of DNA breaks will results in translocations (the hallmark genetic mutations of ALL) and this is a key step in pathogenesis of ALL. Previous studies have shown that some DNA viruses' infection, notably herpes viruses, Epstein-Barr virus (EBV) and Hepatitis B virus (HBV) interferes with cellular DNA repair mechanisms [5–7] and could lead to these characteristic genetic changes. Previous studies tried to determine the association between prenatal virus infections and development of pre-leukemic cells containing characteristic translocations; however they showed no association between infection with polyoma viruses [8], herpesviruses [9, 10], or parvovirus B19 [11] and childhood ALL. We thereby performed this study in order to investigate whether HSV, EBV and HBV infections could be correlated with ALL in southern Iran.

Materials and Methods

In this cross-sectional case control study, a total of 90 patients were enrolled with a bone marrow biopsy-confirmed ALL admitted to Nemazee Hospital of Shiraz University of Medical Sciences, Shiraz, Iran from 2007 to 2008. The patients transfused in the last 6 months were excluded. Also, major surgery, tooth extraction, parenteral treatments, ongoing viral infections at admission to hospital, congenital abnormalities, other malignancies and serious diseases like blood culture positive sepsis, endocrine or hepatic disorders, and immune deficiency state were exclusion criteria. An age-sex matched control group of 90 healthy children was selected from those who referred to Motahari clinic (Shiraz, Iran) for routine check-ups. Those with ongoing viral infections, malignancies, congenital abnormalities, tooth extraction, previous major surgeries and parenteral therapies were excluded from the study. Written informed consent was obtained from all patients, controls and parents. The local ethics committee of the Shiraz University of Medical Sciences approved the study protocol.

A blood sample was drawn from each patient at the time of diagnosis and was transferred to Shiraz Institute for cancer Research for further analysis. For the HBV, EBV and HSV infection screening, a third-generation, sandwich enzyme-linked immunosorbent assay (ELISA) was used. All kits were purchased from Bioprobes Srl, Milan, Italy. The measurements were performed according to the manufacture's guidelines.

With this method we measured the antibodies (IgG) against HBsAg, HSV1 and HSV2 surface antigens, Ebstein-Barr nuclear antigen-1 (EBNA-1), viral capsid antigen (VCA) and early antigen (EA).

Statistical Analysis

All statistical analyses were performed with the Statistical Package for Social Sciences version 15.0 (SPSS Inc., Chicago, IL, USA). The results are expressed as mean value ± standard deviation. Chi-square test was used for comparison between groups. A two-tailed *P*-value less than 0.05 was considered statistically significant.

Results

There were 54 (60%) male and 36 (40%) female in both study groups. The mean age was 8.47±3.61 and 8.61± 2.84 years in case and control group respectively (*P= 0.812*). The seroprevalence of study viruses in both groups is shown in Table 1.

Forty four patients (48.8%) in the case group tested positive for HBs Ab while 22 (24.4%) of controls were found to be positive (*P= 0.002*). In the same way patients had higher prevalences of antibodies against VCA (*P= 0.021*) and EA (*P< 0.0001*) antigens of EBV as well as HSV1 (*P< 0.0001*). There was no difference regarding the prevalence of EBV (EBNA-1) IgG (*P= 0.061*) and HSV2 between two groups (*P= 0.129*).

Table 1 The seroprevalence of HBV, EBV and HSV in patients and controls

	Cases (*n*=90)	Controls (*n*=90)	*P*-value
HBs Ab	44 (48.8%)	22 (24.4%)	0.002
EBV (EBNA-1) IgG	40 (44.4%)	44 (48.8%)	0.061
EBV (VCA) IgG	28 (31.1%)	13 (14.4%)	0.021
EBV (EA) IgG	10 (11.1%)	1 (1.1%)	<0.0001
HSV1 IgG	74 (82.2%)	49 (54.4%)	<0.0001
HSV2 IgG	0 (0%)	2 (2.2%)	0.129

Discussion

In this study we investigated the relationship between the previous infection with three viruses including HSV, HBV and EBV and later ALL. These three DNA viruses have already shown to play an important role in the pathogenesis of different cancers through their interference with cellular DNA repair mechanisms [5–7]. We found that the prevalence of HBV, HSV1 and EBV was significantly higher in those who were diagnosed to have ALL. In the other hands, HSV2 and the antibodies against EBNA-1 antigen of EBV were not significantly different among cases and controls. This shows that HBV, HSV1 and EBV may play a role in the pathogenesis of ALL. However it is not known that these infections are acquired or congenital. It is hypothesized that in utero infections with potentially oncogenic viruses could be involved in pathogenesis of ALL by inducing genomic instability in B lymphocytes. Studies on twins suggest that a postnatal molecular event or exposure is required to expand pre-leukemic clone. Infectious agents especially DNA viruses can increase the stress on lymphocyte precursor proliferation and thus accelerate the transition of pre-leukemic clones to leukemic ones [12, 13].

Kinlen in two separate studies showed that the incidence of leukemia will rise in populations that have been composed of previously separated urban and rural populations by facilitating transmission of infective agents [14, 15]. Most authors use space-time clustering at diagnosis [16, 17], while others prefer space-time clustering around the time of birth [18–20]. While this may reflect exposures operating in utero, the leukemogenic infection may occur after birth since addresses used in space-time clustering studies often apply well after birth. Knox et al. have

suggested another model for virus transmission during pregnancy by observing maternal transmission of leukemia in cats [21]. This maternal transmission can be a result of either primary maternal infection or reactivation of the latent infection.

In this regard Bogdanovic and colleagues [9] used the Guthrie cards of neonates who later developed ALL to detect human herpes virus 6 (HHV-6) and EBV. But all the subjects tested negative for these two viruses. They concluded that childhood ALL is unlikely to be associated with an in utero infection with EBV or HHV-6. In the same way Gustafsson and colleagues [22] detected adenovirus DNA in 13 of 49 neonatal blood spots from ALL patients but only in 3 of 47 controls ($P= 0.012$) suggesting a correlation between prenatal adenovirus infection and the development of ALL. However, in a study which was performed in Egypt, Loutfy and colleagues [23] found a higher exposure of HSV1 and HSV2 both primary infection and reactivation among ALL patients. But they couldn't correlate the ALL with EBV and CMV.

EBV has close relationship with the endemic form of Burkitt lymphoma, the most common childhood cancer in equatorial Africa and it was first discovered because of this relationship [24, 25]. Lethal lymphoproliferative disease in patients undergoing organ and stem cell transplantation is a result of EBV reactivation [19] and this demonstrates its onclogical potential. Besides the transplant setting, EBV is also frequently reactivated during pregnancy [26]. Lehtinen and colleagues [27] showed that reactivation of EBV during the first trimester of pregnancy, increases the risk of ALL in the offspring.

In conclusion we showed that the seroprevalence of EBV, HSV1 and HBV was higher in patients with ALL. Hence, we could not exclude a connection between these viral infections and later leukemogenesis in childhood ALL, although nor latent infection nor congenital infection cannot be excluded by this method. In view of the epidemiological evidence for a relation between childhood ALL and infection, the search for a virus etiology must continue.

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