Journal of Leukemia
ISSN: 2329-6917

Zareifar et al., J Leuk 2016, 4:3
DOI: 10.4172/2329-6917.1000213

Research Article Open access

Detection of Epstein–Barr virus in Pediatric Lymphoma: A Single Center Study

Zareifar S[1*], **Kazemi B**[2], **Arzanian MT**[3] **and Bandehpour M**[2]

[1]*Hematology Research Center, Pediatric Hematology/Oncology Department, Shiraz University of Medical Sciences, Shiraz, Iran*

[2]*Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[3]*Department of Pediatric Hematology, Oncology, Mofid Children's Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran*

[*]**Corresponding author:** Soheila Zareifar, Pediatric Hematologist/Oncologist, Shiraz University of Medical Sciences, Shiraz, Iran, Tel: +9891771787827; E-mail: zareifars@sums.ac

Received date: Jul 26, 2016; **Accepted date**: Aug 22, 2016; **Published date**: Aug 30, 2016

Abstract

Background: Efforts were made in order to identify the etiologic factors in pediatric cancers. Several studies have suggested a probable etiologic association between lymphoma and Epstein-Barr virus (EBV); therefore, the aim of this study was to investigate the association of EBV in childhood lymphoma.

Materials and method: Paraffin block of 63 pediatric patients with lymphoma was studied for detection of EBV latent membrane protein 1 (LMP-1). The clinical data regarding age, sex, type of lymphoma and histology, stage of the disease of the patients treated in a retrospective consecutive manner for 5 years were used.

Results: Sixty-three eligible patients including 41 (65%) patients with non-Hodgkin lymphoma (NHL) and 22 (34.9%) of Hodgkin lymphoma (HL) were assessed. The male to female ratio was 3.84/1. Regarding the gender, the overall difference between NHL and HL was statistically significant. With respect to age, the difference between HL and NHL was not significant. EBV LMP1 gene transcripts were found in 65.8% of children with NHL and 59% of children with HL. Regarding the type of lymphoma, LMP1 positivity was not statistically significant (P=0.087).

Conclusion: EBV infection may be a factor involved in the high incidence of pediatric lymphoma; our study suggests a positive influence of EBV infection in pediatric lymphomas.

Keywords: Epstein barr virus, LMP-1; Non hodgkin lymphoma; Hodgkin lymphoma; Pediatric

Introduction

Epstein-Barr virus (EBV), known as human herpes virus 8, is a unique virus. The virus has infected more than 95% of the world population. EBV virus mainly targets B cells, lymphocytes and nasopharyngeal epithelial cells [1]. In developing countries, Epstein-Barr virus infection occurs often in poor communities in infancy and early childhood. The Epstein-Barr virus infection in industrialized countries is generally more common in childhood, but about a third of cases occur during adolescence and young adulthood [2-5]. Like other herpes family viruses, EBV after primary infection stays dormant throughout the life of the infected person. In the oropharynx, latent virus is carried in B-lymphocytes and epithelial cells as multiple nuclear episomes. Overall, after infection, EBV remains consistently in resting memory B cells [3,5].

In infected B cells which are in latent state, six nuclear proteins, two membrane proteins and two non-translated RNA are expressed. During latency, the EBV limits gene expression and therefore reduces viral proteins expression; thus, the infected cells cannot be identified by cytotoxic T cells. EBV LMP-1 is a viral protein that acts as an oncogene [6,7] and the expression of this protein in transgenic strains can cause B cell lymphoma. LMP-1 protein also causes a signal in a manner similar to an active form of cell surface molecule CD40 on B cells [8-10].

Epidemiological studies indicate that the EBV may be involved in development of African Burkitt lymphoma. Ugandan children also have high titers of antibodies against structural proteins of the virus and are considerable risk for Burkitt lymphoma [11]. Patients with Hodgkin's disease usually have higher titers of antibodies against structural proteins of the EBV before and after developing lymphomas, when compared to the normal population [12,13].

Material and Methods

The aim of the present cross-sectional retrospective study was to detect EBV genome (latent membrane protein 1, LMP1) in paraffin block samples of children with lymphoma aged less than 18-years-old in a large tertiary-care referral pediatric oncology center during 10 years, in Tehran, Iran. The medical records of all patients who were diagnosed with NHL, were retrieved from archives of Hematopathology ward in Mofid children's hospital affiliated to Shahid Beheshti University of Medical Sciences. The study enrolled 63 patients including 41 NHL cases and 22 HL cases who had undergone courses of chemotherapy. All cases of the lymphoma had been diagnosed according to the latest WHO classification. Diagnosis was based on morphology and immunohistochemistry (IHC) panel of the paraffin blocks by two pathologists. The paraffin blocks of these patients were used for EBV DNA extraction and PCR in Cell and Molecular Research Department. To investigate EBV frequency, IHC was used for identification of LMP-1 (latent membrane protein, monoclonal antibodies clones CS. 1-4, DAKO).

Variables included age, sex, type of lymphoma, and detection of EBV virus genome, LMP1 protein. According to hospital records in archives, the demographic data of children with lymphomas were recorded on the questionnaire form. The study was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences.

Statistical analysis

After data collection, the data was entered as special codes. Statistical analysis was performed using SPSS software (version 15, Chicago, Illinois, USA) and analyzed using Fisher's analysis. Based on the objectives of the study, the frequency and percentage was achieved. Both groups of variables/lymphoma's variables were assessed using T-test. P values less than 0.05 were considered as statistically significant.

Results

Of 75 patients who entered this study, the DNA was extracted from 63 patients including 41(65%) patients with NHL and 22 (34.9%) of HL samples. Due to insufficient DNA extraction, 12 patients were excluded. The characteristics of patients with lymphoma and EBV infection are shown in Table 1.

	Total patients N=63	Hodgkin disease N=23	Non Hodgkin lymphoma N=41	P value
Age	9.21 ± 3.22	9.72 ± 3.70	8.8 ± 2.88	0.095
Male/female	3.84/1	2.4/1		0.095
LMP1	40	13	27	0.087

Table 1: The characteristics of patient with lymphoma and EBV infection

Sex

The total 63 patients with lymphoma, 50 cases (79.4%) were male and 13 (20.6%) were female. Generally, the male to female ratio was 3.84/1. The NHL subgroup included 35 boys (85.4%) and 6 (14.6%) girls and male to female ratio was 5.8/1. In children with HD, the male to female ratio was calculated 2.4/1, including 15 (68.2%) males and 7 (31.8%) females. Regarding gender, the overall difference between NHL and HL was statistically significant (P=0.02).

Age

In this study, the mean age of the patients was 9.21 ± 3.22 years (2.5-15 years). Children were divided by age into three groups: less than 5, 5-10, and older than 10 years. Thirty-two patients (50.8%) were aged less than 5 years, 23 patients (36.5%) 5-10 years and 8 patients (12.7%) were older than 10 years. Most of our cases were less than 10 years (87.3%).

In NHL subtype, the mean age of children was recorded 8.8 ± 2.88 years (2.5-13 year range). In these patients, 25 children (61%) were under 5 years, 12 children (29.3%) were 5-10 years and 4 (8.9%) were in the age group above 10 years. About 90.3% of NHL cases were also less than 10 years. The average age in the HL group was calculated 9.72 ± 3.70 years (4-15 years). In terms of age distribution in HL, 7 children (31.8%) were less than 5 years, 11 children (50%) 5-10 years and 4 children (18.2%) were aged above 10 years. In the HL group also, most of the cases were less than 10 years (81.8%). Regarding age, the difference between NHL and HL was not statistically significant (P=0.095).

LMP1

The DNA was extracted from paraffin blocks of 63 samples, and PCR was performed. LMP1 antigen was detected in 40 patients (62.5%) including 27 (67.5%) cases with NHL and 13 (32.5%) patients with HL. It means that 65.8% of children with NHL and 59% with HL were positive for Epstein-Barr virus (antigen LMP1). Regarding the type of lymphoma, LMP1 positivity was not statistically significant (P=0.087). Twenty-four children under 5 years-old (60%) were positive for Epstein-Barr virus. 11 cases (27.5%) were 5-10 years and 5 children (12.5%) were in the older than 10 years-old age group.

In the NHL group, LMP1 was positive in 19 (70.37%) cases less than 5 years of age, 5 patients (18.5%) aged 5-10 years, and 3 of them (11.11%) aged older than 10 years. There was a negative correlation between age and LMP1 positivity in the NHL group which was statistically significant (P=0.02). From 13 patients with HL who were positive for LMP1, 5 children (38.5%) were in the age group less than 5 years, 6 cases (46.2%) in 5-10 years-old group and 2 children(15.4%) in the age group above 10 years.

Relationship between lymphoma and EBV

The DNA was extracted from 63 paraffin block samples and tested by PCR for LMP1 antigen. LMP1 was positive in 40 cases (62.5%) including 27 NHL (67.5%) and 13 HL patients (32.5%). It can be concluded that 65.8% of children with NHL and 59% of those with HL were positive for Epstein-Barr virus LMP-1 antigen. Regarding the LMP1 positivity, the difference between NHL and HL was not statistically significant (P=0.087).

Lymphoma subtypes

The patients with NHL were categorized into B cell group (n=30, 73.1%) and T cell NHL (n=11, 26.8%). LMP1 was detected in 22 B cells NHL and 5 T cells NHL. This difference was statistically significant (P=0.001). The histopathology diagnosis of 22 cases with HL revealed three subtypes including mixed cellularity (MC) (n=10, 40.9%), nodular sclerosis (NS) (n=8,36.3%), and lymphocytic predominance (LP) (n=4, 18.1%). LMP 1 was detected in MC (n=7, 53.8%), NS (n=5, 38.46%) and LP (n=1, 7.7%), respectively. In our study, no cases of lymphocytic depleted HL were observed.

Discussion

The role of Epstein-Barr virus in the pathogenesis of lymphomas is not clear, nor is it evident whether the association of virus is essential in cancer development.

In the present study, the frequency of NHL and HL was 65% and 35%, respectively; the results are comparable with some developing countries. In a study by Castella et al. in the United Arab Emirates, 208 cases were evaluated; of these, 41% were diagnosed as non-Hodgkin's lymphomas and 59% of the sample group was HL [14]. This was different from our study in which NHL was more frequent than HL.

A research in Oman revealed that the frequency of NHL and HL was 65% and 35%, respectively; 59% NHL and 41% HL were reported, which is roughly similar to our study [15].

A study by Al-Salam S showed that EBV was seen in 38% of cases of HL and it was predominately seen in the MC subtype followed by NS, LD and LR subtypes, respectively. This result of this study was similar to our research. EBV was more frequently expressed in HL in the pediatric age group than the adult age group. This study suggested that all cases of HL should be assessed for EBV status, because its presence might have a significant impact on prognosis and response to therapy [16]. Zhou et al. in an investigation showed that EBV was identified in 72% of HD cases, and pediatric HD was also associated with EBV, irrespective of histological subtype [17]. It was different from the rate of our patients (35%). This study found that virus detection in Chinese children was more than adults [17]. Almasri showed that in Jordan, EBV was detected in 47% of HD cases and similar to our cases they were mostly in mixed cellularity and nodular sclerosis subtypes. Epstein-Barr virus was seen in 73% of HD cases in children under 15 years of age as opposed to 34% of the young adult group [18]. It is comparable with our results, in which most of our cases were less than 10 years of age. This finding confirms the fact that, in most underdeveloped countries, the initial contact with Epstein Barr virus (EBV) usually happens in the first decade of life and results in an asymptomatic infection, whereas in developed areas, primary infection in adolescence or adulthood is accompanied by infectious mononucleosis in 50% of cases [19]. Nerurkar et al. showed that 60.8% of Indian children with HD carried EBV genome, which differs from our study results [20].

In our study, most of our HL cases were less than 10 years which is similar to the results of De Matteo's study [21]. In a study in Argentina, EBERs was expressed in 37% of pediatric Burkitt's lymphoma (29% of immunocompetent and 100% of immunosuppressed patients). EBV-positive cases were observed exclusively in patients younger than 5 years old [22]. In none of these studies was the relationship between sex and EBV detection evaluated. An evaluation of NHL in Malaya revealed that Epstein-Barr virus association was more prevalent in B-cell than T-cell lymphomas. The most common EBV-associated tumor was Burkitt's lymphoma, and there was an increased risk of EBV association for Burkitt's lymphoma in Chinese patients [23]. Roa et al. showed that association of Burkitt's lymphoma with EBV and their clinical signs based on geographical area in developing countries varies from 25- 80%. In this study, similar to our study, the LMP-1 was used for virus detection [24].

In another study on 54 patients with Burkitt's lymphoma in Brazil, the virus positivity rate of 87% in native and 20% in German patients was reported [25]. In another study in Turkey, this is geographically similar to Iran, independent histological subtypes of HD, EBV were detected in 30% of abdominal tumors [26].

In our study, the occurrence of NHL and HD in male patients was more than the female cases. Paraffin block samples of all patients to Epstein-Barr virus DNA and LMP-1 gene was evaluated by sensitive PCR assay. A considerable proportion of our patients (62.5%) were positive for LMP-1 gene. The LMP1 gene frequency in the NHL population was higher than in HD group (67.5% vs 32.5%). These results are consistent with those of some studies in other countries [27]. Most of the Epstein-Barr virus-positive patients in our study were categorized in younger age groups (under 5 years).

Conclusion

The results of the present study were similar to some previous studies indicating the relationship of Epstein-Barr virus and its protein products with pathogenesis of lymphoma, particularly NHL. Regarding high incidence of lymphoma in our country, other risk factors should also be considered.

Acknowledgement

We would like to express our special thanks to Sheryl Nikpour for English editing of the manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Papesch M, Watkins R (2001) Epstein-Barr virus infectious mononucleosis. Clinical Otolaryngology & Allied Sciences 26: 3-8.
2. Miller, George (2006) Epstein–Barr Virus. NEJM 355: 2708-2709.
3. Luzuriaga K, Sullivan JL (2010) Infectious mononucleosis. NEJM 362: 1993-2000.
4. Maeda E, Akahane M, Kiryu S, Kato N, Yoshikawa T, et al. (2009) Spectrum of Epstein–Barr virus-related diseases: a pictorial review. Jpn J Radiol 27: 4-19.
5. Prince HN, Prince DL (2001) Principles of viral control and transmission. In S. S. Block (Ed.), Disinfection, sterilization and preservation pp: 543-571.
6. Ratt Z, Zhang J, Sugden B (2012) The latent membrane protein 1 (LMP1) oncogene of Epstein–Barr virus can simultaneously induce and inhibit apoptosis in B cells. J Virol 86: 4380-4393.
7. Gupta (2011) Latent Membrane Protein 1 as a molecular adjuvant for single-cycle lentiviral vaccines. Retrovirology 8: 1.
8. Li H, Chang Y (2003) Epstein–barr virus latent membrane protein 1: Structure and functions. J Biomed Sci 10: 490-504.
9. Rastelli J, Homig-Holzel C, Seagal J, Mulle W, Hermann AC, et al. (2008) LMP signaling can replace CD40 signaling in B cells in vivo and has unique features of inducing class-switch recombination to IgG1. Blood 111: 1448-1455.
10. Soni V, Cahir-McFarland E, Kieff E (2007) LMP1 TRAF ficking activates growth and survival pathways. Adv Exp Med Bio 597: 173-87.
11. Shah KM, Young LS (2009) Epstein-Barr virus and carcinogenesis beyond Burkitt's lymphoma. CMI 15: 982-988.
12. Herling M, Rassidakis GZ, Medeiros LJ, Vassilakopoulos TP, Kliche KO, et al. (2003) Expression of Epstein–Barr virus latent membrane protein-1 in Hodgkin and Reed-Sternberg cells of classical Hodgkin's lymphoma: associations with presenting features, serum interleukin 10 levels, and clinical outcome. Clin Cancer Res 9: 2114–2120.
13. Andersson J (2006) Epstein–Barr virus and Hodgkin's lymphoma. Herpes 13: 12-16.
14. Castella A, Joshi S, Raaschou T, Mason N (2001) Pattern of malignant lymphoma in the United Arab Emirates--a histopathologic and immunologic study in 208 native patients. Acta Oncol 40: 660-664.
15. Bamanikar S, Thunold S, Devi KR, Bamanikar A (1995) The pattern of malignant lymphoma in Oman. J Trop Med Hyg 98: 351-354.
16. Al-Salam S, John A, Daoud S, Chong SM, Castella A, et al. (2008) Expression of Epstein-Barr virus in Hodgkin lymphoma in a population of United Arab Emirates nationals. Leuk Lymphoma 49: 1769-1777.
17. Zhou XG, Sandvej K, Li PJ, Ji XL, Yan QH, et al. (2001) Epstein-Barr virus (EBV) in Chinese pediatric Hodgkin disease: Hodgkin disease in young children is an EBV-related lymphoma. Cancer 92: 1621-1631.
18. Almasri NM, Khalidi HS (2004) Epstein-Barr virus expression in Hodgkin's disease in Jordan. Saudi Med J 25: 770-775.
19. Chabay PA, Preciado MV (2013) EBV primary infection in childhood and its relation to B-cell lymphoma development: a mini-review from a developing region. Int J Cancer 133: 1286-1292.

20. Nerurkar AY, Vijayan P, Srinivas V, Soman CS, Dinshaw KA, et al. (2000) Discrepancies in Epstein-Barr virus association at presentation and relapse of classical Hodgkin's disease: impact on pathogenesis. Ann Oncol 11: 475-478.
21. De Matteo E, Baron AV, Chabay P, Porta J, Dragosky M, et al. (2003) Comparison of Epstein-Barr virus presence in Hodgkin lymphoma in pediatric versus adult Argentine patients. Arch Pathol Lab Med 127: 1325-1329.
22. Lara J, Cohen M, De Matteo E, Aversa L, Preciado MV, et al. (2014) Epstein-Barr virus (EBV) association and latency profile in pediatric Burkitt's lymphoma: experience of a single institution in Argentina. J Med Virol 86: 845-850.
23. Peh SC, Nadarajah VS, Tai YC, Kim LH, Abdullah WA, et al. (2014) Pattern of Epstein-Barr virus association in childhood non-Hodgkin's lymphoma: experience of university of malaya medical center. Pathol Int 54: 151-157.
24. Rao CR, Gutierrez MI, Bhatia K, Fend F, Franklin J, et al. (2000) Association of Burkitt's lymphoma with the Epstein-Barr virus in two developing countries. Leuk Lymphoma 39: 329-337.
25. Araujo I, Foss HD, Bittencourt A, Hummel M, Demel G, et al. (1996) Expression of Epstein-Barr virus-gene products in Burkitt's lymphoma in Northeast Brazil. Blood 87: 5279-5286.
26. Cavdar AO, Yavuz G, Babacan E, Gözdasoglu S, Unal E, et al. (2003) Burkitt's lymphoma in Turkish children: clinical, viral [EBV] and molecular studies. Leuk Lymphoma 14: 323-330.
27. Vassallo J, Metze K, Traina F, de Souza CA, Lorand-Metze I (2001) Expression of Epstein-Barr virus in classical Hodgkin's lymphomas in Brazilian adult patients. Haematologica 86: 1227-1228.