Detection and Genotyping of Epstein-Bar Virus Among Paraffin Embedded Tissues of Hodgkin and Non-Hodgkin's Lymphoma Patients in Ahvaz, Iran

Ala Habibian[1,2], Manoochehr Makvandi[1,2], Alireza Samarbaf-Zadeh[1,2], Niloofar Neisi[1], Rahim Soleimani-Jelodar[1], Kamyar Makvandi[3], Shima Izadi[1]

[1] Infectious and Tropical Diseases Research Center, Health Research Institute, Jundishapur University of Medical Sciences, Ahvaz, Iran

[2] Department of Virology, Jundishapur University of Medical Sciences, Ahvaz, Iran

[3] Department of Orthopedic Surgery, Taleghani Hospital Research Development Unit, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Received: 16 Jul. 2017; Accepted: 18 Dec. 2017

Abstract- Epstein-Barr virus (EBV) is a DNA virus which belongs to the Herpesviridae family, has been infected with 90% of the world's population. EBV is transmitted through saliva and associated with different diseases such as Infectious Mononucleosis, Nasopharyngeal Carcinoma, Burkitt`s lymphoma, Hodgkin and Non-Hodgkin`s lymphoma. This study was designed to determine the frequency of EBV DNA and genotyping of this virus in histological tissues of Hodgkin and Non-Hodgkin's lymphoma in Ahvaz, Iran. In this study, 12 samples of Hodgkin and 29 samples of Non-Hodgkin`s lymphoma were examined from Ahvaz Imam Khomeini Hospital. After deparaffinization and DNA extraction, Nested-PCR technique was carried out on Epstein-Barr nuclear antigen 1 (EBNA-1) and Epstein-Barr nuclear antigen 3C (EBNA-3C) regions in order to detect EBV genome and genotype in tumoral tissues. Among 12 cases of Hodgkin`s lymphoma, 3 (25%) cases and among 29 cases of Non-Hodgkin's lymphoma, 14 (48%) cases were positive for EBV. All 3 EBV positive cases of Hodgkin`s lymphoma and a number of 8 (57%) cases of Non-Hodgkin`s lymphoma belonged to the adolescence age group which proves an association between age and EBV positive in Hodgkin and Non-Hodgkin`s lymphoma according to Fisher`s exact test ($P$=0.03). All EBV positive cases in Hodgkin and Non-Hodgkin`s lymphoma were EBV-1. The results of this study revealed that EBV played a possible important role in Hodgkin and Non-Hodgkin`s lymphoma especially in adults. Also, the main genotype of virus in the current study was EBV-1.

Acta Med Iran 2018;56(7):434-440.

Keywords: EBNA-1; EBNA-3C; Epstein-Barr virus; Hodgkin lymphoma; Non-hodgkin lymphoma

Introduction

Epstein-Barr virus (EBV), or human herpesvirus 4, is a lymphotropic herpesvirus which belongs to the subfamily of γ-herpesvirus in Herpesviridae family (1). EBV is the causative agent of Infectious Mononucleosis (IM) as a primary infection, and it may be subclinical in early childhood (2,3). EBV is widespread in the human population. Its antibodies are found in most serums of children all over the world, and by adulthood, more than 90% of human populations are infected with EBV (1,4). After primary infection, the genome of virus is established latency in B-lymphocytes and then it can be expressed after a while as some malignant disorders like Hodgkin`s lymphoma (HL), Nasopharyngeal carcinoma, Gastric carcinoma, Burkitt`s lymphoma (BL), B and T cell non-Hodgkin`s lymphoma which were shown to be associated with EBV (4-6).

There are three different types of latency in the pattern of EBV gene expression. In fact, virus infection is described by a state of viral latency as an episome in association with host chromosome (3,7). During latency infection time, the virus can express some limited genes such as six EBV Nuclear Antigens (EBNA-1,EBNA-2,EBNA-3A,EBNA-3B,EBNA-3C and EBNA-LP), three Latent Membrane Proteins (LMP-1,LMP-2A, and LMP-2B) and two EBV encoded RNAs (EBER-1 and EBER-2) (8). Expression of all latent genes is observed in Lymphoproliferative Disease (LPD) in immunocompromised hosts and IM patients. This pattern refers to the third type of latency (latency III). In Hodgkin`s lymphoma and Nasopharyngeal Carcinoma

Corresponding Author: M. Makvandi
Infectious and Tropical Diseases Research Center, Health Research Institute, Jundishapur University of Medical Sciences, Ahvaz, Iran
Tel: +98 61 33354389, Fax: +98 61 33361544, E-mail address: manoochehrmakvandi299@gmail.com

(NPC) which are seen in the second program (latency II), some restricted genes such as EBNA-1, LMP-1, LMP-2, and EBERs are expressed. In the first pattern of latency (latency I) EBNA-1 and two EBERs are expressed. This type of latency is associated with (BL) (3,7).

Polymerase Chain Reaction (PCR) method is used for detection and strain distinction (9). Based on this method, there are two different types of EBV, A and B or 1 and 2, sequencing of EBNA-3A, EBNA-3B, and EBNA-3C, are known to be targeted for genotyping of EBV. The alleles of two types are different in predicted primary sequence by 16%, 20%, and 28%, respectively. Type 1 of EBV is predominant in the developed countries, while type 2 of EBV is common in Africa and New Guinea (3,10). EBNA-3C plays an important role in the transformation of B lymphocyte to lymphoblastoid cell lines (LCLs) (3,11).

EBNA-1 has a critical role in maintaining EBV in the infected cells, and it is essential for replication of the genome in the latently infected cells. It has also activator role for transcription (8,12). It has been considered to have a role in cell growth and survival (3).

Materials and Methods

In this cross-sectional study, 29 non-Hodgkin`s lymphoma and 12 Hodgkin`s disease paraffin-embedded tissue blocks were collected during March 2005 to March 2012, from the Imam Khomeini Hospital of Ahwaz. Their diagnoses and histological classifications were confirmed in a pathological part of the hospital, and their immunohistochemical assay was approved at the Pars laboratory in Ahvaz.

Removal of paraffin

Deparaffinization was done using of xylene and ethanol. 10 µm slices were put on the 1.5 ml autoclaved microcentrifuge tubes. Each tube of samples exposed to 1 ml xylene for 15 minutes for twice and then centrifuged at 14000 rpm for 1 minute after each stage and the supernatant aspirated. Then added 1ml absolute ethanol for 10 minutes, centrifuged at 14000 rpm for 1 minute and discard its supernatant. Also added 70% Ethanol in the same condition, discarded supernatant and took the pellet to use as a tissue for DNA extraction.

DNA extraction

High Pure PCR Template Preparation kit (Roche, Germany, Code No: 11796828001) was used for the extraction of DNA, according to the manufacturers` instruction.

Detection of EBV by PCR for EBNA-1

PCR for the region of EBNA-1 gene was carried out using extracted DNA from tissue in a 25µl mixture, containing 2.5 µl PCR Buffer (Roche), 0.5 µl deoxynucleotide triphosphate (Roche), 0.2 µl Taq Polymerase (Roche), 0.2 µl of each primer was used. The thermal conditions used in two steps PCR described in table 1.

Table 1. Primers of the EBNA-1 used for PCR and thermal cycling conditions

Primer	Sequence	PCR thermal cycling conditions
First cycle	5`-GTAGAAGGCCATTTTTCCAC-3` 5`- CTCCATCGTCAAAGCTGCA -3`	94°C for 5 min: 1 cycle 94°C for 45 s, 54.5°C for 45 s, 72°C for 45 s: 35 cycles 72°C for 10 min; final extension
Nested cycle	5`- AGATGACCCAGGAGAAGGCCCAAGC -3` 5`- CAAAGGGGAGACGACTCAATGGTGT -3`	94°C for 5 min: 1 cycle 94°C for 45 s, 58.5°C for 45 s, 72°C for 45 s: 35 cycles 72°C for 10 min; final extension

Nested PCR

The PCR conditions were same as described aforementioned with the set of Nested primers and different annealing temperature which are reported in Table 1.

The positive control was extracted DNA from an EBV cell line, B95.8, and the negative control was a mixture of samples without DNA.

The products of PCR were separated on a 2% agarose gel and developed by Safe Stain under voltage at 100 V. The results were observed under ultraviolet transilluminator. The sizes of bands were compared with 100 bp Ladder (Fermentas). Cell line B95.8 was used as a positive control to detect EBV DNA (13). Amplification with EBNA-1 primers resulted in production of 300 base-pair fragments (10).

EBV typing by PCR for EBNA-3C

In order to determine the type of EBV, PCR was performed by amplifying a strain-specific sequence in the EBV 3C Nuclear Antigen (EBNA-3C). As described aforementioned, all PCR reactions were performed in a total volume of 25 µl which contained 2.5 µl PCR Buffer (Roche), 0.5 µl, deoxynucleotide triphosphate (Roche), 0.2 µl Taq Polymerase (Roche), 0.2 µl of each primer sequence and PCR condition which are reported in Table 2.

Table 2. Primers of the EBNA-3C used for PCR and thermal cycling conditions

Primer	Sequence	PCR thermal cycling conditions
First cycle	5`-GAGAAGGGGAGCGTGTGTTGT-3` 5`-GGCTCGTTTTTGACGTCGGC-3`	94°C for 5 min: 1 cycle 94°C for 45 s, 53°C for 45 s, 72°C for 45 s: 35 cycles 72°C for 10 min; final extension
Nested cycle	5`-TCATAGAGGTGATTGATGTT-3` 5`-ATGTTTCCGATGTGGCTTAT-3`	94°C for 5 min: 1 cycle 94°C for 45 s, 47°C for 45 s, 72°C for 45 s: 35 cycles 72°C for 10 min; final extension

Nested PCR

The PCR conditions were the same as described previously with the set of Nested primer and different annealing temperature which are reported in table 2.

Gel electrophoresis and PCR product purification

8 µl of each product was analyzed using the electrophoresis. The results of amplification reactions were expected 246 and 153 base-pair for EBV type 2 and EBV type 1 respectively. In the following round, the amplified fragment by nested PCR was 75 base-pair for EBV-1 and 168 base-pair for EBV-2.

Sequencing

The second cycle of PCR products were sent out to Korean Bioneer Company to sequence DNA. After use of Bioinformatics program in www.ncbi.gov, EBV genotype was surveyed, and the mentioned results were confirmed.

Statistical analysis

Results were analyzed by the version 17 of SPSS software, and the role of age and sex on positive cases were surveyed by the Fisher`s exact and *Chi*-square test.

Results

Out of 41 paraffin-embedded tissue blocks, 12 (29.26%) samples were Hodgkin`s lymphoma, and 29 (70.73%) of them were non-Hodgkin`s lymphoma.

Nested-PCR results were demonstrated three of 12 (25%) Hodgkin`s lymphoma, and fourteen of 29 (48%) non-Hodgkin`s lymphoma were positive for EBNA-1, and they were associated with Epstein-Barr Virus (Figure 1).

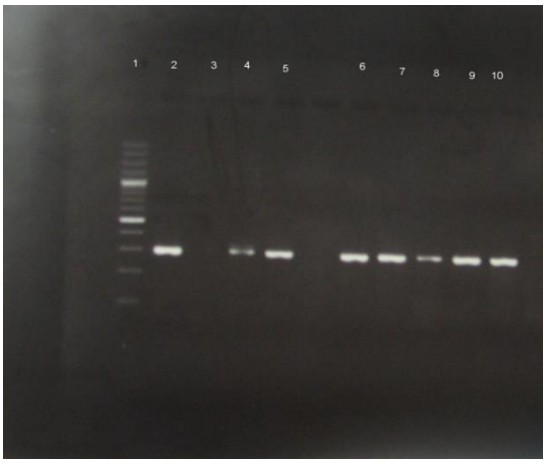

Figure 1. Analysis of EBV using EBNA-1 (positive band 300 bp). Lane 1: 100-bp size marker, Lane 2: Positive control (B95.8 cell line), Lane 3: Negative Control, Lanes 4, 5: Hodgkin`s lymphoma sample`s EBV positive, Lanes 6-10: Non-Hodgkin`s lymphoma sample`s EBV positive

Single and nested-PCR were performed in all positive cases for amplifying the EBNA-3C gene. The frequency of type 1 and 2 of EBV infection was determined that all of 3 EBV positive Hodgkin`s lymphomas were EBV type 1. Also among 14 EBV positive of non-Hodgkin`s lymphomas, all cases were EBV type 1 (Figure 2).

Out of 3/12(25%) cases of Hodgkin`s lymphoma which were associated with EBV, 2/12 (16.66%) patients were male, and 1/12 (8.3%) was female. There is no significant difference between EBV positive cases and sex ($P$=0.626). All 3 cases belonged to Mixed Cellularity subtype of Hodgkin`s lymphoma, and all of them belonged to the age of 15-34 years (Table 3).

Among 29 cases of Non-Hodgkin's lymphoma, 14 (48%) cases were positive for EBV. Out of 14 cases of non-Hodgkin`s lymphoma, 10 (71.4%) were male, and 4

(28.5%) were female. The frequency of Epstein-Barr virus was observed 42% in children and 57% in adults. The most prevalent belonged to the adults who prove association between age and EBV positive Non-Hodgkin`s lymphoma according to Fisher`s exact test (*P*=0.03).

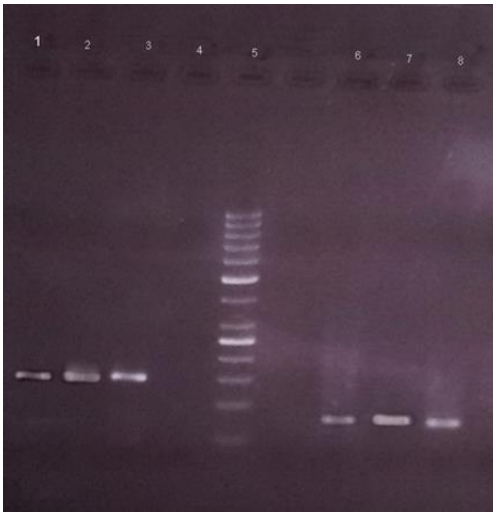

Figure 2. Analysis of EBV using EBNA-3C (positive band, left: 153bp, right: 75bp). Lane 1: Positive control (B95.8 cell line), Lanes 1- 3: EBV positive Lane 4: Negative Control, Lane 5: 50-bp size marker, Lanes 6-8: EBV positive

Table 3. Age classification in Hodgkin and Non-Hodgkin`s lymphoma

Age group	Lymphoma type	
	Hodgkin	**Non-Hodgkin**
Children (under 14-year-old)	0	6 (42.8%)
Adults (above 14-year-old)	3 (100%)	8 (57.1%)

Discussion

EBV is an important pathogen which causes of variety of malignancies, such as Hodgkin`s lymphoma, Non-Hodgkin`s lymphoma, post-transplant lymphoproliferative disorder and nasopharyngeal carcinoma (14,15). Since most of the children are infected by EBV during childhood, more than 90% of populations are lifelong carriers of this virus and incidence latent infection up to two decades after the diagnosis of Infectious Mononucleosis (16,17).

Recent studies have shown the association of molecular epidemiology between EBV and Hodgkin`s lymphoma, Burkitt`s lymphoma, and the other types of Non-Hodgkin`s lymphomas. This evidence were confirmed by some techniques like polymerase chain reaction in latent infections of tumors (18,19).

Many of the mentioned tumors are only sometimes associated with EBV, with the determinants of association varying from cofactors such as malaria (Burkitt's lymphoma) or other factors (Hodgkin's lymphoma). In fact, epidemiological factors such as age, sex, race, geographical region and social level play an important role in pathogenicity (1). Whereas EBV positive Hodgkin`s lymphoma is less common in developed countries, the percentage of prevalence is variable from 25% to 50% in European cases (20), 31% in America (21), and much higher rates in underdeveloped countries like Peru and Kenya (11).

It has been demonstrated by American researchers on patients with Hodgkin`s lymphoma, that Asians are in low encounter with this disease (22,23). In point of fact, the most frequent incidence rate of Hodgkin and Non-Hodgkin`s lymphomas are in Africa because of some mosquito-transmitted disease such as malaria (11).

In the current study, we detected 3 (25%) cases from 12 Hodgkin`s lymphoma, and 14 (48%) cases from 29 Non-Hodgkin`s lymphoma were positive for EBV DNA.

According to MacMahon hypothesis, Hodgkin`s lymphoma was classified on the basis of age. This classification is involved in three different age groups: children, Adult and elderly persons that were distinguished 0-14, 15-34 and 50 year and above, respectively (20).

Hodgkin`s lymphoma is a bimodal distribution in association with age which the first peak is in childhood

period (0-14 year) and the second peak is in above 50; although these peaks vary according to the socioeconomic condition. The incidence of Hodgkin`s lymphoma is in adolescence (15-35 year) in developed countries and is in childhood (0-14 year) in undeveloped countries (24).

Hodgkin`s lymphoma, used to be called Hodgkin`s disease, is classified into 2 main groups by World Health Organization (WHO) according to how the cells look under a microscope: Classical Hodgkin`s lymphoma and Nodular lymphocyte predominant Hodgkin`s lymphoma. There are 4 types of classical Hodgkin`s lymphoma on the basis of abnormal cells called Reed-Sternberg cell which is essential to the diagnosis of Hodgkin lymphoma may be seen under the microscope. The 4 types of Classical Hodgkin`s lymphoma are 1. Nodular Sclerosing (NS), 2. Mixed Cellularity (MC), 3. Lymphocyte Rich (LR), and 4. Lymphocyte Depleted (LD) (25,26). Using EBER as a target gene and Chromogenic In Situ Hybridization test and immunohistochemistry to identify latent membrane protein-1 (LMP1) expression, Najafipour *et al*., detected EBV markers were presented in 30 cases (55%) among 55 Hodgkin`s lymphoma (27). Using primers designed from EBNA-1 gene, Kim *et al.*, reported that EBV DNA was detected in 85% cases of 20 histological samples by PCR (10). Expression of Epstein-Barr virus in Hodgkin lymphoma in a population of United Arab Emirates nationals revealed that 17 of 45 (38%) cases of Hodgkin`s lymphoma were EBV positive and MC subtype predominately seen in this cases (28). Moreover, the results of other studies reported the association of Hodgkin`s lymphoma with EBV infection 44.8% in Brazil (29), 34% in the Netherlands (30), 85% in China (31). Although the occurrence of EBV in Hodgkin`s lymphoma varies from country to country, but it has been reported to be higher in developing countries than in developed countries (32,33).

Najafipour and *et al*., demonstrated that EBV was found in age group 1-14 and above 50 and were mainly confined to the mixed cellularity (MC) and nodular sclerosis (NS) subtypes (27); meanwhile in the current study all of these 3 EBV positive cases belong to the age of 15-34 years, and there are no EBV positive cases among two other age groups; also all 3 cases were in the Mixed Cellularity type. In fact, our result reveals changing and transforming in socioeconomic conditions in Iran as a developing country.

In our study EBV frequency using EBNA-1 as a target gene in PCR technique revealed that only 3 (25%) Hodgkin`s lymphoma patients among 12 cases were EBV positive; 2 (66.6%) patients were male, and 1 (33.3%) was female.

Non-Hodgkin`s lymphoma which is known as a fifth most commonly occurring cancer in both men and women is more common among men than women and this increases while growing old. The highest prevalence of this disease has been observed in developed countries such as the United States, and the least frequent incidence rate is in East Asia (2 per 100,000 people) (34-37).

In the current study, Non-Hodgkin`s lymphoma patients were classified into two different age groups, children, and adults. In this study among 29 cases of Non-Hodgkin's lymphoma, 14 (48%) cases were positive for EBV. The frequency of EBV was observed 42% in children and 57% in adults. The most prevalent belonged to the adults who prove association between age and EBV positive Non-Hodgkin`s lymphoma according to Fisher`s exact test (P=0.03).

Using EBNA-1 as a target gene and PCR test, Kasprzak *et al*., reported that EBV DNA was detected in 12 (46%) cases from 26 paraffin-embedded tissue of Non-Hodgkin`s lymphoma (38). Using primers designed from EBNA-2 gene, PourAkbari *et al*., reported that EBV DNA was detected in 10.5% cases of 19 histological samples by Nested-PCR (39). Using EBER as a target gene and Chromogenic In Situ Hybridization test, Kosari *et al*., detected EBV DNA in 8 (16%) cases from 50 histological cases of Non-Hodgkin`s lymphoma and there was an association between age and presence of EBV DNA (40). Moreover, the result of other studies reported the association of Non-Hodgkin`s lymphoma with EBV infection 71.5% in Thailand (41), 40% in Argentina (42) and 70% in Egypt (43).

In the current study, there was no significant difference between EBV positive cases and sex (P>0.05). There were no any significant differences between the presence of EBV DNA in Non-Hodgkin`s lymphoma and sex in the previous studies (40,41).

Different prevalence of EBV in patients with Non-Hodgkin`s lymphoma maybe because of the sensitivity of the applied methods in studies. Some factors such as used samples (blood, fresh tissues, paraffin-embedded tissues), experimented region of the genome may be effective on the results of the study (43). Nested-PCR is one of the most strong and best techniques used for detection of special segments of DNA in tissues. Only limited amount of the template is needed for this method (44).

EBNA-1 is the only protein that is persistently expressed in all malignancies which were associated with EBV. EBNA-1 is expressed latency phase, it may maintain EBV in infected cells and promote replication of episome (8,45,46). In the current study, researchers

have used EBNA-1 protein to detect and found the frequency of EBV in Hodgkin`s and Non-Hodgkin`s lymphoma. They have applied EBNA-3C to identify the EBV genotype in all samples.

Two different types of EBV, EBV-1, and EBV-2 were indicated by amplification of DNA from EBNA-3A, 3B, and 3C. The predominant type in Asia , Europe, and the United States is EBV-1 whereas EBV-2 is distinguished in Africa, New Guinea and in individuals who are HIV seropositive even in regions in addition of Africa (10,47).

In the present study, EBV genotyping study using sequencing of positive samples revealed that all EBV positive in both Hodgkin`s and Non-Hodgkin`s lymphoma were belonged to the type 1 and were EBV-1.

Acknowledgments

Sincere gratitude is extended to indefinite efforts of the honorable supervisor of the department of pathology of the Imam Khomeini Hospital of Ahvaz. This project with registration No 91103 was financially supported by Health Research Institute Infectious and Tropical Diseases Research Centre, Jundishapur University of Medical Sciences, Ahvaz, Iran.

References

1. Arvin A, Campadelli G, Mocarski E, Moore PRB, Whitley R, Yamanishi K. Human Herpes Virus Arvin A, Campadelli G, Mocarski E, Moore PRB, Whitley R, Yamanishi K, eds. New York: Cambridge University, 2007.
2. Brink A, Vervoort M, Middeldorp J, Meijer C, van den Brule A. Nucleic Acid Sequence-Based Amplification, a New Method for Analysis of Spliced and Unspliced Epstein-Barr Virus Latent Transcripts, and Its Comparison with Reverse Transcriptase PCR. J Clin Microbiol 1998;36:3164-9.
3. Fields BN, Knipe DM, Howley PM. Fields Virology. 6th ed. Cohen JI, Griffin DE, Lamb RA, Martin MA, Racaniello VR, Roizman B, eds. Philadelphia: Lippincott Williams & Wilkins, 2013.
4. Pereira de Lima MA, Pitombeira Ferreira MV, Pessoa Barros MA, Inês de Moura Campos Pardini M, Camargo Ferrasi A, Barem Rabenhorst SH. Epstein-Barr virus-associated gastric carcinoma in Brazil: comparison between in situ hybridization and polymerase chain reaction detection. Braz J Microbiol 2012;43:393-404.
5. Klein G, Klein E, Kashuba E. Interaction of Epstein-Barr virus (EBV) with human B-lymphocytes. Biochem Biophys Res Commun 2010;396:67-73.
6. Stevens SJ, Vervoort MB, van den Brule A, Meenhorst PL, Meijer C, Middeldorp J. Monitoring of Epstein-Barr Virus DNA Load in Peripheral Blood by Quantitative Competitive PCR. J Clin Microbiol 1999,37:2852-7.
7. Carbone A, Gloghini A, Dotti G. EBV-Associated Lymphoproliferative Disorders: Classification and Treatment. Oncologist 2008;13:577-85.
8. Ai J, Xie Z, Liu C, Huang Z, Xu J. Analysis of EBNA-1 and LMP-1 variants in diseases associated with EBV infection in Chinese children. Virol J 2012;9:13.
9. Hassan R, White LR, Gustavo Stefanoff C, Elgui de Oliveira D, Felisbino F, Esteves Klumb C, et al. Epstein-Barr Virus (EBV) detection and typing by PCR: a contribution to diagnostic screening of EBV-positive Burkitt's lymphoma. Diagn Pathol 2006;1:17.
10. Kim I, Park ER, Park SH, Lin Z, Kim YS. Characteristics of Epstein-Barr Virus Isolated From The Malignant Lymphomas in Korea. J Med Virol 2002;67:59-66.
11. Ocheni S, Olusina DB, Oyekunle AA, Ibegbulam OG, Kröger N, Bacher U, et al. EBV-Associated Malignancies. Open Infect Dis J 2010;4:101-12.
12. Wang WY, Chien YC, Jan JS, Chueh CM, Lin JC. Consistent Sequence Variation of Epstein-Barr Virus Nuclear Antigen 1 in Primary Tumor and Peripheral Blood Cells of Patients with Nasopharyngeal Carcinoma. Clin Cancer Res 2002;8:2586-90.
13. Skare J, Edson C, Farley J, Strominger J. The B95-8 isolate of Epstein-Barr virus arose from an isolate with a standard genome. J Virol 1982;44:1088-91.
14. Carbone A, Cesarman E, Spina M, Gloghini A, Schulz T. HIV-associated lymphomas and gamma-herpesviruses. Blood 2009;113:1213-24.
15. Gulley ML. Molecular Diagnosis of Epstein-Barr Virus-Related Diseases. J Mol Diagn 2001;3:1-10.
16. Tumwine LK, Orem J, Kerchan P, Byarugaba W, Pileri SA. EBV,HHV8 and HIV in B cell non Hodgkin lymphoma in Kampala, Uganda. Infect Agent Cancer 2010;5:1-7.
17. Hjalgrim H, Askling J, Madsen M, Rosdahl N, Storm HH. Risk of Hodgkin's Disease and Other Cancers After Infectious Mononucleosis. J Natl Cancer I 2000;92:1522-8.
18. Farrell K, Jarrett RF. The molecular pathogenesis of Hodgkin Lymphoma. Histopathology 2011;58:15-25.
19. Čičkušić E, Mustedanagić-Mujanović J, Iljazović E, Karasalihović Z, Škaljić I. Association of Hodgkin's Lymphoma with Epstein Barr Virus Infection. Bosn J Basic Med Sci 2007;7:58-65.
20. Flavell KJ, Murray PG. Hodgkin's disease and the Epstein-Barr virus. J Clin Pathol 2000;53:262-9.
21. Levin LI, Chang ET, Ambinder RF, Lennette ET, Rubertone MV, Mann RB, et al. Atypical prediagnosis Epstein-Barr virus serology restricted to EBV-positive Hodgkin lymphoma. Blood 2012;120:3750-5.

22. Glaser SL, Hsu JL. Hodgkin's disease in Asians: Incidence patterns and risk factors in population-based data. Leukemia Res 2002;26:261-9.
23. Shenoy P, Maggioncalda A, Malik N, Flowers C. Incidence Patterns and Outcomes for Hodgkin Lymphoma Patients in the United States. Adv Hematol 2011;2011:725219.
24. Macsween KF, Crawford DH. Epstein-Barr virus—recent advances. Lancet Infect Dis 2003;3:131-40.
25. Bakshi N, Maghfoor I. The Current Lymphoma Classification: New Concepts and Practical Applications—Triumphs and Woes. Ann Saudi Med 2012;32:296-305.
26. Steidl C, Connors JM, Gascoyne RD. Molecular Pathogenesis of Hodgkin's Lymphoma: Increasing Evidence of the Importance of the Microenvironment. J Clin Oncol 2011;29:812-26.
27. Najafipour S, Mokhtari Azad T, Kousari F, Mahmoodi M, Murray PG, Nategh R. Association of Epstein-Barr virus and hodgkin disease. Acta Med Iran 2003;41:1-10.
28. Al-Salam S, John A, Daoud S, Chong S, Castella A. Expression of Epstein-Barr virus in Hodgkin lymphoma in a population of United Arab Emirates nationals. Leuk Lymphoma 2008;49:1769-77.
29. Barros MH, Hassan R, Niedobitek G. Disease patterns in pediatric classical Hodgkin lymphoma: a report from a developing area in Brazil. Hematol Oncol 2011;29:190-5.
30. Diepstra A, Van Imhoff GW, Schaapveld M, Karim-Kos H, van den Berg A, Vellenga E, et al. Latent Epstein-Barr virus infection of tumor cells in classical Hodgkin's lymphoma predicts adverse outcome in older adult patients. J Clin Oncol 2009;27:3815-21.
31. Zhao P, Lu Y, Liu L, Zhong M. Aberrant cytoplasmic expression of cyclin B1 protein and its correlation with EBV-LMP1, P53 and P16(INK4A) in classical Hodgkin lymphoma in China. Pathol Oncol Res 2011;17: 369-73.
32. Zhou XG, Hamilton-Dutoit SJ, Yan QH, Pallesen G. The association between Epstein-Barr virus and Chinese Hodgkin's disease. Int J Cancer 1993;55:359-63.
33. Zarate-Osorno A, Roman LN, Kingma DW, Meneses-Garcia A, Jaffe ES. Hodgkin's disease in Mexico. Prevalence of Epstein-Barr virus sequences and correlations with histologic subtype. Cancer 1995;75:1360-6.
34. Beiderbeck AB, Holly EA, Sturkenboom MCJM, Coebergh JWW, Stricker BHC, Leufkens HGM. Prescription Medications Associated with a Decreased Risk of Non-Hodgkin's Lymphoma. Am J Epidemiol 2003;157:510-6.
35. Basiratnia M, Baradaran-Heravi A, Yavarian M, Geramizadeh B, Karimi M. Non-Hodgkin Lymphoma in a Child with Schimke Immuno-Osseous Dysplasia. Iran J Med Sci 2011;36:222-5.
36. Fisher SG, Fisher RI. The epidemiology of non-Hodgkin's lymphoma. Oncogene 2004;23:6524-34.
37. Evens AM, Winter JN, Gordon LI, Chiu BC, Tsang R, Rosen ST. CancerNetwork home of the journal oncology. (Accessed January 2018, 12, at 2011 http://www.cancernetwork.com/cancer-management/non-hodgkin lymphoma/article/10165/1802748).
38. Kasprzak A, Spachacz R, Wachowiak J, Stefañska K, Zabel M. Epstein-Barr virus (EBV) infection in B-cell non-Hodgkin's lymphomas in children: virus latency and its correlation with CD21 and CD23 molecules. Folia Histochem Cyto 2007;45:169-79.
39. Pourakbari B, mamishi S, Pajand O, Naji AR, Mahjoub F, Kouchakzadeh L, et al. Detection of Epstein- Barr virus infection in lymphoma: ELISA and PCR method. Tehran Univ Med J 2010;67:787-92.
40. Kosari F, Yarigarravesh HR, Rezvan M. Determining the correlation of Epstein-Barr virus with diffuse large B-cell lymphoma by chromogenic in situ hybridization. Tehran Univ Med J 2012;70:351-6.
41. Mitarnun W, Pradutkanchana J, Takao S, Saechan V, Suwiwat S, Ishida T. Epstein-barr virus-associated non-Hodgkin's lymphoma of B-cell origin, Hodgkin's disease, acute leukemia, and systemic lupus erythematosus: a serologic and molecular analysis. J Med Assoc Thai 2002;85:552-9.
42. Chabay P, Lara J, Lorenzetti M, Cambra P, Acosta Haab G, Aversa L, et al. Epstein Barr virus in relation to apoptosis markers and patients' outcome in pediatric B-cell non-Hodgkin lymphoma. Cancer Lett 2011;307:221-6.
43. Bahnassy AA, Zekri ARN, El-Houssini S, Khalid HM, Sedky LM, Mokhtar NM. epstein barr virus in head and neck extranodal non-hodgkin Lymphoma in Egypt. J Egypt Natl Canc Inst 2003;15:349-62.
44. Umar CS. New Developments in Epstein-Barr Virus Research. New York: Nova Publishers, 2006.
45. Frappier L. The Epstein-Barr Virus EBNA1 Protein. Scientifica 2012;2012:1-15.
46. Frappier LD. Details about NEW EBNA1 and Epstein-Barr Virus Associated Tumours. (Accessed January 2018, 15, at http://link.springer.com/book/10.1007%2F978-1-4614-6886-8).
47. Robaina TF, Valladares CP, Tavares DS, Napolitano WC, Silva LE, Dias EP, et al. Polymerase chain reaction genotyping of Epstein-Barr virus in scraping samples of the tongue lateral border in HIV-1 seropositive patients. Mem Inst Oswaldo Cruz 2008;103:326-31.