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Original Article *Özgün Araştırma*

DETECTION OF EPSTEIN-BARR VIRUS GENOME IN PEDIATRIC PATIENTS WITH LEUKEMIA AND HODGKIN LYMPHOMA: VIRAL ETIOLOGY IN PEDIATRIC CANCERS IN TÜRKİYE

Lösemi ve Hodgkin Lenfomalı Pediatrik Hastalarda Epstein-Barr Virüsü Genomunun Tespiti: Türkiye'de Pediatrik Kanserlerde Viral Etiyoloji

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ABSTRACT

Objective: Cancer is a pervasive disease characterized by its widespread occurrence and challenging treatment process. While numerous agents, including viruses, have been identified as potential causes of cancer in both adults and children, the complete pathogenesis of cancers remains incompletely elucidated. This study aimed to examine the presence of four viral agents, namely Human T-cell Lymphotropic Virus Type 1 (HTLV-1), Epstein-Barr Virus (EBV), Kaposi's Sarcoma-Associated Herpesvirus (KSHV), and Human Parvovirus B19 (HPV B19), in blood samples obtained from pediatric patients (n=64) diagnosed with B cell acute lymphoblastic leukemia (ALL), T cell ALL, Hodgkin lymphoma, and patients with relapsed leukemia and lymphoma.

Material and Methods: The whole blood samples collected from the patients during the pre-treatment and post-treatment periods underwent polymerase chain reaction (PCR) and real-time PCR to identify the presence of the viral genomes of HTLV-1, EBV, KSHV, and HPV B19. The samples that tested positive were subsequently subjected to Sanger sequencing, followed by phylogenetic analysis.

Results: Among a total of 64 samples analyzed, HTLV-1, KSHV, and HPV B19 were found to be negative. However, EBV genome was detected in six samples (9.37%) from patients with ALL and Hodgkin lymphoma, comprising both pre-treatment (n=3) and post-treatment (n=3) cases. Subsequent sequencing and alignment of the positive EBV samples with other EBV sequences deposited in GenBank revealed a high degree of similarity.

Conclusion: Our findings suggest that EBV may be one of the viral agents implicated in pediatric cancer cases involving leukemia and Hodgkin lymphoma. Therefore, it is recommended to consider testing for the presence of EBV genome in these patient populations within the context of Türkiye. This information contributes to a better understanding of the viral etiology underlying pediatric cancers, enabling the development of targeted diagnostic and therapeutic strategies in the future.

Keywords: *Human T-cell lymphotropic virus type 1, Epstein-Barr virus, Kaposi's sarcoma associated herpesvirus, human parvovirus B19, acute lymphoblastic leukemia, Hodgkin lymphoma*

ÖZ

Amaç: Kanser, yaygın görülmesi ve zorlu tedavi süreci ile karakterize yaygın bir hastalıktır. Virüsler de dahil olmak üzere birçok etken hem yetişkinlerde hem de çocuklarda kanserin potansiyel nedenleri olarak tanımlanmış olsa da kanserlerin patogenezi tam olarak aydınlatılamamıştır. Bu çalışma, B hücreli akut lenfoblastik lösemi (ALL), T hücreli ALL, Hodgkin lenfoma tanısı almış ve lösemi ile lenfoma nükseden pediatrik hastalardan elde edilen kan örneklerinde (n=64), insan T-hücreli lenfotropik virüs tip 1 (HTLV-1), Epstein-Barr virüsü (EBV), Kaposi sarkom ilişkili herpesvirüs (KSHV) ve insan parvovirus B19'un (HPV B19) varlığını incelemeyi amaçlamıştır.

Gereç ve Yöntemler: Hastalardan hem tedavi öncesi hem tedavi sonrası dönemlerinde tam kan örnekleri alınmış ve bu örneklerde HTLV-1, EBV, KSHV ve HPV B19 viral genom varlığını tespit etmek için polimeraz zincir reaksiyonu (PCR) ve gerçek zamanlı PCR yapılmıştır. Pozitif örneklerden Sanger sekanslama ile sekans analizi yapılmış ve filogenetik analizler gerçekleştirilmiştir.

Bulgular: Toplam 64 örnek analiz edildiğinde, HTLV-1, KSHV ve HPV B19'un negatif olduğu belirlenmiştir. Ancak, EBV genomu ALL ve Hodgkin lenfoma hastalarından altı örnekte (%9.37) tespit edilmiştir; bu örnekler hem tedavi öncesi (n=3) hem de tedavi sonrası (n=3) vakalarda saptanmıştır. Pozitif EBV örneklerinin sekanslama sonrasında ve GenBank'ta bulunan diğer EBV dizileri ile hizalanması, birbirleri ile yüksek derecede benzerlik gösterdiğini ortaya koymuştur.

Sonuç: Bulgularımız, EBV'nin, lösemi ve Hodgkin lenfomalı pediatrik kanser vakalarında etkili olan viral ajanlardan biri olabileceğini düşündürmektedir. Bu nedenle, Türkiye'deki bu hasta popülasyonunda EBV genomunun varlığını test edilmesi önemli olabilecektir. Bu veri, pediatrik kanserlerin temelindeki viral etiyolojiyi daha iyi anlamamıza ve gelecekte hedefe yönelik tanı ve tedavi stratejilerinin geliştirilmesine yardımcı olacaktır.

Anahtar Kelimeler: *İnsan T-hücreli lenfotropik virüs tip 1, Epstein-Barr virüsü, Kaposi sarkom ilişkili herpesvirus, insan parvovirus B19, akut lenfoblastik lösemi, Hodgkin lenfoma*

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INTRODUCTION

Childhood cancer stands as a prominent cause of mortality within the pediatric population, and globally, leukemia, lymphoma, and central nervous system tumors emerge as the most prevalent malignancies among children (1). According to the Centers for Disease Control and Prevention, malignant neoplasms account for 18.8% and 16.2% of total deaths among children aged 5-9 and 10-14 years, respectively, in the United States in 2010 (2). The etiology of cancer involves a significant contribution of various viruses, including Human T-cell leukemia virus type 1 (HTLV-1), Epstein-Barr virus (EBV), Kaposi's sarcoma-associated herpesvirus (KSHV), human papillomaviruses, hepatitis C virus, hepatitis B virus, simian vacuolating virus 40, BK virus, and JC virus (3). Furthermore, human parvovirus B19 (HPV B19) has been associated with specific cancer types including acute leukemias (4).

HTLV-1 is correlated with a spectrum of clinical presentations, encompassing adult T-cell lymphoma (ATL), lymphoma, HTLV-1-associated myelopathy (HAM), tropical spastic paraparesis (TSP), distinctive dermatological lesions, lymphadenopathy, hepatosplenomegaly, and uveitis (5,6). EBV is responsible for a spectrum of illnesses, encompassing Hodgkin's lymphoma, stomach cancer, nasopharyngeal carcinoma (NPC), Burkitt lymphoma, diffuse large B-cell lymphomas, infectious mononucleosis, and a variety of other malignancies originating from both lymphoid and epithelial sources (7). KSHV is linked to conditions including Kaposi sarcoma, non-Hodgkin B-cell lymphomas, and multicentric Castleman's disease (8). HPV B19 significantly impacts the erythroid progenitor cells in human bone marrow, leading to temporary erythropoiesis suppression and severe complications in individuals with hematologic disorders and immunodeficiency. Chronic HPV B19 infection is observed in both adult and pediatric patients with acute lymphocytic leukemia (ALL) (4,9). These viruses are associated with various diseases, including lymphomas and other malignancies. Their infection can lead to the development of cancerous conditions in affected individuals.

The principal aim of this study was to assess the existence of HTLV-1, EBV, KSHV, and HPV B19 within whole blood samples collected from pediatric cancer patients in Türkiye. Furthermore, we aimed to explore the potential influence of immunosuppressive cancer treatments on the presence of viral genomes.

MATERIALS AND METHODS

Samples

Children between the ages of 2-16, applied to the Department of Hematology of Ankara Children's Health and Diseases Hematology Oncology Training and Research Hospital between the years of 2010-2017, who have symptoms such as paleness, fever, vomiting, excessive fatigue, loss of appetite and excessive weight loss were taken in the present study. A total of 32 patients were included to the study, who are diagnosed B cell acute lymphoblastic leukemia (ALL) (n=9), T cell ALL (n=6), Hodgkin lymphoma (n=8), relapsed from leukemia and lymphoma disease (n=9). The laboratory diagnosis of ALL and Hodgkin's lymphoma is conducted through routine diagnostic procedures such as staining, immunohistochemistry and flow cytometry at the Ankara Children's Health and Diseases Hematology Oncology Training and Research Hospital (10-12). Leukemia patients were tested for the EBV by ELISA or PCR in an external private diagnostic laboratory and Hodgkin lymphoma patients were subjected to EBV assessment using immuno-histochemistry in the hospital. All results of the patients were negative for EBV (data not shown). When the diagnosis was confirmed, two sets of whole blood samples were collected from each of the 32 patients: the initial sampling occurred prior to the commencement of treatment when patients were newly diagnosed (pre-treatment period, n=32), while the subsequent sampling took place on the 33rd day of the treatment regimen (post-treatment period, n=32). The therapies of the ALL and Hodgkin lymphoma patients are maintained according to the ALL IC-BFM 2009 protocol, and

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ABVE-PC protocol, respectively (13,14). The study protocol was approved by the Clinical Research Ethics Committee of the Ankara Children's Health and Diseases Hematology Oncology Training and Research Hospital (Date:21.03.2016, Number:2016-011).

DNA Isolation

DNA isolation from samples was performed using the PureLink Genomic DNA Mini Kit (K182002, Invitrogen, CA, USA). In brief, 200 µl of whole blood sample, 20 µl proteinase K and 20 µl RNase were mixed in a sterile microcentrifuge tube. The mixture was slightly vortexed and allowed to stand at the room temperature for 2 minutes. Then 200 µl lysis solution is added to the mixture, vortexed and incubated at 55 °C for 10 minutes in a water bath (BM15, Nüve, Türkiye). 200 µl of 100% ethanol was added to the mixture and vortexed. The prepared lysate was transferred to the purification column. The tubes were centrifuged at 10,000 × g for 1 minute (5417R, Eppendorf, Germany). After centrifugation, the column was placed in a new collection tube. 500 µl of wash solution 1 was added into the column and centrifuged at 10,000 × g for 1 minute. After centrifugation, the column was placed in a new collection tube. 500 µl of wash solution 2 was added into the column and centrifuged for 3 min at 13,000 × g. The column was placed in a sterile 1.5 ml microcentrifuge tube and 200 µl elution buffer was added to the column tube and incubated for 1-2 min at room temperature. Thereafter, the column tube was centrifuged at 10,000 × g for 1 min. DNA samples collected in the microcentrifuge were stored at -20 °C for further analyses.

Polymerase Chain Reaction (PCR)

To investigate the presence of EBV, KSHV, HPV B19, and HTLV-1 genomes in the samples, PCR was performed. The human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as a housekeeping gene in PCR. To amplify the viral genes and GAPDH, the primer pairs shown in Table 1 were used and the PCR mix was optimized as containing DNA sample, 10 pmol forward and reverse primer, 1.25 mM dNTP, 5× PCR buffer, 50 mM $MgCl_2$, 2.5 U Taq DNA polymerase in total 50 µl volume. The PCR conditions used for amplification are summarized in Table 2. The PCR products were analyzed by 1.2% agarose gel electrophoresis including ethidium bromide and visualized by UV transilluminator (Illuminx, Nyxtechnik, USA). The positive DNA controls for each virus were kindly provided by Düzen Laboratory (Ankara, Türkiye).

Table 1: Targeted genes and virus-specific primer sequences investigated in the study

Target / Gene	Sequence	Product	Reference
KSHV / ORF73	F: 5'-GGTGATGTTCTGAGTACATAGCGG-3' R: 5'-CCGAGGACGAAATGGAAGTG-3'	143 bp	(34)
EBV / gp220	F: 5'-CCTTAGGAGGAACAAGTCCC-3' R: 5'-GGCTGGTGTCACCTGTGTTA-3'	239 bp	(35)
HPV B19 / NS1	F: 5'-CCACTATGAAAACTGGGCAATA-3' R: 5'-GCTGCTTTCACTGAGTTCTTCA-3'	154 bp	(36)
HTLV-1 / Pol	F: 5'-CCCTACAATCCAACCAGCTCAG-3' R: 5'-TGGAGTAACTTACTAGGTTAG-3'	668 bp	(37)
Human / GAPDH	F: 5'-AGGGCTGCTTTTAACTCTGGT-3' R: 5'-CCCCACTTGATTTTGGAGGGA-3'	204 bp	(38)

KSHV: Kaposi's sarcoma associated herpesvirus, EBV: Epstein-Barr virus, HPV B19: Human parvovirus B19, HTLV-1: Human T lymphotropic virus, GAPDH: Glyceraldehyde-3-phosphate dehydrogenase, F: Forward, R: Reverse, bp: Base pair.

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Table 2: The PCR conditions optimized for detection of viral agents

Virus	Denaturation	Amplification	Final elongation	No of amplification cycles
KSHV	95 °C 2 min	95 °C 30 s 54 °C 30 s 72 °C 30 s	72 °C 5 min	× 40
HPV B19	95 °C 2 min	95 °C 30 s 53 °C 30 s 72 °C 30 s	72 °C 10 min	× 35
EBV	95 °C 2 min	95 °C 20 s 59 °C 20 s 72 °C 20 s	72 °C 5 min	× 40
HTLV	95 °C 2 min	95 °C 20 s 58 °C 20 s 72 °C 20 s	72 °C 10 min	× 36

KSHV: Kaposi's sarcoma associated herpesvirus, EBV: Epstein-Barr virus, HPV B19: Human parvovirus B19, HTLV-1: Human T lymphotropic virus.

Real-time PCR

Real-time PCR mix contained DNA, 2× SYBR Green master mix (04707516001, Roche, Germany), 0.5 pmol forward and reverse primers, and PCR grade water up to a volume of 20 µl. The reactions were carried out on LightCycler 96 real-time PCR instrument (Roche, Mannheim, Germany) and each reaction condition were optimized for the viral agents (Table 3). A total of 38 cycle amplifications were performed in all reactions. After the amplification, Cq values, amplification curves and melting curve analysis of the samples were evaluated using LightCycler 96 software 1.1.

Table 3: The real-time PCR conditions optimized for detection of viral agents

Viruses	Preincubation	3-step amplification	Melting
KSHV	95 °C 10 min	95 °C 10 s 54 °C 10 s 72 °C 10 s	95 °C 10 s 65 °C 60 s 97 °C 1 s
HPV B19	95 °C 10 min	95 °C 10 s 53 °C 10 s 72 °C 10 s	95 °C 10 s 65 °C 60 s 97 °C 1 s
EBV	95 °C 10 min	95 °C 10 s 59 °C 10 s 72 °C 10 s	95 °C 10 s 65 °C 60 s 97 °C 1 s
HTLV	95 °C 10 min	95 °C 10 s 54 °C 10 s 72 °C 10 s	95 °C 10 s 65 °C 60 s 97 °C 1 s

KSHV: Kaposi's sarcoma associated herpesvirus, EBV: Epstein-Barr virus, HPV B19: Human parvovirus B19, HTLV-1: Human T lymphotropic virus

Cloning and plasmid isolation

To validate the EBV presence and acquire the viral genomic sequence, EBV gp220 gene was initially cloned using the TA cloning kit (K2040, Invitrogen, CA, USA) according to manufacturers' instructions. Transformation of the recombinant plasmid DNA into *E. coli* was done using the heat shock method. Each media containing transformed *E. coli* was inoculated on

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LB agar containing 100 µg/ml ampicillin and the plates were incubated overnight at 37°C. Colonies on LB agar were checked by PCR screening method to determine whether EBV gp220 gene was successfully cloned (15,16). The plasmid DNAs from positive colonies were isolated using the PureLink HiPure Plasmid Midiprep kit (K210005, Invitrogen, California, USA) according to manufacturers' instructions. The isolated plasmid DNA was suspended in 200 µl of TE buffer and stored at -20 °C.

Sequencing and Phylogenetic Analysis

Three out of the positive 6 samples and one EBV positive control sample were sequenced by RefGen Biotechnology (Ankara, Türkiye) with Sanger sequencing using EBV-specific primers (Table 1). The consensus viral sequences and other EBV genome sequences from the GenBank database were aligned using the MUSCLE algorithm in MEGA 7 software. Phylogenetic analysis was performed using MEGA 7 software and a phylogenetic tree was created using the neighbor-joining method, the p-distance nucleotide substitution model, and bootstrap analysis with 500 replicates.

Statistical Analysis

The McNemar test was used for statistical analyses in IBM SPSS Statistics 21 software (Chicago, U.S.A.) to assess pre- and post-treatment EBV positivity status within groups and in all patients. Results were considered statistically significant at $p<0.05$.

RESULTS

A total of 64 whole blood samples of 32 patients who were diagnosed as B cell ALL (n=9), T cell ALL (n=6), Hodgkin lymphoma (n=8), relapsed from leukemia and lymphoma disease (n=9) were investigated for genome presence of HTLV-1, EBV, KSHV and HPV B19 by both conventional PCR and real-time PCR. According to PCR and real-time PCR results, HTLV-1, KSHV and HPV B19 were not detected in any of the samples.

Among investigated viral agents, only EBV was positive in 6 patients by both PCR and real-time PCR (Figure 1). EBV positivity was determined in 2 patients who had ALL, 1 patient who had Hodgkin's lymphoma, and 3 patients who had relapses (Table 4). Whole blood samples (n=64) were taken in both pre-treatment (n=32) and post-treatment (n=32) periods from all patients. Out of the 6 patients with a positive EBV genome, 3 were during the pre-treatment phase, and the remaining 3 were in the post-treatment phase. However, none of these patients showed positivity in both the pre-treatment and post-treatment periods (Table 4). No statistically significant difference was found ($p>0.05$) for pre- and post-treatment EBV positivity status within groups and in all patients.

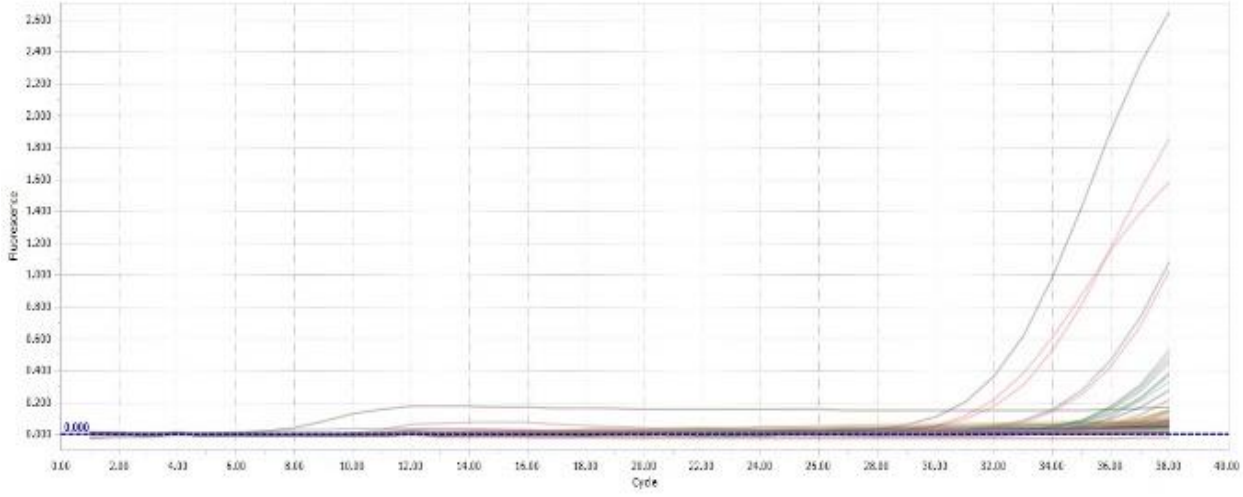

Figure 1: Real-time PCR amplification curve graphic of Epstein-Barr virus positive samples and positive controls.

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Table 4: Diagnosis, sampling time, and EBV-positivity status of the patients

Diagnosis	Patients	Pre-treatment	Post-treatment
T cell ALL	1	Negative	Negative
	2	Negative	Negative
	3	Negative	Negative
	4	Negative	**Positive**
	5	Negative	Negative
	6	Negative	Negative
B cell ALL	7	Negative	Negative
	8	Negative	Negative
	9	Negative	Negative
	10	Negative	Negative
	11	Negative	Negative
	12	Negative	Negative
	13	Negative	Negative
	14	**Positive**	Negative
	15	Negative	Negative
Hodgkin's lymphoma	16	Negative	Negative
	17	Negative	Negative
	18	**Positive**	Negative
	19	Negative	Negative
	20	Negative	Negative
	21	Negative	Negative
	22	Negative	Negative
	23	Negative	Negative
Leukemia or lymphoma relapses	**24**	Negative	**Positive**
	25	Negative	Negative
	26	Negative	**Positive**
	27	Negative	Negative
	28	Negative	Negative
	29	Negative	Negative
	30	**Positive**	Negative
	31	Negative	Negative
	32	Negative	Negative

The cloning of the gp220 gene of the EBV isolates obtained from this study was confirmed by PCR and real-time PCR (Supplementary Figure 1). After confirmation of cloning the samples were sequenced. The EBV gp220 sequences obtained from this study are deposited to GenBank with accession numbers of MT445990-MT445992 for EBV isolates Azkur/Turkiye/2, Azkur/Turkiye/3, and Azkur/Turkiye/4, respectively. Azkur/Turkiye/1 (GenBank accession number MT445989) is the EBV positive control for sequencing and confirmation.

Sequence and phylogenetic analysis of the EBV positive 3 samples revealed that the obtained isolates were similar. The samples (namely; Azkur/Turkiye/2, Azkur/Turkiye/3, and Azkur/Turkiye/4) have similarity between 91.9-98.03% to each other (Table 5).

Table 5: The percentage matrices of the Epstein-Barr virus isolates obtained from this study. The matrices were conducted with Clustal Omega 2.1.

	Türkiye_3	Türkiye_2	Türkiye_4
Türkiye_3	100.00	93.60	91.90
Türkiye_2	93.60	100.00	98.03
Türkiye_4	91.90	98.03	100.00

EBV Türkiye isolates were phylogenetically close to Chinese EBV isolate which is from gastric carcinoma (KX674064.1), and three EBV saliva isolates from United Kingdom (MG298920.1, MG298921.1, MG298927.1). EBV Türkiye isolates have phylogenetically distance to Canadian EBV isolates which are gained from gastric adenocarcinoma (MG021308.1, MG021310.1, MG021311.1) (Figure 2).

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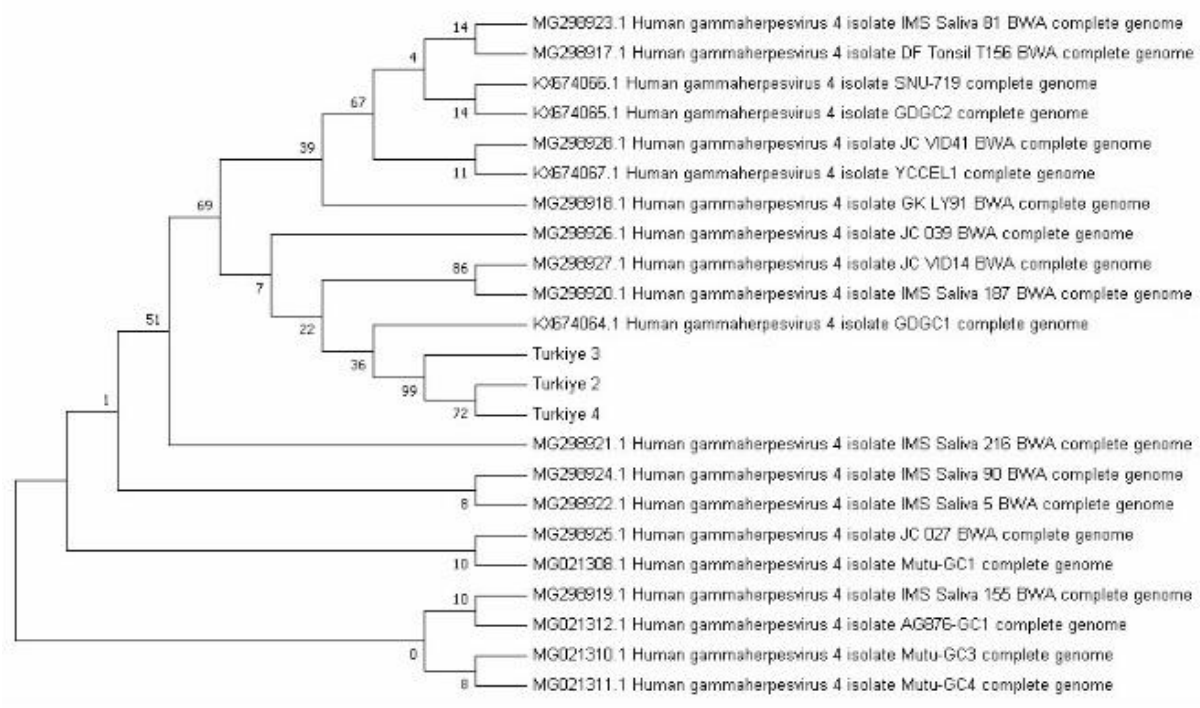

Figure 2: The phylogenetic tree of EBV positive samples obtained from this study.

DISCUSSION

The present investigation encompassed four viral agents, utilizing whole blood samples gathered from pediatric cancer patients in both the pre-treatment and post-treatment periods. This selection of viral agents was informed by scientific literature and International Agency for Research on Cancer (IARC) reports, classifying HTLV-1, EBV, and KSHV as Group 1 agents due to their recognized potential to cause cancer in humans (17). Our findings suggest a potential involvement of EBV as a viral agent in pediatric cancer cases, encompassing leukemia and Hodgkin lymphoma. Considering the Turkish context, it is recommended to consider assessing the presence of the EBV genome.

Despite sporadic instances of HTLV-specific antibody responses detected in merely two healthy blood donors, the genomic presence of HTLV has yet to be ascertained within the afflicted patient population in Türkiye. In Türkiye, Kaposi's sarcoma presence has been only shown in pediatric liver transplant recipients (18-20). HPV B19 seroprevalence in central Türkiye was reported as 20.7% in pediatric patients (21). HPV B19 DNA had been detected in the bone marrow aspiration sample of an 8-year-old patient with pre-B cell ALL and anti-HPV B19 IgM positivity had been reported in two children with pre-B cell ALL in Türkiye (22,23). Our study revealed no indications of HTLV, KSHV and HPV B19 genome presence in pediatric individuals diagnosed with ALL or lymphoma, highlighting the need for future research efforts to encompass a more diverse and extensive patient cohort within the Turkish context to enhance our understanding of these viral infections in pediatric cancer patients.

In Türkiye, EBV positivity had been reported in pediatric patients with Burkitt's lymphoma and non-Hodgkin lymphomas (24,25). In the current study, out of the 64 samples analyzed, the EBV genome was identified in 6 samples (9.37%). Specifically, one patient with T-cell ALL, one patient with B-cell ALL, one patient with Hodgkin's lymphoma, and three patients with relapses demonstrated positive EBV results in both PCR and real-time PCR analyses (Table 4). In a study conducted in Türkiye, EBV positivity was reported as 30.2% in pediatric patients with primary immunodeficiency and most of them were diagnosed with lymphoma (26). The relatively small patient cohort in this study may explain the lower EBV positivity among pediatric cancer patients in Türkiye.

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Sequence and phylogenetic analysis of the EBV samples from this study have high similarity (91.9-98.03%) (Table 5). According to the phylogenetic tree, EBV Türkiye isolates were close to Chinese and English EBV isolates which are from gastric carcinoma and saliva samples (Figure 2). The EBV isolates in this study were from whole blood samples of pediatric cancer patients. On the other hand, only the gp220 gene of EBV was cloned and sequenced in the present study. Sequences of other genes or whole genome of EBV isolates could be gained in further studies.

Studies have demonstrated that elevated plasma levels of EBV DNA have predictive utility in assessing NPC risk, treatment response to EBV-associated non-Hodgkin lymphoma, prediction of biological and clinical characteristics of Hodgkin lymphoma, and persistent infection in the early treatment of lymphoma (27-31). It is demonstrated that the quantitative surveillance of EBV-DNA levels, commencing at the outset and continuing throughout EBV-associated lymphoproliferative disease (EBV-LPD) therapy, has the potential to serve as a potent instrument for fine-tuning and choosing treatments in individuals with EBV-LPD (32). In this study, we did not perform quantitative real-time PCR to measure EBV DNA levels in plasma samples. Nonetheless, the findings of the aforementioned researches suggest a requirement for extensive investigations aimed at determining the plasma EBV DNA load across diverse cohorts of cancer patients in Türkiye.

EBV reactivation can be induced by various factors, with immunosuppressants being the most relevant reason for viral reactivation (33). In our study, three patients who tested negative during the pre-treatment period became EBV-positive after receiving treatment that included immunosuppressant agents. This observation could be interpreted as viral reactivation attributed to the use of immunosuppressants. Nevertheless, the underlying cause behind the transition from pre-treatment EBV positivity to post-treatment EBV negativity in the three patients remains elusive.

The results of this study indicated that vigilant monitoring of viral agents, particularly EBV, is imperative throughout the pre-treatment and post-treatment phases in pediatric cancer patients in Türkiye to comprehensively assess the treatment process and establish effective treatment protocols. Given the sample size of this study, it is advisable that future research endeavors encompass a broader patient cohort, potentially involving diverse cancer types, to further enhance the scope and depth of the investigations.

*Conflict of Interest***:** The authors have indicated no conflicts of interest regarding the content of this article.

Researchers' Contribution Rate Statement: Concept/Design: AKA, YK; Analysis/Interpretation: AKA, YK, HMÖ; Data Collection: AKA, YK, HMÖ, EA; Writer: AKA, YK, HMÖ, EA; Critical Review: AKA; Approver: AKA, YK, HMÖ, EA

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Ethics Committee Approval: The study protocol was approved by the Clinical Research Ethics Committee of the Ankara Children's Health and Diseases Hematology Oncology Training and Research Hospital (Date:21.03.2016, Number:2016-011).

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