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Original Investigation

Relationship between Epstein-Barr Virus (EBV) Infection and Viral Load in Immunosuppressive Patients

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Abstract

Introduction: The aim of this retrospective study is to investigate the relationship between clinic and presence of Epstein-Barr virus (EBV) DNA and viral load by real-time polymerase chain reaction (PCR) in patients with high risk.

Methods: A total of 168 samples obtained from 160 patients, hospitalized in Gazi University Hospital between March 2014 and May 2015, were included in the study. EBV antibodies were investigated by ELISA (Dia.Pro, Milano, Italy) in clinical samples of patients, and serological profiles of patients were determined. Nucleic acid isolation was performed by High Pure Viral Nucleic Acid Kit (Roche, Germany). Isolated DNAs were amplified by LightCycler® EBV Quantitative Kit (Roche, Germany) in LightCycler 2.0 (Roche, Germany) device, and results were evaluated quantitatively.

Results: EBV DNA was positive for 4.2% (7/168) of the samples. The distributions of positive rates were 14.2% (1/7) for oncology, 12.5% (1/8) for intensive care units, 4.8% (4/42) for pediatric gastroenterology, and 4.1% (1/24) for pediatric hematology. Three of the patients that EBV DNA detected had liver transplant, one had non-Hodgkin's lymphoma, one had Burkitt's lymphoma, one had acute renal failure, and one had gingivostomatitis and pharyngotonsillitis while follow-up. In 6/7 of the samples, EBV DNA detected 104 copies/ml, and 1/7 of the samples 105 copies/ml. The patient whose EBV DNA load was 105 copies/ml had Burkitt's lymphoma. EBV DNA and viral capsid antigen IgM positive were detected simultaneously only in one patient (17%).

Conclusion: Early diagnosis by real-time PCR is of great importance in terms of follow-up of patients by monitoring DNA amounts and prognosis. Therefore, in immunosuppressive patients who have high levels of EBV DNA, Burkitt's lymphoma disease should be considered.

Keywords: Epstein-Barr virus (EBV), viral load, immunosuppressive patient

Introduction

Epstein-Barr virus (EBV; human herpesvirus 4) is an enveloped, icosahedral symmetrical, double-stranded DNA virus belonging to the Gammaherpesvirinae subfamily of the Herpesviridae family. Double-stranded DNA is linear in virion, and when it is latent in infected cells, it is circular. EBV was first isolated in 1964 by the researchers Epstein, Barr, and Achong from the tumor samples of African children (1).

Epstein-Barr virus first infects the oropharynx epithelial cells, then nasopharynx, salivary glands, and sensitive B lymphocytes in the larynx lymphoid tissues. While the virus does not cause cytopathic effect in the infected cell, the viral genome-carrying cell acquires the ability to reproduce continuously. Humoral and cellular immune response occurs, but with the various mechanisms that it develops, the virus causes latent infections by remaining in infected B lymphocytes (2).

Epstein-Barr virus is an infectious mononucleosis agent, especially in children. EBV is found to be associated with nasopharyngeal carcinoma, Burkitt's lymphoma, and Hodgkin's lymphoma, and it causes posttransplant lymphoproliferative disease (PTLD) in immunosuppressive patients such as in organ and tissue transplant recipients (2, 3).

It has been reported that 80%-95% of the adult population in our country is EBV seropositive (4, 5). Soylu et al. (4) found the EBV seropositivity at 81% in our country, and Aydemir et al. (5) found it in the range of between 70% and 99.4%. It is reported that 90% of adults worldwide have anti-EBV antibodies (3, 6).

The EBV-specific serologic tests are used to diagnose the EBV infections, and IgG and/or IgM antibodies developing against the major antigens of the virus (nuclear antigen [EBNA]), viral capsid antigen (VCA), and early antigen (EA) are being investigated. With serological tests, it is possible to distinguish between an acute and past infection in patients with normal immune system. While the absence of EBNA-IgG in the presence of VCA-IgM and VCA-IgG indicates an acute infection, the VCA-IgM negativity in the presence of VCA-IgG and EBNA-IgG indicates past infection. However,

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suspicious diagnoses may arise in cases where the EBV serology cannot be interpreted due to the inadequacy of an immune response, especially in patients in whom the immune system is not normal, and molecular methods are required (3).

Because routine serologic tests for an EBV infection may be insufficient in immunosuppressed patients, it is important to determine the presence and amount of viral DNA by the real-time polymerase chain reaction (Real-Time PCR). The aim of this study is to retrospectively investigate the presence of the EBV DNA with the Real-Time PCR in immunosuppressed patients at risk for the EBV infection and to correlate it with a clinical and viral load.

Methods

Between March 2014 and May 2015, 168 clinical specimens (146 serum, 16 cerebrospinal fluid [CSF], two bronchial alveolar lavage [BAL], two pleural fluids, one vitreous fluid, and one pericardial fluid) that were sent to Gazi University School of Medicine Hospital Molecular Microbiology Laboratory were analyzed by a Real-Time PCR.

A total of 168 samples of 94 male patients (58.8%) aged between 9 months and 74 years, and 66 female patients (41.2%) were included in the study. The number and age ranges of the patients according to the clinics to which the samples were sent are shown in Table 1.

Blood samples were centrifuged at 3000 rpm for 5 minutes, and the serum fraction was separated and stored at -80°C until the time of the study. Other clinical specimens were aliquoted and stored at -80°C until the time of the study.

Serological Analyse

In patient samples, the VCA IgM, VCA IgG, EBNA IgM, EBNA IgG, and EA IgG antibodies were studied in accordance with the manufacturer's instructions by the enzyme-linked immunosorbent assay (DIA.PRO, Milano, Germany). At the end of the study, the microplate was scanned in a spectrophotometer (TECAN, Switzerland) at 450 nm wavelength, and the obtained optical density (OD) results were evaluated. According to the manufacturer's instructions, if the negative control was OD<0.1 for the VCA IgM, VCA IgG, EBNA IgG, and EA IgG, and the positive control (calibrator 100 arbiU/mL) was OD>1.0, the test was considered to be correct. The cut-off value for the VCA IgM and EBNA IgG tests is Cal (Calibrator) 10 arbU/mL, and it is Cal (Calibrator) 5 arbU/mL for the VCA IgG and EA IgG tests. For the EBNA IgM test, if the negative control was OD<0.150, and the positive control was OD>0.500, it was considered that the test worked correctly. The cut-off value was calculated by adding 0.250 to the OD of the negative control, and the values below the cut-off value were considered as negative, whereas the values above the cut-off value were considered as positive. It is stated in the kit prospectus that the method used does not show cross-reactivity, and its sensitivity and specificity is >98%.

Nucleic Acid Isolation and DNA Replication

Viral DNA isolation from clinical specimens was performed through the "Spin-Column" method using the "High Pure Viral Nucleic Acid Kit" (Roche, Germany). Viral DNA was obtained in accordance with the manufacturer's protocol. The DNAs obtained were stored at -80 °C until amplification.

The amplification of the isolated viral DNAs was performed through the Real-Time PCR method using a Light Cycler® EBV Quantitative Kit (Roche, Germany) in the Light Cycler® 2.0 device (Roche Applied Science, Germany).

Primers sequences amplifying the 213 bp portion of the highly conserved EBNA1 gene region of the EBV genome were used for the amplification, and the results were determined numerically.

Table 1. Evaluation of the number and age ranges of the patients according to the clinics

Clinic	Male Patients	Median (Min.-Max.)	Mean±SD	Female Patients	Median (Min,-Max,)	Mean±SD	Total number of patients	Median (Min,-Max,)	Mean±SD
Pediatric gastroenterology	23	6.0 (0.7-17)	6.8±4.7	19	7.0 (2-19)	8.9±5.2	42	6.5 (.7-19)	7.8±5.0
Pediatric hematology	19	7.0 (1-17)	8.3±5.7	5	4.0 (2-7)	4.4±1.8	24	5.5 (1-17)	7.5±5.3
Pediatric nephrology	12	16.0 (12-19)	15.5±2.3	7	15.0 (13-18)	15.5±1.8	19	15.0 (12-19)	15.5±2.1
Neurology	8	14.0 (3-18)	11.8±5.8	7	10.0 (2-60)	16.5±20	15	13.9 (2-60)	14.0±13.9
BMT unit	7	34.0 (4-65)	37.7±22	5	31.0 (8-63)	33.6±21.5	12	32.5 (4-65)	36.0±21.2
Child health and diseases	5	2.0 (1-16)	6.6±7.3	6	9.0 (1-14)	8.3±4.6	11	7.0 (1-16)	7.5±5.7
Adult hematology	5	60.0 (21-74)	50.2±212	5	37 (24-56)	41.2±13.5	10	45.5 (21-74)	45.7±17.4
Intensive care	3	14.0 (14-23)	17.0±5.1	4	16.0 (2-35)	17.2±13.5	7	16.0 (2-35)	17.1±10.0
Pediatric oncology	6	16.5 (2-36)	16.3±11	1	17.0 (17-17)	17.0±0	7	17.0 (2-36)	16.4±11.4
Child infection	3	3 .0 (1-14)	6.0±7.0	1	3.0 (3-3)	3.0±0	4	3.0 (1-14)	5.2±5.9
Cardiology	1	19.0 (19-19)	19.0±0	3	18.0 (9-73)	33.3±34.6	4	18.5 (9-73)	29.7±29.1
Eye diseases	-	-	-	2	36.5 (21-52)	36.5±21.9	2	36.5 (21-52)	36.5±21.9
Pediatric chest diseases	1	6.0 (6-6)	6.0±0	1	3.0 (3-3)	3.0±0	2	4.5 (3-6)	4.5±2.1
Pediatric surgery	1	2.0 (2-2)	2.0±0	-	-	-	1	2.0 (2-2)	2.0±0
Total	94	13 (0.7-74)	14.2±14	66	11.5 (1-73)	16.7±16.5	160	12.5 (0.7-74)	15.2±15.5

BMT: bone marrow transplantation; SD: standard deviation

Five standard and one negative controls, each containing a different number of EBV DNA (102-106 copies/ml), were used for an EBV analysis. The amplification curves of the standards were evaluated through an absolute quantification analysis in the 530 channel of LightCycler 2.0, and the EBV positivity and results were determined numerically.

Capillary tubes were each loaded with 10 µL of master mix and 10 µl of isolated DNA samples to obtain a total reaction volume of 20 µL. The capillaries were centrifuged at 2000 rpm for 10 seconds and were loaded in the Light Cycler 2.0 device.

No peaks were seen in the curves of the negative controls used in the analyses. Evaluation of the negative results and the control of the validity of the analyses were provided with the internal control in an absolute quantification analysis in the 610 channel of the LightCycler 2.0 device.

Statistical Analysis

Statistical analyses were performed using the SPSS (Statistical Package for Social Sciences) version 20.0 (IBM Corp.; Armonk, NY, USA) computer program. The Mann-Whitney U and Chi-squared test were used to evaluate the data, and $p<0.05$ was considered significant in the analyses.

Results

In our study, a total of 168 clinical specimens were examined, including 146 sera, 16 CSF, two BAL, two pleural fluids, one vitreous fluid, and one pericardial fluid, and the distribution of the studied specimens is shown in Table 2 according to the clinics where they were sent.

It was found that 20.6% (33/160) of the patients who were included in the study were liver transplantation patients, 11.8% (19/160) were

Table 2. Distribution of samples included in the study according to the clinics

Clinic	Serum Number (%)	BAL Number (%)	BOS Number (%)	Vitreous Fluid Number (%)	Pericardial Fluid Number (%)	Pleural Fluid Number (%)
Pediatric gastroenterology						
n: 42	42 (100)					
Pediatric hematology						
n: 24						
	24 (100)					
Child nephrology						
n: 20	20 (100)					
Neurology						
n: 18	7 (38.8)		11 (61.2)			
Child health						
n: 13	11 (84.6)		2 (15.4)			
BMT unit						
n:1 2	11 (92.3)	1 (7.7)				
Adult hematology						
n: 10	10 (100)					
Intensive care						
n: 8	7 (87.5)		1 (12.5)			
Oncology						
n: 8	6 (85.8)					2 (14.2)
Pediatric infectious diseases						
n: 4	4 (100)					
Cardiology						
n: 4	1 (25)		2 (50)		1 (25)	
Eye diseases						
n: 2	1 (50)			1 (50)		
Pediatric chest diseases						
n: 2	1 (50)	1 (50)				
Pediatric surgery						
n: 1	1 (100)					
Total						
n: 168	146 (87)	2 (1.2)	16 (9.5)	1 (0.6)	1 (0.6)	2 (1.2)

BMT: bone marrow transplantation; CSF: cerebrospinal fluid; BAL: bronchoalveolar lavage; %: calculated over the number of samples in the clinic; n: number of samples

kidney transplant patients, 10.6% (17/160) had leukemia, 10% (16/160) were bone marrow transplant patients, 5% (8/160) were suffering from non-Hodgkin's lymphoma, and 2.9% (5/160) were stem cell transplantation patients. Of the patients, 2.5% (4/160) were diagnosed with renal failure, 1.9% (3/160) with herpesvirus infection, 1.2% (2/160) with Guillain-Barre syndrome, 0.6% (1/160) with infectious mononucleosis, 0.6% (1/160) with Burkitt's lymphoma, and 0.6% (1/160) with ALL (gingivostomatitis and pharyngotonsillitis developed during the follow-up), and 48 (28.5%) of them were the patients who were regularly sent to our laboratory for the examinations of the EBV DNA.

Serological results were evaluated together with the Real-Time PCR results of the patients. The EBV DNA positivity was detected in 4.2% (7/168) of all clinical samples. Of these patients, 85.8% (6/7) were followed up by weekly controls in terms of the EBV DNA positivity. VCA IgM was negative in 83% (5/6) of the EBV DNA positive patients, and VCA IGM, a sign of an acute infection, was found to be positive in 17% (1/6) of the patients (the patient in whom gingivostomatitis and pharyngotonsillitis developed during the follow-up with the diagnosis of ALL). In 2 patients with non-Hodgkin's lymphoma and autoimmune hemolytic anemia, the VCA-IgM negativity was detected in the presence of VCA IgG and EBNA IgG. In three patients with liver transplantation referred from pediatric gastroenterology clinic, only VCA IgM, which is an indication of acute infection, was studied, and VCA IgM was found to be negative in these three patients. No serologic analysis results were found in the fourth patient referred from the pediatric gastroenterology clinic; this patient had undergone liver transplantation in an external center 1 year ago and was referred to our hospital with the complaints of fever and rash; the presence of the EBV DNA was investigated through the Real-Time PCR method in our hospital. The EBV DNA positivity was detected in the patient, but the patient did not participate in the treatment and the follow-up process and did not come to the controls. Serology and the Real Time PCR results of the patients are shown in Table 3.

When the EBV DNA positivity rates are analyzed according to the clinics where the samples were sent, the EBV DNA positivity was detected in 14.2% (1/7) (non-Hodgkin's lymphoma) of the samples from the pediatric oncology service, in 12.5% (1/8) (autoimmune hemolytic anemia) in the samples from an intensive care unit, in 4.8% (4/42) (three liver transplants, one Burkitt lymphoma) of the samples from the pediatric gastroenterology service, and in 4.1% (1/24) (gingivostomatitis and pharyngotonsillitis in the period when followed up with the diagnosis of ALL) of the samples from the pediatric hematology unit. The EBV DNA positivity was not detected in the samples from the pediatric nephrology, neurology, bone marrow transplantation unit, child health and adult hematology unit, pediatric infectious diseases, cardiology, pediatric chest diseases, eye diseases, and pediatric surgery. When the distribution of the EBV DNA positivity was examined according to clinics, there was no statistically significant difference ($p>0.05$).

When the samples included in the study were evaluated in terms of the EBV DNA positivity, it was seen that 85.8% (6/7) of the positive samples were serum, and 14.2% (1/7) were the CSF specimens. The EBV DNA positivity was not detected in BAL, vitreous fluid, pericardial fluid, and pleural fluid samples. When the distribution of the EBV DNA positivity was examined according to clinical specimens, statistically significant difference was not observed ($p>0.05$).

In seven of the six samples (85.8%) in which the EBV DNA positivity was detected; EBV DNA was 10^4 copies/ml and 10^5 copies/ml in one sample (14.2%), and it was seen that the patient in whom the amount of the EBV DNA was found to be 10^5 was diagnosed with Burkitt's lymphoma. When we examined the relationship between the amount of the EBV DNA and the clinics where samples were sent, it was found that the EBV DNA$>10^4$ copies/ml was detected in more samples from the pediatric gastroenterology unit than the other clinics, but no statistically significant difference was observed ($p>0.05$). Three of the patients with the EBV DNA positivity had liver transplantation, non-Hodgkin's lymphoma was detected in 1 patient, Burkitt's lymphoma was found in 1 patient, gingivostomatitis and pharyngotonsillitis which developed during the follow-up with ALL diagnosis was found in 1 patient, and 1 patient was observed to have been diagnosed with autoimmune hemolytic anemia. The amount of DNA detected in the EBV DNA positive samples and the distribution of samples according to the clinics and underlying diseases are shown in Table 4.

Discussion

Epstein-Barr Virüs is reported to be associated with cancers such as Burkitt's lymphoma, Hodgkin's lymphoma, and nasopharyngeal carcinoma; to cause infectious mononucleosis in children; and to be the cause of PTLD in immunosuppressed subjects (6, 7).

Table 3. Relationship between the EBV serology and Real-Time PCR results

	Real-Time PCR Positive					
	VCA IgM	VCA IgG	EBNA IgM	EBNA IgG	EA IgG	Underlying Disease
Patient 1	(-)	-	-	-	-	Liver transplantation
Patient 2	(-)	-	-	-	-	Liver transplantation
Patient 3	(-)	-	-	-	-	Liver transplantation
Patient 4 *	-	-	-	-	-	Liver transplantation
Patient 5	(+)	-	-	-	-	ALL
Patient 6	(-)	(+)	(-)	(+)	-	Non-Hodgkin lymphoma
Patient 7		(-)	(+)	-	(+)-	Autoimmune hemolytic anemia

VCA: viral capsid antigen; IgM: immunoglobulin M; IgG: immunoglobulin G; EBNA: nuclear antigen; EA: early antigen; *: The patient had liver transplantation in an external center and consulted with the complaints of fever and rash to our hospital. The EBV DNA was detected by the Real-Time PCR method. Since the patient was not one of our follow-up patients, serology results are not available.

The EBV seropositivity is reported to be 80%-95% in our country (4, 5). Soylu et al. (4) investigated the seropositivity of EBV in serum samples of 7363 patients and found the incidence of EBV at a rate of 81% in our population. Aydemir et al. (5) reported in field surveys that the EBV seropositivity ranged between 70% and 99.4% in the adult age group.

In patients with immune system deficiency, it is important to detect the presence and amount of viral DNA by Real Time PCR in those cases where the EBV serology cannot be interpreted because of an insufficient immunological response (3). In our study, the presence of EBV DNA was investigated through Real-Time PCR in the samples of immunosuppressive patients, and the distribution of samples according to the clinics, and the relationship between the amount of EBV DNA and the clinic was examined.

In our study, the EBV DNA positivity was detected at a rate of 4.2% (7/168) through the Real-Time PCR method. Niesters et al. (8) reported a positivity at a rate of 19.2% in immunosuppressed patients with the Real-Time PCR. Karadağ Geçgel et al. (9) found 6% (6/99) of positivity in immunosuppressed patients. The 4.2% EBV DNA positivity we found in our study was seen to be consistent with the study of Karadağ Geçgel et al. (9) conducted in our country.

When the distribution of the EBV DNA positivity according to the clinics was examined in our study, the highest EBV DNA positivity was found as 14.2% in the samples from the pediatric oncology service (1/7). It was seen that the patient with the EBV DNA positivity was diagnosed with non-Hodgkin's lymphoma. In the thesis study, Bağır (10) investigated the incidence of EBV in 87 children, 51 of whom had non-Hodgkin's lymphoma, and 36 of whom had Hodgkin's lymphoma, and reported a positivity at a rate of 27.4% in patients with non-Hodgkin's lymphoma. Karadağ Geçgel et al. (9) found 8.1% (3/37) of the EBV DNA in patients referred from the pediatric oncology department and reported that 2 of the 3 patients with positivity were diagnosed with Hodgkin's lymphoma, and 1 patient was diagnosed with malignant histiocytosis. It is known that EBV is associated with various lymphomas and cancers, and EBV is often isolated in immunosuppressed patients.

In our study, a 12.5% (1/8) of the EBV DNA positivity was detected in the samples coming from the intensive care unit, and it was seen that the patient with positivity was diagnosed with autoimmune hemolytic anemia. Fadeyi et al. (11) reported a male patient who had autoimmune hemolytic anemia and died as a result of the EBV infection, and they pointed out that autoimmune hemolytic anemia may occur after the treatment of the disease in EBV infections, or may even be the precursor of relapse. Because EBV is among the viral agents that accompany autoimmune hemolytic anemia or play a role in its pathogenesis, the EBV infection should be considered in patients who are diagnosed with autoimmune hemolytic anemia (11-13).

In our study, 4.8% (4/42) of the EBV DNA positivity was detected in the samples from the pediatric gastroenterology service, and it was seen that 3 patients had liver transplantation and that PTLD did not develop in the patients. However, since PTLD is the most common malignancy after transplantation due to the EBV reactivation, patients are continued to be followed up. Lee et al. (14) examined 73 patients who had liver transplantation and EBV positivity, and they reported that there was a statistically significant relationship between an EBV reactivation and the development of PTLD in patients with liver transplantation. Frequent occurrence of the EBV reactivation and the PTLD development in organ recipients suggests that the EBV infection should be considered in these patients, and they should regularly be monitored in terms of EBV.

In our study, the EBV DNA was detected 16 months after the transplantation in the fourth patient coming from the pediatric gastroenterology department, and Burkitt's lymphoma developed in the 26th month of the process continuing with the treatment and re-infections; it was seen that the patient was in the 4th stage when diagnosed. Chemotherapy and acyclovir treatment were started in the patient. High fever, candidiasis, and bacterial and viral infections developed in the patient within the treatment process, and after 25 months, the patient died due to Burkitt's lymphoma. Burkitt's lymphoma is the first neoplasm that was found to be associated with EBV (15-17). Taçyıldız et al. (18) reported a 93% (28/30) of the EBV positivity through PCR in patients with Burkitt's lymphoma, and they drew attention to its association with Burkitt's lymphoma. Yılmaz et al. (19) reported a case who was serologically EBV negative before the transplantation and in whom 7x10^6 copies/ml EBV DNA was found in the plasma sample 32 months after the transplantation, and they emphasized the necessity of the PCR monitoring for early diagnosis and treatment of the infections developing due to the EBV reactivation after transplantation. Özkan

Table 4. The amount of DNA detected in the EBV DNA positive samples, the distribution of samples according to clinics, and underlying diseases

Clinic	Serum (copies/ml)	CSF (copies/mL)	Underlying Disease	Total
Pediatric gastroenterology	1.2x10^4			
	1.3x104			4
	5.0x104		Liver transplantation	
	6.5x105		Burkitt's lymphoma	
Pediatric hematology	1.2x104		Acute lymphoblastic leukemia	1
Oncology	4.3x104		Non-Hodgkin's lymphoma	1
Intensive care unit	5.2x104	Autoimmune hemolytic anemia	1	
Total	6	1		7

CSF: Cerebrospinal fluid
p>0.05

et al. (20) reported that the diagnosis of Burkitt's lymphoma was made after 17 months in a patient in whom the EBV DNA was detected after liver transplantation, and they emphasized the need for a regular follow-up of the patients with liver transplantation due to an EBV reactivation and the success of the Real-Time PCR method for early diagnosis and treatment. The EBV reactivation detected in our study, which resulted in the death of the patient with Burkitt's lymphoma, is valuable in terms of demonstrating the association between Burkitt's lymphoma and EBV.

A total of 4.1% (1/24) of the EBV DNA positivity was detected in the samples from the pediatric hematology unit. EBV-induced gingivostomatitis and pharyngotonsillitis were seen to develop during the period when the patient with positivity was followed up with ALL diagnosis. Ahmed et al. (21) found a 36.3% (29/80) of positivity in their study in which they investigated the EBV incidence in pediatric patients with leukemia and reported that 79.3% of the patients were those who received the treatment due to ALL, and the highest level of the EBV positivity was seen in ALL-diagnosed patients. In our study, similarly, the development of pharyngotonsillitis and gingivostomatitis and the detection of EBV as a factor in the patient followed up with the diagnosis of ALL show that especially the immunosuppressed children should be monitored in terms of the EBV infection that could occur depending on the EBV reactivation, and EBV should be investigated as a factor in cases of pharyngotonsillitis, oropharyngitis, and gingivostomatitis.

In our study, the amount of EBV DNA was 10^4 copies/ml (liver transplant, non-Hodgkin lymphoma, ALL, autoimmune hemolytic anemia) in 6 samples (85.8%) and was 10^5 copies/ml in 1 sample (14.2%) (Burkitt's lymphoma). In various studies to understand the relationship between the amount of the EBV DNA and clinic; Visco et al. (22) detected $\geq 10^3$ copies/ml EBV DNA in approximately 20% of patients with leukemia. In their study in which they investigated the presence of the EBV DNA in 109 immunosuppressed patients, 7 lymphoma, and 2 AIDS patients, Gartzonika et al. (23) detected the EBV DNA positivity in 56.7% of the patients and reported that the viral load ranged between 10^2 and 10^4. Özçay et al. (24) reported the viral load as 10^4 copies/mL on average in patients in whom the EBV infection developed after liver transplantation. The amount of 10^4 copies/ml EBV DNA detected in immunosuppressed patients in our study was similar to other studies performed.

In our study, the highest amount of the EBV DNA was detected in a patient with Burkitt's lymphoma with $6.5x10^5$ copies/ml. Stevens et al. (25) found the amount of the EBV DNA in serum samples of the patients with Burkitt's lymphoma as 10^3-10^6 copies/ml. Tang et al. (26) reported the viral load in Burkitt's lymphoma patients as 10^6-10^7 copies/mL. Yılmaz et al. (19) reported a patient of Burkitt's lymphoma in whom 10^6 copies/ml EBV DNA was detected in the plasma sample. In our study, it was seen that the amount of 10^5 copies/ml EBV DNA detected in the patient followed up with the diagnosis of Burkitt's lymphoma was consistent with the studies performed, and the fact that the highest EBV DNA amount was found in the patient diagnosed with Burkitt lymphoma is thought-provoking.

In cases when the immune response is inadequate, and the EBV serology cannot be interpreted in patients with abnormal immune system, the use of molecular methods is required (27). In our study, VCA IgM, an indicator of acute infection, was also positive in only 1 of the EBV DNA positive patients (17%). The fact that the serology was unknown in 1 of the 7 patients who were detected as positive through the Real-Time PCR method and that VCA IgM positivity was detected in only 1 of the 6 patients was interpreted by us as an indication of an inadequate immunological response in immunosuppressed patients. Geçgel et al. (9) reported that the serological tests alone may be insufficient in immunosuppressive patients, and the diagnosis should be supported by VCA-IgG avidity and the Real-Time PCR tests. Consistent with the conducted studies, our study has shown that, especially in immunosuppressed patients, serologic tests may be inadequate in detecting the EBV reactivation; since Real-Time PCR can detect the viral DNA before the symptoms occur and enables preemptive therapy, quantitative EBV DNA testing in such patients is crucial in determining the presence and amount of the viral DNA, and the patient's prognosis and follow-up (14, 28, 29).

Conclusion

As a result, EBV causes various cancers and is frequently isolated in immunosuppressed patients, such as organ recipients. Detection of the presence of the EBV DNA through Real-Time PCR in immunosuppressive patients as well as the regular follow-up of the EBV DNA amount will help to detect the viremia levels and increased viral load, and to assess the changes in early diagnosis and in clinical course. The amount of the EBV DNA detected through Real-Time PCR in immunosuppressed patients was found in high titre ($\geq 10^5$ copies/mL) in Burkitt's lymphoma cases; we think that immunosuppressed patients with high levels of the EBV DNA should be examined in terms of Burkitt's lymphoma.

Ethics Committee Approval: Ethics Committee Approval is not obtained due to the retrospective nature of this study.

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