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Epstein-Barr virus and JC virus Reactivation in Dermatological Patients Receiving Immunosuppressive Therapy

İmmünosüpressif Tedavi Alan Dermatolojik Hastalarda Epstein-Barr virus ve JC virus Reaktivasyonu

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Abstract

Immunosuppressive medications are the mainstay treatment of many severe cutaneous diseases. The objective of this study was to investigate the reactivation of certain viral agents (Epstein-Barr virus, human cytomegalovirus, BK virus, JC virus, B19 virus, and herpes simplex virus) in dermatology patients who underwent immunosuppressive treatment. The study included 57 patients and they were divided into three groups based on the treatments; patients not receiving any immunosuppressive therapy (control group), patients treated with phototherapy only (phototherapy group), and patients treated with systemic immunosuppressive agents (systemic immunosuppressive therapy group). Saliva, blood, and urine samples were collected from all patients, and these samples were analyzed for the presence of virus-specific DNA by TaqMan-based real-time polymerase chain reaction. In the overall study population, the positivity rate for any viral reactivation in at least one of the investigated specimens was 31.6% (18/57). The frequencies of viral reactivation in the control, phototherapy, and systemic immunosuppressive treatment groups were 9.5% (2/21), 18.8% (3/16), and 65.0% (13/20), respectively. The viral reactivation rate in patients receiving systemic immunosuppressive treatments was significantly higher than in the other two groups (p=0.00022 and p=0.00544), but there was no statistically significant difference between the phototherapy and control groups. EBV (15 patients, 26.3%) and JC virus (7 patients, 12.3%) were the most frequently detected viruses. Viral reactivation is a potential risk for dermatology patients receiving immunosuppressive treatments and it can be considered as a parameter of disease control and management. Our study results also reveal that the safety profile of phototherapy is not different from the control group in terms of viral reactivation.

Keywords: Phototherapy, Systemic immunosuppressive, Epstein-Barr virus, Human cytomegalovirus, BK virus, JC virus, B19 virus, Herpes simplex virus.

Özet

İmmünosüpressif ilaçlar, birçok ciddi dermatolojik hastalıkta temel tedavi yaklaşımıdır. Bu çalışmanın amacı, immünosüpressif tedavi alan dermatoloji hastalarında belirli viral etkenlerin (Epstein-Barr virus, insan sitomegalovirus, BK virus, JC virus, B19 virus ve herpes simpleks virus) reaktivasyonunun araştırılmasıdır. Çalışma grubu 57 hastayı içeriyordu ve hastalar uygulanan tedaviler baz alınarak üç gruba ayrıldı; herhangi bir immünosüpressif tedavi almayan hastalar (kontrol grubu), sadece fototerapi uygulanan hastalar (fototerapi grubu) ve sistemik immünosüpressif ilaçlarla tedavi edilen hastalar (sistemik immünosüpressif tedavi grubu). Tüm hastalardan tükürük, kan ve idrar örnekleri toplandı ve bu örnekler TaqMan tabanlı gerçek zamanlı polimeraz zincir reaksiyonu ile ilgili virüs spesifik DNA'ların varlığı yönünden incelendi. Çalışma popülasyonu genelinde incelenen örneklerinden en az birinde herhangi bir viral reaktivasyon için pozitiflik saptanma oranı %31.6 (18/57) idi. Kontrol, fototerapi ve sistemik immünosüpressif tedavi gruplarında viral reaktivasyon sıklığı ise sırasıyla %9.5 (2/21), %18.8 (3/16) ve %65.0 (13/20) olarak bulundu. Sistemik immünosüpressif tedavi alan hastalarda viral reaktivasyon oranı diğer iki gruba göre anlamlı derecede yüksekti (p=0.00022 ve p=0.00544), ancak fototerapi ve kontrol grupları arasında istatistiksel olarak anlamlı farklılık yoktu. EBV (15 hasta, %26.3) ve JC virus (7 hasta, %12.3) en sık saptanan virüslerdi. Viral reaktivasyon, immünosüpressif tedavi alan dermatoloji hastaları için potansiyel bir risktir ve bu nedenle hastalık kontrolü ve yönetiminin bir parametresi olarak kabul edilebilir. Çalışma sonuçlarımız ayrıca, fototerapinin güvenlik profilinin, viral reaktivasyon açısından, kontrol grubundan farklı olmadığını ortaya koymaktadır.

Anahtar Kelimeler: Fototerapi, Sistemik immünosüpressif, Epstein-Barr virus, İnsan sitomegalovirus, BK virus, JC virus, B19 virus, Herpes simpleks virus.

An abstract of this study was presented as an oral presentation at the 5. International Trakya Family Medicine Congress, Balkan Congress Center, Edirne, Turkey (16-20 March 2016).

Introduction

The immune system plays an important role in the pathogenesis of many dermatological diseases including psoriasis, pemphigus, atopic dermatitis, drug eruptions, vitiligo, and alopecia. Therefore, immunosuppressive treatments are generally needed for these indications, and sometimes systemic treatment options. Anti-TNF drugs (e.g., adalimumab and etanercept), topical and systemic steroids, methotrexate, cyclosporine, purine synthesis inhibitors (e.g., azathioprine) are act by targeting the immunological mechanisms and are used successfully for the treatment of these diseases and disorders. Immunosuppressive treatments not only increase the risk of new infections, but also reactivate the preexisting infections of latent viruses [1]. Moreover, impaired cutaneous and/or mucosal integrity due to the indicated skin diseases can contribute to increased susceptibility.

Herpes simplex virus (HSV)-1, HSV-2, varicella zoster virus (VZV), Human adenoviruses, Epstein-Barr virus (EBV), human cytomegalovirus (CMV), BK virus, JC virus, and B19 virus are examples of viruses that cause typical persistent infections [2]. EBV, CMV, BK virus, JC virus, B19 virus, and HSV are most frequently transmitted to humans through oral-respiratory secretions, and the infections caused by these viruses usually occur in early childhood and adolescence and are often asymptomatic [3-9]. After primary infection, these viruses have the ability to establish latent infections and can cause recurrent infections in different organs and systems especially at times of immunosuppression [3].

This preliminary study aimed to investigate asymptomatic reactivation of six different viruses associated with latent infection in dermatology patients receiving immunosuppressive treatment. Certain viral agents considered to have clinical importance were investigated separately in different specimens of patients: EBV, CMV, BK virus, JC virus, B19 virus, and HSV. Reactivation in patients receiving phototherapy treatment was also investigated due to the treatment's immunosuppressive properties and clinical importance in dermatology practice.

Material and Method

Fifty-seven dermatology patients were included in this study on a voluntary basis

(informed consent was obtained from all study subjects). The patients were divided into three groups according to their immunosuppressive therapy status. Control group: Not receiving any immunosuppressive therapy (21 patients). Phototherapy group: Treated with phototherapy only (16 patients). Systemic immunosuppressive therapy group: Treated with systemic immunosuppressive agents (20 patients). The study was conducted in a single center; clinical samples were obtained from the Dermatology Department and studied in the Medical Virology Laboratory of the Gulhane Training and Research Hospital.

Compliance with ethical standards

The study was performed upon approval by the local ethical committee (Gulhane Military Medical Academy in Ankara, Turkey. Decision number: 1491-41-16/1648.4-63) and after informed consent was obtained from the study participants. This article does not contain any studies with animals performed by any of the authors. In addition, this study was not funded by any organization or grant. The authors declare that they have no conflict of interest.

Sample collection

Samples were collected one time from each patient for use in molecular analyses. A saliva specimen was collected from all patients by oral swabbing (buccal swabbing) using a sterile polyester fiber-tipped swab. In addition, urine and blood specimens were collected from all patients. Demographic characteristics and the previous health status of all patients were evaluated retrospectively.

DNA isolation and real-time polymerase chain reaction (PCR) analyses

Single nucleic acid extraction protocol was applied for all viruses, because all viral agents investigated in this study (EBV, CMV, BK virus, JC virus, B19 virus, and HSV) were deoxyribonucleic acid (DNA) viruses. Template DNA was extracted from clinical specimens by means of the standard phenol-chloroform-isoamyl alcohol method [10]. To ensure reproducibility, a specific protocol was used for all clinical specimens. Buccal swabs containing approximately 100-200 µl of saliva, urine samples (100 µl), and blood samples (50 µl) were suspended separately in 500 µl of TE buffer (10 mM Tris hydrochloride, 1 mM EDTA, pH 8) and homogenized through vigorous mixing on a vortex. There was an approximate two-fold range in the amount of saliva collected via swab. When the viral load of various saliva specimens was measured, this did not logarithmically affect the test results and thus was ignored. A 10-µl aliquot of protease solution (65 mg/ml) (Sigma-Aldrich Corp., St. Louis, MO, USA) and 250 µl of K buffer were added to 250 µl of mixed specimen and incubated for 60 minutes at 45°C. Following centrifugation at 10,000 g for 10 minutes at 12°C, DNA was extracted from the supernatant, using a mixture of 250 µl alkali phenol and 250 µl chloroform-isoamyl alcohol (24:1), and then precipitated using 500 µl isopropyl alcohol. The DNA was washed in 75% ethyl alcohol, centrifuged at 10,000 g for 5 minutes at 4°C, air-dried at 37°C, and dissolved in 100 µl distilled water [11].

The presence of different viruses and the viral DNA copy numbers were investigated in saliva, blood, and urine samples. Four viruses (EBV, CMV, B19 virus, and HSV) were investigated in saliva samples, five viruses (EBV, CMV, BK virus, JC virus, and B19 virus) were investigated in blood samples, and three viruses (CMV, BK virus, and JC virus) were investigated in urine samples of all patients. Real-time PCR reactions and quantitative analyses were performed based on the previously described methods [3,11,12].

The reaction mixture was prepared as follows: 1.25 U Hot Start Taq DNA polymerase (Bioron, Germany), 10 pmol of each primer, 2.5 pmol TaqMan probe, 2.5 mM MgCl2, and 0.2 mM dNTP mix. PCR amplifications were conducted after the addition of 5 µL of the sample containing the template DNA in a final volume of 25 µL. The PCR amplification cycles were as follows: Initial denaturation and at hot-start Taq DNA polymerase activation at 95°C for 10 minutes, followed by 40 amplification cycles at 95°C for 15 seconds and at 60°C for 1 minutes (annealing-extension step). The TaqMan probes were labeled with a fluorescent reporter dye (FAM; 6-carboxy fluorescein) at the 5' end and with a black hole quencher (BHQ) as the non-fluorescent quencher at the 3' end. The human glyceraldehyde-3-

phosphate dehydrogenase (GAPDH) gene was used as an internal control [3,11], and the PCR mixture without template DNA was used as a negative control in all PCR reactions. Viral isolates used as a positive control were originated from the strains of our laboratory. All viruses and GAPDH amplicons were cloned into plasmid vectors using a TOPO TA cloning system (Invitrogen, USA), and the detection sensitivities of the PCR assays were analyzed using serial plasmid dilutions (10^8-10^1 copies/ml). The primers and probes were designed using the OligoYap 4.0 software program [13]. All primer and probe sequences (Table 1) were analyzed with the GenBank BLAST database for specificity and were synthesized (MWG-Biotech, Ebersberg, Germany). All PCR reactions were performed on an ABI Prism 7500 Sequence Detection system (Applied Biosystems, USA). By using plasmid dilutions, the detection sensitivities of TaqMan-based PCR assays for all viruses were determined as 10^1 copies/test.

Table 1. Primers and probes used in real-time PCR assays.

Virus / internal control	Target gene	Primer/prob sequences	Amplicon size
CMV	UL20 type 1 membrane protein gene	F: 5'-ggaagtagcgtcggtgttttatg-3'	118 bp
		R: 5'-gccacaacggcatctacgatc-3'	
		P: 5'-FAM-cagcgtcgtcgtcactcgtggc-BHQ-3'	
HSV (common primers/probe for HSV-1 and HSV-2)	Thymidine kinase UL23 gene	F: 5'-gcataaggcrtgcycattgtta-3'	178 bp
		R: 5'-cgcgcgacratatcgtctac-3'	
		P (antisense): 5'-FAM-ccgagccgatgacttactggcrggt-BHQ-3'	
JC virus	Large T antigen gene	F: 5'-catttyttcatggcaaaacaggtytt-3'	97 bp
		R: 5'-tttgtaggtgccaacctatggaa-3'	
		P: 5'-FAM-acttctcattaaatgtattccaccaggat-BHQ-3'	
BK virus	Major capsid protein VP1 gene	F: 5'-gagtgtccaggggcagctc-3'	126 bp
		R: 5'-gcattctacctctgtwatagcatc-3'	
		P: 5'-FAM-aggaacccgtgcaagtgccaaaactac-BHQ-3'	
EBV	Major tegument protein, BNRF1/p140 gene region	F: 5'-gaacctggtcatcctttgcca-3'	102 bp
		R: 5'-ccagtgcttcgttatagccgta-3'	
		P: 5'-FAM-agtacgagtgcctgcgaccagatc-BHQ-3'	
B19 virus	Minor capsid protein VP1 gene	F: 5'-tacacaagcctgggcawgtt-3'	105 bp
		R: 5'-cagcactgtcaacagcactt-3'	
		P: 5'-FAM-actacccggtactaactatgttggg-BHQ-3'	
GAPDH	Human GAPDH gene	F: 5'-tcctgcaccaccaactgcttag-3'	145 bp
		R: 5'-catcacrccacagyttyccagag-3'	
		P: 5'-FAM-aggtcatccatgacaactttggyatcg-BHQ-3'	

Abbreviations: F: forward primer, R: reverse primer, and P: probe. R, W, and Y codes indicate degenerate bases; R=(A/G), W=(A/T), and Y=(C/T).

Statistical analysis

Statistical significance was calculated by means of an independent samples z-test for a difference in two percentages in independent groups, with p values <0.05 considered significant. All reported p values were two-sided. Measures of central tendency were calculated using the Statistical Package for the Social Sciences (SPSS) 15.0 software (SPSS, Inc., Chicago, IL, USA).

Results

The study group consisted of 57 patients with a mean age of 31 years (±15.82), median age of 23, and distribution range of 11-82. 63.2% of the patients were young adults between the ages of 20-26 (36/57), see Figure 1. The groups were not statistically different in terms of age and gender. 18 patients (31.6%) had psoriasis, seven patients (12.3%) had atopic dermatitis, and six patients (10.5%) had pemphigus (Table 2).

All patients in phototherapy group were treated with narrow-band UVB phototherapy. Treatment agents and number of patients in systemic immunosuppressive therapy group were as follows: systemic steroid (11), cyclosporine (4), anti-tumor necrosis factor-alpha (anti-TNF-α) (3), azathioprine (1), and methotrexate (1). In the overall patient sample, the positivity rate for at least one of the six viruses in any of the three specimens was 31.6% (18/57). None of the patients had viral DNA positivity in the blood samples. CMV and B19 viruses could not be detected in any patients. Six patients were affected by multiple viruses. The distribution of the detected viruses was as follows: EBV in 15 patients (26.3%), JC virus in 7 patients (12.3%), BK virus in two patients (3.5%), and HSV in one patient (1.8%). Patients with positive viral samples, treatments they received, and treatment periods are shown in Table 3.

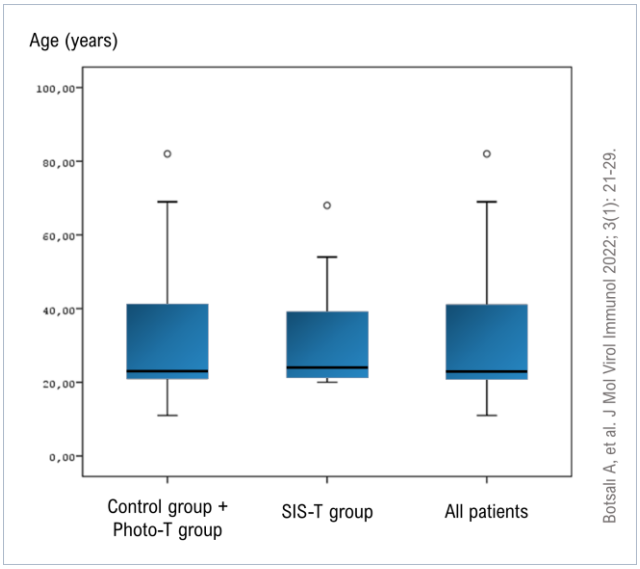

Figure 1. Box plot of age distribution, showing 25th, 50th, and 75th centiles. Most of (63.2%) the study patients consist of young adults (who have aged between 20-26). Photo-T: Phototherapy. SIS-T: Systemic immunosuppressive therapy.

Table 2. Primary diseases and number of patients affected in each group and details of immunosuppressive treatments in systemic immunosuppressive therapy group.

No	Primary disease	Control group	Phototherapy group	Systemic immunosuppressive therapy group and their treatments		Total
1	Psoriasis	3	8	7	adalimumab (2 pt), etanercept (1 pt), cyclosporine (3pt), methotrexate (1 pt)	18
2	Atopic dermatitis	1	2	4	steroid (3 pt), steroid + cyclosporine (1 pt)	7
3	Pemphigus	2		4	steroid (3 pt), steroid + azathioprine (1 pt)	6
4	Drug eruption	4		3	steroid (3 pt)	7
5	Alopecia areata or universalis	1		2	steroid (2 pt)	3
6	Vitiligo		3			3
7	Darier disease	2				2
8	Hidradenitis suppurativa	1				1
9	Ichthyosis	1				1
10	Jessner's lymphocytic infiltration	1				1
11	Keratoderma	1				1
12	Lichen planus		1			1
13	Lipoid proteinosis	1				1
14	Mycosis fungoides		1			1
15	Lichen simplex chronicus		1			1
16	Perforating folliculitis	1				1
17	Squamous cell carcinoma	1				1
18	Tinea barbae	1				1
	Total	**21**	**16**	**20**		**57**

*Steroid treatment; ≥ -40 mg/day methylprednisolone or equivalent. pt: patients.

The frequencies of any viral reactivation in the control, phototherapy, and systemic immunosuppressive therapy groups were 9.5%, 18.8%, and 65.0% respectively. According to these results, the systemic immunosuppressive therapy group revealed a statistically significant difference in comparison to both the control and phototherapy groups (p=0.00022 and p=0.00544, respectively). However, there was no statistical difference between control and phototherapy groups (p=0.41794). The frequencies of EBV, JC virus, and overall viral reactivations within the three groups are shown in Table 4.

Table 3. Patient characteristics with any positive clinical samples.

No.	Patient group	Dermatological diseases	Age	Gender	Saliva sample (copy/ml)	Urine sample (copy/ml)	Immunosuppressive treatment	Duration of treatment
1	Control	Drug eruption	21	male		JC virus: 4.3×10^3	none	-
2	Control	Pemphigus	44	female	EBV: 9.2×10^4	JC virus: 3.0×10^4	none	-
3	Photo-T	Vitiligo	62	male	EBV: 7.7×10^4		phototherapy	8 months
4	Photo-T	Psoriasis	11	female	EBV: 7.2×10^3		phototherapy	1 month
5	Photo-T	Atopic dermatitis	26	male	EBV: 6.8×10^6		phototherapy	2 months
6	SIS-T	Psoriasis	22	male	EBV: 8.7×10^7		cyclosporine	40 days
7	SIS-T	Atopic dermatitis	32	male	EBV: 8.9×10^5		steroid	1 month
8	SIS-T	Psoriasis	36	female		BK virus: 4.2×10^3	cyclosporine	45 days
9	SIS-T	Psoriasis	45	male	EBV: 6.0×10^5		adalimumab	10 days
10	SIS-T	Psoriasis	42	female		JC virus: 8.6×10^4	cyclosporine	1 month
11	SIS-T	Atopic dermatitis	21	male	EBV: 7.3×10^4		steroid / cyclosporine	15 days / 2 months
12	SIS-T	Pemphigus	20	male	EBV: 3.0×10^6		steroid	6 months
13	SIS-T	Drug eruption	30	male	EBV: 7.0×10^4		steroid	1 months
14	SIS-T	Alopecia universalis	25	male	EBV: 8.0×10^4	BK virus: 4.0×10^3	steroid	2 months
15	SIS-T	Pemphigus	23	male	EBV: 6.2×10^5	JC virus: 5.8×10^5	steroid	3 months
16	SIS-T	Pemphigus	23	male	EBV: 2.0×10^5	JC virus: 8.3×10^6	Steroid + azathioprine	1 year
17	SIS-T	Psoriasis	20	male	EBV: 2.0×10^5	JC virus: 4.0×10^3	methotrexate	45 days
18	SIS-T	Drug eruption	54	female	EBV: 2.0×10^6 HSV: 3.0×10^5	JC virus: 5.2×10^7	steroid	2 months

Photo-T: Phototherapy. SIS-T: Systemic immunosuppressive therapy.

Table 4: Significance test for difference of two percentages in independent groups-Z test results.

	Groups compared	Viral reactivation	EBV reactivation	JC virus reactivation
1	Control group vs. Photo-T group	2/21 vs. 3/16 Z-Score=0.8133. P=0.41794.	1/21 vs. 3/16 Z-Score=1.3575. P=0.17384.	2/21 vs. 0/16 Z-Score=1.2692. P=0.20408.
2	Control group vs. SIS-T group	2/21 vs. 13/20 Z-Score=3.6863. **P=0.00022.**	1/21 vs. 11/20 Z-Score=3.5339. **P=0.00042.**	2/21 vs. 5/20 Z-Score=1.3164. P=0.18684.
3	Photo-T group vs. SIS-T group	3/16 vs. 13/20 Z-Score=2.775. **P=0.00544.**	3/16 vs. 11/20 Z-Score=2.217. **P=0.02642.**	0/16 vs. 5/20 Z-Score=2.1553. **P=0.03078.**
4	Control group + Photo-T group vs. SIS-T group	5/37 vs. 13/20 Z-Score=3.991. **P=0.00006.**	4/37 vs. 11/20 Z-Score=3.6158. **P=0.0003.**	2/37 vs. 5/20 Z-Score=2.1511. **P=0.03156.**

The result is significant at p <0.05 and P values are two-sided. Photo-T: Phototherapy. SIS-T: Systemic immunosuppressive therapy.

Discussion

Autoimmune and immune mediated diseases comprise a considerable portion of dermatologic diseases and generally require immune suppression. During immunosuppressive treatments, reactivation of a latent viral infection appears to be an expected result, as in HSV and VZV reactivations [14]. Similarly, human papilloma virus and molluscum contagiosum lesions associated with immunosuppressive treatments have been described [15]. However, in some cases life threatening viral reactivations with high morbidity may occur in association with immunosuppressive drugs. In a study, EBV-associated primary cutaneous lymphoma case was determined in a patients treated with prednisolone and azathioprine for dermatomyositis [16]. In another case, HSV reactivation reported in a patient receiving adalimumab for rheumatoid arthritis [17]. Other examples are that subclinical reactivation of JC virus in natalizumab-treated patients with multiple sclerosis and JC virus associated progressive multifocal leukoencephalopathy (PML) in psoriasis patients with efalizumab use and the researchers suggest that prolonged efalizumab therapy is a risk factor for PML [18,19]. Viral reactivation is a potential risk for patients receiving immunosuppressive treatments. However, it appears this potential risk has not been investigated specifically for dermatological diseases. In this study, the positivity rate for any viral reactivation in at least one of the investigated specimens was 31.6% in the overall study population and 65.0% in systemic immunosuppressive therapy group. In addition, EBV (15 patients, 26.3%) and JC virus (7 patients, 12.3%) were the most frequently detected viruses. High (10^7) viral copy numbers were detected in some patients and this finding is remarkable.

Although EBV establishes a life-long, persistent infection in over 90% of adults worldwide, seasonal shedding of the virus has been related with transient immunosuppression [20,21]. Moreover, failures of EBV-specific immunity may play a role in the pathogenesis of a subgroup of lymphoid and epithelial malignancies [21]. Several case reports of EBV-associated malignancies related to immunosuppressive treatments and recommendations for preventive approaches exist in the literature [16,22,23]. In this study, the frequency of EBV reactivation was significantly higher in systemic immunosuppressive therapy group than in control group ($p=0.00042$) or phototherapy group ($p=0.02642$) patients. Accordingly, a positive correlation was found between the reactivation of EBV and systemic immunosuppressive treatments.

Another viral agent leading to significant morbidity due to reactivation is JC virus which is linked to PML. Different immunosuppressive drugs, including corticosteroids, leflunomide, methotrexate, and cyclophosphamide, have been reported previously in association with PML [24]. In recent years, concerns regarding the risk of JC virus reactivations associated with immunosuppressed populations have led to the promotion of alternative biologic treatments. However, safety concerns related to immune suppression and subsequent PML development have also arisen against biologic agents mainly after reports for rituximab, natalizumab, and efalizumab [18,19,25]. Insomuch that, reported cases of efalizumab-associated PML leading to fatality resulted in withdrawal of the drug from the market [25]. The JC virus is very common in the general population and infecting 70 to 90 percent of humans. It has been shown in different studies that urinary JC virus excretion can be seen in healthy individuals and associated with increasing age [20,26]. Nevertheless, most (63.2%) of the study patients consist of young adults who have aged between 20-26 (Figure 1). Therefore, we can say that the present data which about status on viral reactivation most likely to be due to the systemic immunosuppressive treatment instead of age or secondary chronic diseases.

This study's findings regarding EBV and JC virus reactivations are parallel to previous studies investigating the latency of viruses in healthy individuals. In a study investigating the presence of EBV, CMV, BK virus, and JC virus; EBV and JC virus were the two most frequently detected viruses in healthy individuals [20]. In that previous study, while 76.9% of the 130 EBV reactivations were EBV alone, 21.5% coexisted

with JC virus, and 1.5% coexisted with CMV [20]. In addition, while 66.3% of the 83 JC virus reactivations were JC virus alone, 33.7% coexisted with EBV [20]. In the present study, 60.0% (9/15) of the EBV reactivations were EBV alone, while 40% (6/15) coexisted with both JC virus and BK virus. In addition, this study showed 71.4% (5/7) of the JC virus reactivations coexisting with EBV, and only 28.6% (2/7) being JC virus alone. We think these results suggest that most of the JC virus reactivations in dermatology patients occur with EBV reactivations, potentially related to immunosuppression mechanisms leading to reactivation of both viruses. The second group of patients considered in this study received only phototherapy. Although the mechanisms of action have not been fully understood, UV-induced immunosuppression could interfere with immune response to the reactivations of viruses [27]. However, this study did not determine any correlation for narrow-band UVB phototherapy with viral reactivation. Comparison of viral positivity between the control and phototherapy groups revealed insignificant differences (p=0.41794), including EBV (p=0.17384) and JC virus (p=0.20408). Thus, this study's results confirm the safety of narrow-band UVB in terms of viral reactivation.

One limitation of this study is that viral DNA positivity was accepted as viral reactivation, without distinguishing new infections by determining the serological status of patients. EBV, CMV, BK virus, JC virus, B19 virus, and HSV infections usually occur in early childhood and adolescence [1-5,7], and all but one of the patients in this study with positive results were over 20 years old, suggesting reactivation of latent, rather than new, viral infections. A small number of seronegative individuals may be present in each group, although it seems to be a low possibility according to the age distribution of patients and the prevalence of infections. However, we think that the effect of this situation on the study results is negligible, because the main objective of this study is that investigation of the relationship between immunosuppressive therapy and viral replication. Traditional serology is the best test for evaluating acute, remote, or reactivated infection in healthy individuals [28]. However, in immunosuppressed individuals (e.g., transplant patients, patients undergoing immunosuppressive therapy, elderly patients, and HIV infected patients) serological assays are discouraged for many reasons, such as dysfunctions in the production and maintenance of antibodies and false negative reactions [29]. On the other hand, serologic responses are delayed and do not necessarily indicate ongoing replicative activity of EBV [30].

Therefore, direct detection of viral nucleic acid or protein is essential to diagnosis of viral infection in immunosuppressed patients have inconsistent humoral responses against EBV [28,30]. One of the most common practices of this approach in clinical virology is that the diagnosis and follow-up of CMV infections in transplant patients in which CMV serology has not importance for the diagnosis of active CMV disease or CMV infection [31].

Conclusion

In conclusion, even though this study involved small sample sizes and different groups of dermatological diseases with various treatment protocols, the findings reveal that the frequency of viral reactivation is significantly higher in immunosuppressed dermatology patients. A high viral DNA positivity rates determined in this study existed even with a relatively short duration of immunosuppression. However, most of these patients are in need of long-term treatment, which increases the risk of virus-related adverse events. More comprehensive follow-up studies are needed to determine the risks associated with viral reactivation in individuals with specific dermatological diseases such as pemphigus, psoriasis, and atopic dermatitis, and to determine whether it is necessary to routinely monitor these patients for viral reactivation during the immunosuppressive therapy period.

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