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Original Article DENTISTRY

Investigation of the presence of Epstein-Barr virus in patients who had Oral Lichen Planus and Oral Lichenoid Contact Lesions with Real-time PCR method in serum, tissue, and saliva samples

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ABSTRACT

OBJECTIVE: Oral Lichen Planus (OLP) is an immune system disease and its cause has not been fully determined yet. Oral Lichenoid Contact Lesions (OLCL) is an allergic condition known to develop because of dental materials. It is considered that some infectious agents (e.g., Epstein-Barr virus (EBV)) play roles in the etiology of OLP and OLCL. The purpose of the present study was to investigate the presence of EBV in different clinical samples of patients who had OLP and OLCL, to show its relationship with OLCL, and to determine its role in etiopathogenesis in these patients.

METHODS: Twenty (20) OLCL, twenty-three (23) OLP, and twenty (20) healthy volunteers who applied to Istanbul University Faculty of Dentistry, Oral and Maxillofacial Surgery were included in the study, regardless of gender. Biopsy samples were taken from patients who had a 5mm punch, including the mucosa containing the lesion along with saliva and blood samples, and all clinical samples were sent to the Department of Medical Microbiology Laboratory under appropriate storage conditions. After the isolation of the DNA from clinical samples, EBV DNA was analyzed on the Light Cycler 480 II device by using Real-time PCR (RT-PCR) tests. The evaluation of the statistical data of the results was made by using the SPSS program.

RESULTS: When the data were evaluated, EBV DNA positivity was detected in 13.04% of the patients who had OLP, 10% of the patients who had OLCL, and 5% of the individuals in the Control Group. In saliva samples, EBV DNA was found positive in 21.74% of individuals with OLP, 15% of individuals with OLCL, and 10% of individuals in the Control Group. In the biopsy samples, EBV DNA was detected positive in 21.74% of the OLP patients, 15% of the OLCL patients, and 10% of the Control Group individuals.

CONCLUSION: Based on the findings of the present study, no significant differences were observed in the presence of EBV DNA or the quantitative viral load between patients with OLP, OLCL, and the Control Group. However, the quantitative EBV DNA results varied depending on the type of clinical sample selected. We believe that comprehensive studies that will include a larger number of samples must be conducted to determine the role of EBV in OLP and OLCL.

Keywords: EBV; Oral Lichen Planus; Oral Lichenoid Contact Lesions; Real-time PCR.

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Oral Liken Planus (OLP) is defined as a non-infectious oral mucosal disease that can be detected frequently in individuals and makes up approximately 9% of all white lesions in the oral mucosa [1, 2]. It is already known that OLP creates a chronic inflammatory condition, affecting the stratified squamous epithelial layer of the mucosa. Usually, oral lesions are bilateral, symmetrical, and frequent on the buccal mucosa, dorsum of the tongue, and gingiva. Its unilateral presentation and the localizations on the palate, lips, and floor of the mouth are atypical. It is emphasized that T-cell activation plays role in the pathogenesis of the disease, and it is also considered a T-cell-mediated autoimmune disease because it is a T-cell-mediated hypersensitivity reaction [3, 4]. Oral Lichenoid Contact Lesions (OLCL) cause an allergic clinical condition considered to develop in relation to dental materials. They are also defined as lesions occurring as a result of irritation, contact allergy, and local toxic effects, with histopathological and clinical characteristics similar to OLP lesions occurring in areas in contact with dental materials. Reactions associated with OLCL occur as a result of allergic contact stomatitis, especially in areas with a direct topographic relationship with amalgam or other dental restorative materials [5, 6]. Although OLP and OLCL are similar in clinical and histopathological terms, it is already known that the diagnosis of OLCL is difficult and the differential diagnostic criteria have not yet been fully identified [3]. It is also known that Epstein-Barr virus (EBV) is widely detected all over the world and the primary mode of transmission is contact with oropharyngeal secretions [7]. Although the etiology of OLP is not known, various factors were associated with it. Possible causes include stress, infection, diabetes, autoimmune diseases, some dental materials, chronic liver and intestinal diseases, hypertension, medications, and cancer. Herpes Simplex virus type 1 (HSV-1), Human Herpes virus type 6 (HHV-6), Human Papilloma virus (HPV), EBV, and Hepatitis-C virus (HCV) are among the known infectious agents in this respect [2, 4]. Although some studies that were conducted in the past on patients who had OLP showed EBV positivity, there are no studies on the subject for patients who had OLP. Studies show that the data on the relationships between OLP, OLCL, and EBV are quite limited [8, 9]. In the present study, the purpose was to determine the presence of EBV DNA with the Real-time PCR method in different clinical samples of patients who had OLP and OLCL and individuals in the healthy Control Group. It was also envisaged to emphasize the importance of the etiopathogenesis of EBV in patients who had OLCL in diagnosis by investigating the relationship between EBV and OLCL.

Highlight key points

- EBV DNA was detected in a higher percentage of patients with OLP and OLCL compared to the control group.
- EBV DNA positivity varied depending on the clinical sample (biopsy, saliva).
- No significant differences were found in the quantitative load of EBV DNA between the groups.
- While EBV may be involved in OLP and OLCL, further studies are needed to establish a definitive causal relationship.
- The role of EBV in these conditions may vary depending on factors such as the clinical sample and individual susceptibility.

MATERIALS AND METHODS

Determining the Patient Groups and Collection of Samples

The study included 20 OLCL and 23 OLP patients and a Control Group (n=20), who applied to the Department of Oral and Maxillofacial Surgery of Istanbul University Faculty of Dentistry and whose consent was obtained without discrimination in terms of gender. The inclusion criteria for patients who had OLP were the presence of a histopathologically and clinically defined OLP lesion in the oral mucosa and negative results of dental material and contact allergy tests. The admission conditions for OLCL patients were the presence of atypical OLP lesions in their oral mucosa, positive allergic reaction test results, and one or more lesions in the mouth associated with dental restoration. Those who had cardiovascular system diseases, rheumatological diseases, those receiving medication because of psychological problems, individuals who had oncological treatments, and pregnant women were not included in the study. Clinical samples were taken from healthy individuals who applied for dental treatment and constituted the Control Group, with their voluntary consent. The present study was conducted after the ethics committee approval was obtained from the Istanbul University Faculty of Dentistry, Clinical Research Ethics Committee (date: 27.07.2016, number: 2016/36). Tissue samples were taken from patients who had OLP and OLCL by using a 5 mm punch, including the active lesion, and from the healthy mucosa of the individuals in the Control Group. Saliva samples from the individuals designated as the Study and Control Groups were collect-

Table 1. The comparison of the age and gender averages of the groups

	Group						p
	OLP		OLCL		Control		
	Average	SD	Average	SD	Average	SD	
Age	46.04	±11.34	42.10	±7.48	39.65	±10.05	0.081
Male	44.78	±12.94	46.43	±5.26	40.50	±9.72	0.264
Woman	46.86	±10.63	39.77	±7.61	38.38	±11.08	0.077

SD: Standard deviation; OLP: Oral Lichen Planus; OLCL: Oral Lichenoid Contact Lesions.

ed into 50 ml sterile falcon tubes and whole blood samples were collected into blood tubes that contained EDTA before the intraoral surgical procedures. The blood, saliva, and tissue samples were delivered to the laboratories of the Department of Medical Microbiology and stored in a suitable environment (-20 °C) until analyses.

DNA Isolation from Biopsy, Saliva, and Blood Samples

DNA isolation was made from the samples from all groups that were included in the study by using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmBH, Mannheim - Germany) in line with the manufacturer's instructions. The DNAs that were isolated from the clinical samples were stored at -20 °C until analyses.

Detection of EBV DNA with Real-Time PCR

To detect EBV DNA in the Study and Control Groups, the Fluorion EBV QNP Kit (Iontek Ltd., Istanbul, Turkiye) was used along with the Light Cycler 480 II (Roche Diagnostics Mannheim, Germany) RT-PCR device following the manufacturer's instructions. The study was conducted in a total volume of 30 µl by adding 12.5 µl DNA from the sample DNA, standard, positive, and negative controls to the wells. The RT-PCR protocol was performed as 15 minutes of enzyme activation at 95 °C, followed by 45 cycles of amplification (30 seconds at 95 °C, and 90 seconds at 54 °C (singular reading)).

Statistical Analysis

Statistical analyses of the data were conducted by using the SPSS 17.0 program. The suitability of the variables to the normal distribution was examined with the Kolmogorov-Smirnov Test. The evaluation of the variables with non-normal distribution between the groups was done with the Kruskal Wallis Test. The relationships between the measurements were examined with the Spearman Correlation Test. Situations with $p<0.05$ were considered statistically significant.

RESULTS

A total of 63 people were included in the study: 23 in the OLP Group (36.51%), 20 in the OLCL Group (31.75%) and 20 in the Control Group (31.75%). The average age of the women, who constituted 55.56% of the individuals who participated in the study, was found to be 46.86±10.63 in those with OLP, 39.77±7.61 in those with OLCL and 38.38±11.08 in the Control Group. The average age of men, who constituted 44.44% of the individuals who participated in the study, was 44.78±12.94 in those with OLP, 46.43±5.26 in those with OLCL, and 40.50±9.72 in the Control Group.

When the results were examined in terms of age distribution and gender, no statistically significant relationships were detected between the groups (Table 1).

According to the comparison of the EBV DNA results determined with the RT-PCR Method in blood, saliva, and biopsy samples between the groups by using the ANOVA Test, no statistically significant differences were detected between the groups in terms of quantitative EBV DNA results in blood, saliva, and biopsy samples (Table 2).

When the results of EBV DNA studied qualitatively in the blood, saliva, and biopsy samples were compared between the groups, no significant relationships were determined between negative results and the groups (Table 3).

Table 2. The comparison of the results of blood, saliva, and biopsy samples between the groups

EBV DNA	Group						p
	OLP		OLCL		Control		
	Average	SD	Average	SD	Average	SD	
Blood (copy/ml)	11.39	±31.74	21.45	±69.77	5.20	±23.26	0.527
Saliva (copy/ml)	41.18	±86.30	37.90	±94.73	19.00	±60.28	0.645
Biopsy (copy/ml)	73.97	±162.76	46.90	±121.25	46.00	±141.60	0.767
Blood (log) (copy/ml)	0.25	±0.66	0.23	±0.71	0.10	±0.45	0.704
Saliva (log) (copy/ml)	0.49	±0.95	0.36	±0.88	0.23	±0.70	0.607
Biopsy (log) (copy/ml)	0.54	±1.05	0.37	±0.91	0.27	±0.82	0.632

EBV: Epstein-Barr virus; SD: Standard deviation; OLP: Oral Lichen Planus; OLCL: Oral Lichenoid Contact Lesions.

Table 3. The comparison of the results of blood, saliva, and biopsy samples between the groups

EBV DNA	Group			p
	OLP %	OLCL %	Control %	
Blood (copy/mL)				0.667
Negative	86.96	90.00	95.00	
Positive	13.04	10.00	5.00	
Saliva (copy/mL)				0.571
Negative	78.26	85.00	90.00	
Positive	21.74	15.00	10.00	
Biopsy (copy/mL)				0.571
Negative	78.26	85.00	90.00	
Positive	21.74	15.00	10.00	

EBV: Epstein-Barr virus; SD: Standard deviation; OLP: Oral Lichen Planus; OLCL: Oral Lichenoid Contact Lesions.

DISCUSSION

OLP is defined as an immune system disease with an unknown cause. However, possible causes include psychological, genetic, and infectious factors. Major infectious factors include HPV, HCV, HSV-1, HHV-6, and EBV [2]. Oral Lichenoid Stomatitis (OLS) may also occur spontaneously or as a re-exacerbation of an existing OLP because of dental materials or medications [3]. Previous studies show that lichenoid reactions cannot be distinguished from idiopathic OLP histopathologically and clinically [3, 10, 11]. Virus-borne infections are generally detected subclinically in childhood period and cause infectious mononucleosis during young adulthood. Long-term EBV infections are associated with various tumors, such as nasopharynx carcinoma and Burkitt lymphoma [12].

Although EBV is known to be a factor in OLP, its status and relationship with OLCL are not known at present. In general, clinical and histopathological examinations as well as allergy test results against dental materials must be evaluated together in diagnosing the lesions. In the presence of OLP, the first step of treatment must be the removal of the relevant dental material. The diagnosis of OLCL is also of great importance in terms of malignant transformation of the lesions. Patients must be informed in detail about these lesions that occur in their mouths and must be monitored at regular intervals [8, 9].

Sand et al. [8] included 23 individuals with OLP and 67 clinically healthy individuals in their study and used Nested PCR, a molecular method, for EBV DNA detection. They found the prevalence of EBV to be 32.1% in individuals with oral diseases and 26.1% in OLP patients. They reported that the results obtained in their study were statistically significant. In the present study, no significant p-value could be detected to speculate that EBV plays direct role in the development of OLP. It is considered that the reason for this may be the number of clinical samples. There is no doubt that more significant results will be obtained by increasing the number of samples.

When Tamer [13] compared the presence of EBV in the OLP patient groups with the Control Group, they found positivity in 4 (7.3%) of 67 patients. They reported in their study that EBV positivity was statistically

significant in OLP patients when compared to healthy individuals (p=0.024). Recent studies report that there is a correlation between the increase in the malignancy of the disease and the increase in EBV prevalence and/or positivity [14–16]. No comparisons were made regarding the correlation between EBV and malignancy because dysplasia was not detected in OLCL and OLP patients in the present study.

When the data from various studies were evaluated, the analysis highlighted the importance of the methods used in the diagnosis of EBV and revealed the necessity of employing specific diagnostic approaches. In this regard, it was reported that EBV could not be detected in studies conducted with Southern Blot PCR and in situ PCR methods, but the virus could be detected with highly sensitive methods such as Nested-PCR and RT-PCR [8, 17]. In the present study, the presence of EBV DNA was investigated with the RT-PCR method.

In their study, Vieira et al. [9] investigated the presence of EBV DNA in different clinical samples of patients who had OLP and in individuals designated as the Control Group with the Nested PCR method. They reported that the samples examined in their study were tissue-containing lesions, saliva, swabs, and whole blood samples. It was reported that no statistically significant EBV positivity could be detected in tissue samples (p=0.899). In the Case and Control Groups, EBV positivity was determined at a rate of 62.5% and 35.3% in fresh tissue samples, 75% and 64.7% in saliva samples, 70.8% and 82.4% in exfoliated cell samples, and 33.3% and 47.1% in blood samples, respectively. No statistically significant differences were detected in terms of EBV DNA positivity between the Case and Control Groups. In the present study, no statistically significant differences were detected between the Case and Control Groups.

In their study, Yildirim et al. [18] investigated the relationship between OLP and infectious agents in 65 cases. HSV positivity was detected to be 9% in 6 cases and no significant correlation was detected between virus positivity and demographic or histopathological characteristics in these cases, except for basal cell degeneration. HPV positivity was found to be 21% in 14 cases, and EBV-positivity was found to be 35% in 23 cases. The results were found to be statistically significant for these two viruses. An increase was detected in the risk of EBV and HPV infection in OLP cases. Considering the oncogenic potential of these viruses, it is important to investigate the presence of viruses in these cases. It was emphasized in the study that regular and long-term follow-up of patients who have lesions with positive results is especially valuable for public health.

Today, blood samples are used widely for analysis in EBV-related studies. Distinct diagnostic results emerge, especially in EBV-related lymphoma or nasopharyngeal carcinoma. In a study conducted by Chan et al. [19], the plasma/serum samples of the patients were subjected to DNase digestion and ultracentrifugation. Circulating EBV DNA molecules were shown to be naked DNA fragments rather than contained in the virions. The results of the study contain fundamental information that may improve the understanding of the release of tumor-derived nucleic acids into the blood of cancer patients. EBV DNA positivity rates in blood samples were found to be between 69% and 96% in oropharyngeal carcinomas [20, 21]. However, this rate was found to be between 7% and 12.2% in patients who did not have any malignancies [22].

Conclusion

As a result of the present study, although no significant differences were detected between patients who had OLP and OLCL and the Control Group in terms of the presence of EBV DNA and quantitative viral load, it was determined that the quantitative EBV DNA results obtained varied depending on the selected clinical sample. In conclusion, we believe that comprehensive studies that will include a larger number of samples must be conducted to define the roles of EBV in OLP and OLCL diseases. We emphasize the importance of knowing the clinical characteristics of the disease because atrophic and erosive form OLP lesions show premalignant characteristics and making a differential diagnosis to follow up patients and apply the correct treatment.

Ethics Committee Approval: The Istanbul University Faculty of Dentistry Clinical Research Ethics Committee granted approval for this study (date: 27.07.2016, number: 2016/36).

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