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Detection of Virus Herpes Simplex Type 1 in Patients with Chronic Periodontal Disease

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Abstract

BACKGROUND: Periodontal disease is an inflammatory-destructive condition of the supporting tissues of the teeth. Microorganisms found in the dental plaque were considered to be the primary local etiologic factor responsible for the periodontal destruction. It is also evident that herpes simplex viruses may have an impact in the etiopathogenesis of periodontal disease.

AIM: This study has been made with the aim to analyse the prevalence of herpes simplex virus type 1 (HSV-1) in the dental plaque (supra- and subgingival) of patients with the chronic periodontal disease.

MATERIAL AND METHODS: The study comprised a total of 89 patients with chronic periodontal disease divided into two groups (patients with moderate and severe periodontitis). Supragingival dental plaque samples were taken with sterile cotton (supragingival), and subgingival dental plaque samples were taken with paper absorbents. Samples were subjected to extraction of DNA and further analysis with multiplex PCR for the presence of herpes viral DNA.

RESULTS: HSV-1 virus was detected In 24.7% of all patients included in the study. HSV-1 was detected in 22.2% of patients with the moderate stage of the disease, of which in all (100%) in the supragingival plaque samples and only 16.7% in subgingival plaque samples. In two patients HSV-1 was concomitantly detected in supra and subgingival plaque samples. In patients with advanced stage of the disease, the HSV-1 virus was detected in 28.6% patients. In two of the patients, HSV-1 was concomitantly detected in supra and subgingival plaque samples. Statistically, a significant difference was found in HSV-1 positive patients with a moderate stage of disease, between the presence of the virus in subgingival (100%) and subgingival (16.7%) dental plaque samples, $p < 0.05$.

CONCLUSION: Herpes simplex viruses type 1 are present in supragingival and subgingival dental plaque.

Introduction

Periodontitis is the most common form of the oral disease in adults; this disease is an inflammatory-destructive condition of the supporting tissues of the teeth, it is considered to be a result of many factors.

For many years microorganisms found in the dental plaque were considered to be the primary local etiologic factor responsible for the periodontal destruction, so a number of putative bacteria as *Porphyromonas gingivalis, Tannerella forsythia* and *Aggregatibacter actinomycetemcomitans* is considered to be associated with the periodontal disease and are used as diagnostic markers [1] [2]. Clinical features of this commonly encountered disease are a result of interaction between microorganisms and the host immune response. It is evident that host immune responses against infection with bacteria and the subsequent production of proinflammatory cytokines are of particular importance in periodontium destruction [3] [4]. Microorganisms initiate inflammatory reactions in the periodontium, which causes in long run loose teeth, destruction of

the connective tissue, and resorption of the alveolar bone.

Some studies have shown that human herpes viruses, especially herpes simplex virus (HSV-1 and 2), cytomegalovirus (HCMV) and Epstein-Barr virus (EBV), play an important role in the pathogenesis of the periodontal disease [5] [6] [7] [8]. Association of herpes viruses with specific pathogenic bacteria can also explain the etiopathogenesis of periodontal disease [5]. Indicating that the development of periodontal disease most probably depends on the interaction between herpes viruses, specific pathogenic bacteria and destructive inflammatory mediators [9] [10].

The present study has been made with the aim to analyse the prevalence of herpes simplex virus type 1 (HSV-1) in the dental plaque (supra- and subgingival) of patients with the chronic periodontal disease, to evidence the possible association between the presence of this virus and the stage of the chronic periodontal disease.

Material and Methods

This study was made at the University Clinic of Mouth and Periodontal Diseases and Genetic Laboratory at the University Clinic for Children's Diseases- Medical Faculty in Skopje. Each patient included in the study signed informed consent for participation in the study. Institutional Ethical committee approved the study.

The study comprised a total of 89 patients divided into two groups:

- Patients who have diagnosed a moderate stage of chronic periodontal disease (clinical attachment loss of 2-5 mm) and
- Patients who were diagnosed a serve stage of chronic periodontal disease (clinical attachment level ≥ 6 mm).

Patients who were included in the examined group had to meet certain inclusion criteria.

Inclusion criteria: patients who were no-smokers, patients who did not take antiviral drugs in the previous six months and patients without systemic diseases such as renal, cardiovascular, respiratory, malignant diseases, diabetes.

Exclusion criteria: patients who take antibiotics over the last three months, patients who are on long-term treatment with drugs that influence on the periodontium (non-steroidal anti-inflammatory drugs), as well as pregnant and breastfeeding women.

In this study first was detected clinical stage of periodontal disease in patients who were included in study by determination of: plaque index-PI (Silness-Löe) [11], gingival index of gingival inflammation-GI (Löe-Silness) [12], periodontal bleeding on probing-BOP (Mühlemann-Son) [13], clinical attachment loss-CAL and measurement of periodontal pocket depth-PPD.

Clinical and laboratory examinations consisted of collecting dental plaque samples supra- and subgingivally in both clinical disease stages.

- *Supragingival dental plaque samples* were taken with a sterile cotton swab by vigorous scrubbing the tooth surface.

- *Subgingival dental plaque samples* were taken with paper absorbents, Absorbent paper points, Vericom, Eazi-Endo, Chuncheon-Sl. Korea, which was applied to the bottom of the periodontal pocket. Usually, 5-6 paper absorbents were used for one patient.

Samples contaminated with blood were not used in the examination.

After collecting the plaque samples, they were put in sterile microbiological plastic tubes-ependorfs with suspended 1ml x 1 x PBS (phosphate buffered saline) buffer (pH = 7.4) (Figure 1) and were transported to the Genetic Laboratory at the University Children's Hospital for further analysis.

Dental plaque samples were immediately subjected to the protocol for DNA digestion and extraction in the Laboratory. The test tubes with the samples were vortexed and centrifuged, after that, the supernatant was decanted and 300-350 μl buffer for digestion was added to the precipitation (0.05 M Tris, 0.001 M EDTA, 1% Tween 20, 1% Nonidet 40, 0.3 mg/ml Proteinase K), after which it was incubated in a water bath overnight at 56^{o}C. Next step was DNA extraction and precipitation by a standard method with phenol-chloroform and ethanol. All extracted DNA samples were stored at -20^{o}C for further analysis.

PCR amplification of HSV-1 virus was performed with the PCR machine-Veriti Thermal Cycler (Applied Biosystems, California, USA) according to the following protocol: an initial denaturation, followed by 40 amplification cycles and a terminal extension. Last product was A total volume of 50 μl reaction mixture contained: 1 μl of extracted DNA, 10pmol of each primer-H1P32 (5'-TGGGACACATGCCTTCTTGG-3') and H1M32 (5'-ACCCTTAGTCAGACTCTGTTACTTACCC-3'), 5 μl of 10 x reaction buffer, 0.2 mM of each (deoxynucleotide triphosphate) d NTP and 2.5 U of cloned pfu DNA polymerase enzyme (G-Biosciences, USA), previously described by Das et al., in 2012 [14].

A 10 μl of the amplified PCR product was taken and analysed with electrophoresis on a 2.5% agarose gel containing 1 mg/ml ethidium bromide in 1 x TBE (Tris/Borate/EDTA) buffer and was visualised under UV transilluminator. Presence of a fragment of

147 bp confirmed the presence of HSV-1 virus in the analysed sample. Angiotensinogen served as a control gene for monitoring the success of PCR amplification of HSV-1 virus by using the pair of primers oligo25/oligo26, resulting in PCR product of 165 bp.

The data analysis is performed in a statistical program Statistica 7.1 for Windows The following methods were used: In the analysis of the series with attribute markers (presence of the HSV1, viruses in supragingival and subgingival dental plaque in patients with moderate and advanced stage of periodontal disease), percentage percentages of the structure (%) were determined;. The difference in the frequency of detected HSV-1 virus between patients with the moderate and advanced stage of periodontal disease, the difference between the presence of the virus in supragingival and subgingival dental plaque samples in positive patients with the moderate and advanced stage of periodontal disease are performed with statistic Difference test.

Results

A total number of 89 patients were examined for the presence of HSV-1 virus. In 60.7% of these patients, a moderate clinical stage of the disease was detected, while in 39.3% an advanced clinical stage was found. In 22 (24.7%) of all 89 patients with moderate and advanced periodontal disease HSV-1 virus was detected in supra- and/or subgingival dental plaque samples (Figure 1).

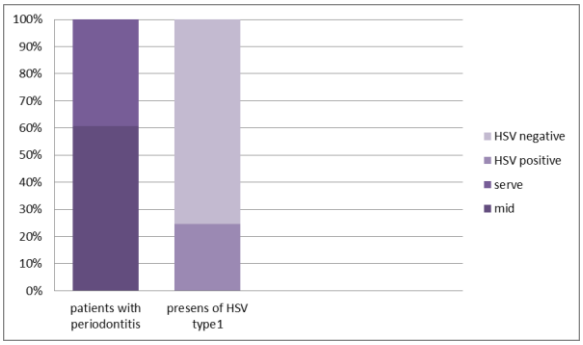

Figure 1: Presentation of patients according to the clinical stage of the disease and the detection of HSV-1 in patients with periodontal disease

Molecular analysis of HSV-1 showed the presence of the virus in 12/54 (22.2%) of patients with the moderate stage of the disease, of which in all 12/12 (100%) in the supragingival plaque samples and only 2/12 (16.7%) in subgingival plaque samples. In two patients HSV-1 was concomitantly detected in supra and subgingival plaque samples (Table 1, Figure 2).

In patients with advanced stage of the disease, the HSV-1 virus was detected in 10/35 (28.6%) patients, of which in 6/10 (60%) supra-gingival samples and 6/10 (60%) sub-gingival plaque samples. In two of the patients, HSV-1 was concomitantly detected in supra and subgingival plaque samples (Table 1, Figure 3)

Table 1: Distribution of HSV-1 in patients with the different clinical stage of the disease

Periodontal disease / HSV 1	Moderate disease stage		Advanced disease stage	
	Patients (n)	%	Patients (n)	%
Negative	42	77.8	25	71.4
Positive	12	22.2	10	28.6
Total	54	100	35	100

Statistical analysis (Difference test-which one) showed no significant difference in the frequency of detected HSV-1 virus between patients with moderate and advanced periodontal disease *(p = 0.4942)*.

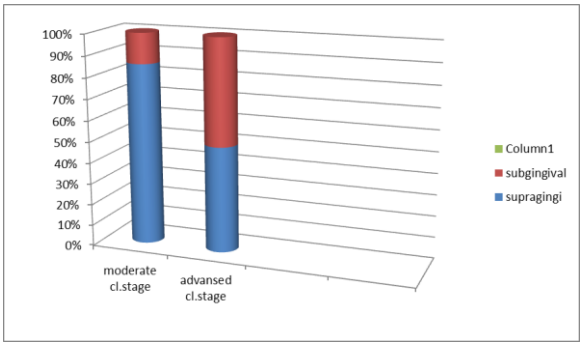

Figure 2: Distribution of HSV-1 virus in supragingival and subgingival plaque samples in virus-positive patients

On the contrary, the statistically significant difference was found in HSV-1 positive patients with a moderate disease stage, between the presence of the virus in supragingival (100%) and subgingival (16.7%) dental plaque samples, $p < 0.05$. No significant difference was observed between the presence of the virus in supragingival and subgingival plaque samples in HSV-1 positive patients with advanced disease stage, $p > 0.05$.

Discussion

Researches about the pathogenesis of periodontal diseases proved that some viruses could also have a role in the progression of periodontal disease. It is also demonstrated that a single site of active periodontal destruction as periodontal pockets may have more than a million copies of herpesvirus genomes [15]. Herpes viruses have been detected in supragingival and subgingival plaque samples, gingival biopsies, and gingival crevicular fluid (GCF) of healthy and periodontitis patients [16] [17] [18]. The gingival sulcus or periodontal pocket has also been

proposed to act as a reservoir between periods of recurrence of herpetic medical infections [19].

Herpes simplex viruses type 1, and 2 (HSV-1 and HSV-2), also known as human herpesviruses 1 and 2 (HHV-1 and HHV-2) are characterised by a short reproductive cycle and the ability to establish latency in the nerve ganglia so that might destroy the cells in the host [20]. Reactivation of herpes simplex virus can be without clinical symptoms, but about one-third of the individuals become prone to clinical recurrences [21]. HSV-1 is a common cause of oral herpes. HSV-1 is usually acquired by direct contact with an infected person, most often by contact with saliva during childhood, but it can also be transmitted vertically from a mother to a child before or during childbirth.

Viral HSV particle consists of a relatively large double-stranded DNA molecule encased within a protein cage called nucleocapsid, which is wrapped in a lipid bilayer cover. Besides lipids, the lipid cover in its structure contains glycoproteins, with a diverse role [22]. Most often these glycoproteins are membrane-bound receptors of cells that are going to be attacked.

Large part of these studies evidenced that HSV-1, EBV and CMV are detected with high prevalence of periodontal lesions, that they directly infect gingival epithelial cells and that viral loads positively correlate with disease severity [14] [23] [24] [25] [26] [27] [28] [29] [30].

It has been proved that the presence and frequency of herpesviruses in periodontal pockets increase with the increase of the periodontal pocket depth [31]. The prevalence of herpesviruses in periodontal pockets may vary according to the type of periodontal disease [7]. Herpes viruses can multiply in gingival tissue, especially in the epithelial cells and fibroblasts of clinically healthy gingiva that is easily subjected to HCV infection [32]. This implies that these cells might be a reservoir of the latent form of this virus. Herpes virus-infected cells can reduce the host defence and give rise to overgrowth of pathogenic bacteria and invade the cells more efficiently [5].

The results obtained from our study about presence of the HSV-1 virus in subgingival dental plaque samples, because of low prevalence of HSV-1 virus are not in agreement with the results of Grenier G et al., [17], Contreras A et al., [33], Sanja Matić Petrović et al., [34] that detected higher prevalence of virus Herpes simplex type 1 (HSV1) in subgingival dental plaque in patients with chronic periodontal disease. Our results are in agreement with the results of Nibaili et al., [35] which did not detect the presence of HSV-1 in the sub-gingival dental plaque.

There are findings that suggest that advanced stage of chronic periodontal disease was more commonly associated with the herpes viruses HSV1 and HSV2. Our findings are not in agreement with the observations that were made by Contreras et al., [31], in their study they detected Herpes simplex virus-1 in subgingival dental plaque in 52% of patients with advanced stage of chronic periodontal disease and herpes simplex virus-2 was detected in subgingival dental plaque in 56% of patients with severe chronic periodontitis this results are higher than that reported by Imbronito et al., [36], Greiner et al., [17],and Grande et al., [37].

In conclusion, in this study, we detected that virus Herpes simplex type 1 is present in supragingival and subgingival dental plaque. Our findings suggest that there is no significant difference in the frequency of detected HSV-1 virus between patients with the moderate and advanced stage of periodontal disease. So, we don't suggest with certainty that the presence of HSV-1 is related to the degree of periodontal tissue damage and manifestation of the different degree of periodontal destruction.

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