Journal of Oral Science, Vol. 51, No. 1, 79-86, 2009

Original

Herpesviruses in chronic and aggressive periodontitis patients in an Indian population

Shivaprasad Bilichodmath[1)], Sachin B. Mangalekar[2)], Dileep C. G. Sharma[3)], Ashok K. Prabhakar[4)], Sridhar B. Reddy[5)], Nagaraj B. Kalburgi[6)], Sudhir R. Patil[6)] and Kishore Bhat[7)]

[1)]Department of Periodontics, Rajarajeshwari Dental College and Hospital, Bangalore, India
[2)]Department of Periodontics, Chattisgarh Dental College and Research Institute, Rajnandgaon, Chattisgarh, India
[3)]Department of Periodontics, College of Dentistry, Al-Fateh University of Medical Sciences, Tripoli, Libya
[4)]Department of Periodontics, SJM Dental College and Hospital, Chitradurga, India
[5)]Department of Periodontics, HKDET Dental College and Hospital, Humnabad, India
[6)]Department of Periodontics, PMNM Dental College and Hospital, Bagalkot, India
[7)]Department of Microbiology, Maratha Mandal Dental College and Hospital, Belgaum, India

(Received 29 July and accepted 15 December 2008)

Abstract: Many recent studies have assessed the prevalence and role of herpesviruses in the etiopathogenesis of periodontal diseases, which has led to the realization of intricate interactions between viruses and bacteria within periodontal pockets. It has also been shown that the occurrence of herpesviruses may vary depending upon the age of the patient and the race of the population studied. Thus, the present study aimed at detecting herpes simplex virus type 1 and 2 (HSV 1 and 2), Epstein-Barr virus (EBV) and human cytomegalovirus (HCMV) in periodontal pockets of Indian patients with chronic and aggressive periodontitis. Subgingival plaque samples (n = 33) were collected from 19 randomly chosen chronic periodontitis and 14 aggressive periodontitis patients. Herpesviruses were detected using multiplex polymerase chain reaction technique. Chronic periodontitis patients revealed presence of HSV-1 in 19 (100%) samples, HSV-2 in 3 (15.7%), EBV in 15 (78.9%) and HCMV in 5 (26.31%) samples. Samples from aggressive periodontitis patients showed the presence of HSV-1 in 8 (57.14%), EBV in 4 (28.57%) and HCMV in 1 (7.14%), whereas HSV-2 was not detected in any specimen. In this population, herpesviruses were found more frequently in chronic periodontitis than in aggressive periodontitis patients and their prevalence may vary according to the age and race of the patient. (J Oral Sci 51, 79-86, 2009)

Keywords: aggressive periodontitis; chronic periodontitis; herpesviruses; polymerase chain reaction.

Correspondence to Dr. Shivaprasad Bilichodmath, Department of Periodontics, Rajarajeshwari Dental College and Hospital, Bangalore, India
Tel: +91-80-26793637
Fax: +91-80-28437468
E-mail: drshivaprasad2008@yahoo.co.in

Introduction

The etiopathogenesis of periodontal disease is a complex process, involving the multifarious interaction between microbial and host factors and a variety of disease-modulating environmental factors. Bacterial etiology alone has not been able to substantiate various aspects such as i) rapid periodontal tissue breakdown with minimal plaque (1), ii) phases of disease activity and quiescence (2), iii) site specificity in periodontal disease (3) and, iv) progression to advanced periodontal destruction which occurs in a fraction of a given population (4).

Classically, microbiological research on human

periodontal disease has focused on bacteria, and to a minor extent on yeasts and parasites. Many recent studies have shown the prevalence of herpes viruses including herpes simplex virus (HSV 1 and 2), Epstein-Barr virus (EBV) and human cytomegalovirus (HCMV) in the periodontal pockets and gingival tissue of periodontitis patients (5-9). Association of herpesviruses with bacteria was also assessed to improve our understanding of the etiopathogenesis of periodontal diseases (10-12). Furthermore, recent studies have quantified the herpesviruses in periodontal pockets to show their positive association with severity of periodontal disease (13). The prevalence and number of herpesviruses in periodontal pockets may vary according to the age (5, 14), ethnicity (14), type of periodontal disease (8), immune status (15) and genetic predisposition (16) of patients.

Thus, further studies in other races from different parts of the world are deemed essential to establish herpesviruses as a recognized etiologic (or co-etiologic) agent for periodontitis. Accordingly, this is the first study conducted in a South Indian population to assess the prevalence of herpesviruses in periodontal pockets of different types of periodontitis. The aim of the present study was to detect HSV-1 and 2, EBV and HCMV in aggressive periodontitis patients and compare their results with those of chronic periodontitis in a South Indian population using a sensitive multiplex polymerase chain reaction (PCR) technique.

Materials and Methods

The study population was selected from patients attending the outpatient section of the Department of Periodontics, PMNM Dental College and Hospital, Bagalkot, Karnataka, India. Subgingival plaque samples were collected from 19 randomly chosen patients with chronic periodontitis (12 men and 7 women, age range 21-57 years, mean age 43 ± 7.3 years) and 14 with aggressive periodontitis (11 men and 3 women, age range 21-29 years, mean age 25 ± 3.1 years).

Chronic and aggressive periodontitis were diagnosed based on the criteria of American Academy of Periodontology classification of periodontal diseases (1999). Chronic periodontitis patients had periodontal pockets greater than 5 mm and clinical attachment loss greater than 3 mm in more than 20 teeth with moderate to severe bone loss. Patients diagnosed as having aggressive periodontitis exhibited probing attachment loss in the excess of 5 mm in more than 14 teeth; at least 3 of which were not first molars or incisors with moderate to severe bone loss. All patients were systemically healthy and had not received periodontal treatment or antibiotics for at least 6 months prior to the clinical examination and sampling.

Subjects who satisfied the inclusion criteria of the study were selected and ethical approval was obtained from the institutional review board and Rajiv Gandhi University of Health Sciences, Karnataka, India. Furthermore, each patient received a detailed explanation regarding the study procedure, and written informed consent was obtained from those who agreed to participate voluntarily in the study.

The subjects for sampling were selected at random from individuals scheduled for a routine oral examination. Periodontal evaluation included the modified gingival index (17), plaque index (18), probing pocket depth, and probing attachment loss. A subgingival plaque sample was taken from the deepest pockets of the dentition.

Subgingival sample collection

Supragingival plaque was gently removed with sterile cotton pellets and sample sites were isolated with cotton rolls and air-dried prior to sampling. Subgingival specimens were collected using a sterile curette with a single stroke after gentle insertion into the bottom of the sampling site. From each periodontitis patient, a pooled specimen was obtained from the two deepest pockets of the dentition (5-10-mm probing depth). The specimens were suspended in 500 µl of TE buffer (10 mM Tris-hydrochloride, 1mM EDTA, pH 8) and homogenized by vigorous mixing on a vortex.

Nucleic acid extraction

Samples were stored at -20°C and centrifuged at 10,000 rpm for 5 min the following morning. The precipitate thus obtained was washed three times with TE buffer. After the third wash, the supernatant was discarded and the precipitate was treated with 500 µl lysis buffer 1 (Tris HCl and Triton x-100) for 5 min, centrifuged, and the supernatant was discarded once again. The precipitate was treated with 100 µl lysis buffer 2 (Tris HCl, Nonidet p-40, Tween 200) with 100 µg/ml freshly prepared proteinase K and kept at 60°C for 2 h followed by 95°C for 10 min and deep frozen at -70°C until amplification (19).

DNA amplification by multiplex PCR technique

The primers used for polymerase chain reaction are listed in Table 1. To optimize the multiplex PCR, a series of titrations of primer concentrations and deoxynucleotide triphosphate (dNTP) levels were performed. Primer concentrations of 10, 25, 50 and 100 pmol from each primer pair were titrated simultaneously with dNTP (0.1, 0.2 and 0.3 mM concentrations of each of the dNTPs).

Amplification was performed with the PCR system

(Corbett research palm cycler version 2.2, imported by J.H. BIO Innovations, Bangalore, India). Forty amplification cycles of 30 s at 94°C, 40 s at 60°C and 50 s at 72°C were carried out in a 50-µl final volume containing 5 µl of ×10 reaction buffer (Bangalore Genei, Bangalore, India), 0.2 mM concentrations of each dNTP, 10 pmol of each of the 12 primers, and 2.5 U of cloned *Pfu* DNA polymerase (Bangalore Genei). Five microliters of appropriate DNA sample was added to the reaction mixture. After the last cycle, the samples were incubated for 15 min at 78°C to complete the extension of primers (20). The molecular weight of the individual PCR product was 147 bp (HSV I), 227 bp (HSV2), 182 bp (EBV), and 256 bp (HCMV).

Ten µl of each amplified product was analyzed by agarose gel electrophoresis on 3.5% agarose (Sigma-Aldrich, Bangalore, India) containing 1µg of ethidium bromide/ml in 1X TBE buffer and was visualized in a UV transilluminator (Bioimaging systems, imported by JH BIO Innovations, Bangalore, India).

Results

Tables 2, 3 and 4 show the distribution of viruses in the study patients. Chronic periodontitis sites revealed HSV-1 in 19 (100%) samples, HSV-2 in 3 (15.7%) samples, EBV in 15 (78.9%) samples and HCMV in 5 (26.31%) samples out of 12 specimens. Aggressive periodontitis sites revealed HSV-1 in 8 (57.14%) samples, EBV in 4 (28.57%) samples and HCMV in 1 (7.14%) sample. HSV-2 was not detected in any specimen from the aggressive periodontitis patients.

Twenty-three males and 10 females were included in the study. All males from the chronic periodontitis group, and 5 males from the aggressive periodontitis were positive for viruses. Herpesvirus (any one or more) was detected in all the samples (n = 10) from females.

The distribution of viruses in 6 chronic periodontitis patients belonging to the 31-40 years age group was as follows: HSV-1 in 6, HSV-2 in 1, EBV in 5, and HCMV in 1 sample. HSV-1 in 9, HSV-2 in 1, EBV in 7 and HCMV in 4 samples were detected from 9 chronic

Table 1 Primers used in polymerase chain reaction to detect human viruses

Primers	Sequence (5'-3')	Virus
H1P32	TGGGACACATGCCTTCTTGG	HSV-1
H1M32	ACCCTTAGTCAGACTCTGTTACTTACCC	
EP5	AACATTGGCAGCAGGTAAGC	EBV
EM3	ACTTACCAAGTGTCC ATAGGAGC	
H2M40	GTACAGACCTTCGGAGG	HSV-2
H2P2	CGCTTCATCATGGGC	
CP15	GTACACGCACGCTGGTTACC	HCMV
CM3	GTAGAAAGCCTCGACATCGC	

Table 2 Frequency of virus detection in periodontal pockets of chronic and aggressive periodontitis patients

Viruses	Chronic periodontitis (n = 19)	Aggressive periodontitis (n = 14)
HSV-1	19 (100%)	8 (57.14%)
HSV-2	3 (15.7%)	Nil
EBV	15 (78.9%)	4 (28.57%)
HCMV	5 (26.31%)	1 (7.14%)

Table 3 Age and sex wise distribution of viruses in chronic periodontitis patients

Sl. No	Age and Sex of the patient	Viruses present
1	38/F	HSV-1, EBV, HCMV
2	45/M	HSV-1, HCMV
3	38/F	HSV-1
4	43/M	HSV-1
5	40/M	HSV-1, HSV-2, EBV
6	52/M	HSV-1, EBV
7	48/F	HSV-1, EBV
8	40/M	HSV-1, EBV
9	42/M	HSV-1, EBV, HCMV
10	42/F	HSV-1, EBV
11	46/F	HSV-1, EBV, HCMV
12	42/F	HSV-1, EBV, HCMV
13	26/M	HSV-1
14	32/F	HSV-1, HSV-2, EBV, HCMV
15	37/M	HSV-1, EBV,
16	57/M	HSV-1, EBV
17	42/M	HSV-1, EBV
18	50/M	HSV-1, HSV-2, EBV
19	52/M	HSV-1, HSV-2, EBV

periodontitis patients of the 41-50 years age group. Out of the 3 chronic periodontitis patients belonging to the 51-60 years age group, HSV-1 was present in 3, HSV-2 in 1, and EBV in 3 samples (Table 5).

In the 11-20 years age group, HSV-1 was only present in the sample from 1 aggressive periodontitis patient. One sample from the lone chronic periodontitis patient in the 21-30 years age group revealed HSV-1. Thirteen patients with aggressive periodontitis in the age group 21-30 years expressed HSV-1 in 7, EBV in 4 and HCMV in 1 sample. In the present study, there were no aggressive periodontitis patients over 31 years of age (Table 5).

A combination of herpesviruses was seen in 16 samples (out of 19) from chronic periodontitis patients. The combination of HSV-1 and 2 was seen in 3 (15.7%) specimens, HSV-1 and EBV in 15 (78.9%) samples, and HSV-1 and HCMV in 6 (31.5%) specimens (Table 6). The combined occurrence of EBV and HCMV was detected

Table 4 Age and sex wise distribution of viruses in aggressive periodontitis patients

Sl. No.	Age and Sex of the patient	Viruses present
1	20/M	HSV-1
2	25/M	NIL
3	28/F	HSV-1
4	22/M	NIL
5	26/M	NIL
6	21/M	HSV-1, HCMV
7	25/M	NIL
8	26/M	HSV-1, EBV
9	24/M	NIL
10	29/F	HSV-1
11	29/F	HSV-1, EBV
12	21/M	HSV-1, EBV
13	28/M	HSV-1, EBV
14	28/M	NIL

Table 5 Distribution of Herpes viruses in different age groups

Age Group (Years)	Chronic periodontitis					Aggressive periodontitis				
	No. of patients	HSV-1	HSV-2	EBV	HCMV	No. of Patients	HSV-1	HSV-2	EBV	HCMV
11-20	NIL	-	-	-	-	1	1	-	-	-
21-30	1	1	-	-	-	13	7	-	4	1
31-40	6	6	1	5	1	NIL	-	-	-	-
41-50	9	9	1	7	4	NIL	-	-	-	-
51-60	3	3	1	3	-	NIL	-	-	-	-

Table 6 Incidence of Herpes virus combination in periodontal pockets

Virus combinations	Chronic periodontitis	Aggressive periodontitis
HSV-1+ HSV-2	3 (15.7%)	NIL
HSV-1 + EBV	15 (78.9%)	4 (28.5%)
HSV-1+HCMV	6 (31.5%)	1 (7.1%)
EBV+ HCMV	4 (21%)	NIL
HSV-1+ EBV+ HCMV	4 (21%)	NIL
HSV-1+ HSV-2 + EBV	3 (15.7%)	NIL
HSV-1+ HSV-2 + EBV + HCMV	NIL	NIL

in 4 (21%) specimens, HSV-1, EBV and HCMV in 4 (21%), and HSV-1, HSV-2 and EBV in 3 (15.7%) samples from chronic periodontitis patients.

Combination of herpesviruses in periodontal pockets of aggressive periodontitis patients was revealed in 5 of 14 samples. HSV-1 and EBV in grouping was found in 4 (28.5%) patients and HSV-1 and HCMV in 1 (7.1%) patient only. The grouping of all the 4 viruses was not found in any subgingival plaque specimen from chronic and aggressive periodontitis patients.

Statistical analysis was done using Yates Chi-square test (Intercooled STATA statistical software Version 9.2, STATA corporation, Lakeway drive, College station, Texas, USA) to compare the status of Herpesviruses in chronic and aggressive periodontitis patients. The presence of HSV-1 ($P = 0.0069$), EBV ($P = 0.0112$) and HCMV ($P = 0.0112$) was statistically significant in chronic periodontitis patient but not in aggressive periodontitis patients (Tables 7 and 8).

Discussion

To the best of our knowledge, this is the first study conducted in a South Indian population regarding detection of herpesviruses in periodontal pockets of chronic periodontitis patients compared with that in aggressive periodontitis patients. Viral identification was done using multiplex-PCR technique with which microorganisms of different species may be detected simultaneously. Primer pairs specific to each intended organisms are engaged in a single-tube amplification process (21,22). PCR is a very sensitive technique that can detect microorganisms in smaller plaque samples than conventional and basic microbiological techniques.

The present study detected a significantly higher prevalence of herpesviruses in subgingival samples of chronic periodontitis patients (HSV-1 100%, HSV-2 15.7%, EBV 78.9%, and HCMV 26.31%) than in that of the aggressive periodontitis patients (HSV-1 57.14%, HSV-2 0%, EBV 28.57%, and HCMV 7.14%). The results were similar to the study conducted by Imbronito et al., in which EBV-1 was detected in 45% and HCMV in 82.5% of subgingival plaque samples (23).

The findings of the present study were in contrast to those of studies conducted by Parra and Slots (5) and Contreras and Slots (7). They demonstrated the presence of HCMV or EBV in 90% of localized juvenile periodontitis lesions (aggressive periodontitis) but only in 40-78% of adult periodontitis lesions (chronic periodontitis). Kubar et al. demonstrated HCMV DNA in 78% of subgingival samples from aggressive periodontitis lesions, but only in 46% of

Table 7 Comparison of status of HSV-1 and HSV-2 in chronic and aggressive periodontitis patients

Groups	HSV-1			HSV-2		
	Detected	Non-detected	Yates-Chi square test	Detected	Non-detected	Yates-Chi square test
AgP	8 (57.14)	6 (42.86)	7.2800	0 (0.00)	14 (100.00)	0.8963
CP	19 (100.00)	0 (0.00)	$P = 0.0069$ S	3 (15.79)	16 (84.21)	$P = 0.3438$ NS

S = statistically significant ($P < 0.05$); NS = statistically not significant AgP = Aggressive periodontitis; CP = Chronic periodontitis

Table 8 Comparison of status of EBV and HCMV in chronic and aggressive periodontitis patients

Groups	EBV			HCMV		
	Detected	Non-detected	Yates-Chi square test	Detected	Non-detected	Yates-Chi square test
AgP	4 (28.57)	10 (71.43)	6.4193	1 (7.14)	13 (92.86)	6.4193
CP	15 (78.95)	4 (21.05)	$P = 0.0112$ S	5 (26.32)	14 (73.68)	$P = 0.0112$ S

S = statistically significant ($P < 0.05$); AgP = Aggressive periodontitis; CP = Chronic periodontitis.

that of chronic periodontitis (8). EBV DNA was identified in 89% of subgingival and 78% of gingival tissue samples from aggressive periodontitis lesions, but only in 46% of both subgingival and gingival tissue samples from chronic periodontitis lesions (8). In another study, Kubar et al. identified HCMV in 68.8% of aggressive periodontitis lesions using real-time PCR (24). Also, Saygun et al. have detected HCMV, EBV-1 and HSV-1 in 72-78% of the subgingival samples from aggressive periodontitis patients (10).

In the present study, herpesviruses were found more frequently in chronic periodontitis than in aggressive periodontitis patients. This variation in the results, compared to previous studies, may be due to the age range of the patients, ethnic differences within the study population, the limited sample size, and difference in the severity of periodontal disease in the selected group. Also, the difference in the methodological approach used to detect herpesviruses plays a vital role in the disparity between the present study and the previous studies.

HSV-1 and 2, EBV and HCMV were found more frequently in patients over 30 years of age (chronic periodontitis) than those with age <30 years (aggressive periodontitis) in the present study. This is in agreement with one of the studies by Slots and Parra, which revealed increased occurrence of CMV with increasing age (>45 years) (1). In contrast to the above findings, a study conducted by Kubar et al (8) observed HCMV more frequently (78%) in samples from aggressive periodontitis lesions (ages 21-34 years). Only 46% of subgingival samples from chronic periodontitis indicated the presence of HCMV DNA.

The difference in the range of patients' age between the previous studies and the present study might be one of the reasons for the variation in the findings. Patient age may have a greater effect on the prevalence of herpesviruses than type of periodontitis. As age increases, the prevalence of herpesviruses in periodontal pockets may increase. If age of the patient is not considered as one of the criteria, the positive relationship between herpesviruses and destructive periodontal disease might be overestimated.

Ling et al. demonstrated that HSV was related to the severity of periodontal diseases in terms of clinical attachment loss (25). In the present study, HSV-1 was found most frequently than any other herpesviruses, in 100% of chronic periodontitis and 57.4% of aggressive periodontitis patients. These results emphasize the presence of HSV-1 in periodontal pockets but the tangible role of HSV-1 in initiation and progression of periodontal diseases has to be ascertained.

All female patients demonstrated the presence of herpesviruses (any). All males except 6 aggressive periodontitis patients revealed the presence of herpesviruses in the subgingival specimens. However, a relationship between sex and the prevalence of herpesviruses in periodontal pockets was not established.

It has been estimated that, worldwide, 90% of the population infected with EBV is asymptomatic. HCMV was found to be prevalent in 50-80% of adolescents and adults in developed countries, and 40-70% of the population acquires antibodies against herpes simplex virus from the first year of life to adolescence (5). These data suggest that herpesviruses are usually present in the body in inactive state. Reactivation of herpesviruses in periodontal sites comprises an important pathogenic event in the development of periodontitis (26).

Herpesviruses may cause direct cytopathic effects on keratinocytes, fibroblasts, endothelial cells and inflammatory cells (27). Contreras et al have detected HCMV in monocytes/macrophages and T lymphocytes, EBV-1 in B lymphocytes, and HSV in T lymphocytes and monocytes/macrophages (28). Infected immune cells may not mount a proper immune response against periodontopathogenic bacteria predisposing to microbial superinfection. Herpesvirus induced defective polymorphonuclear neutrophils can increase the risk for destructive periodontal disease. The study found herpesviruses infecting various types of inflammatory cells in periodontitis lesions (28). It was not known whether the infected cells carry functional or latent herpesviruses. Active herpesvirus infections would potentially be more detrimental to the periodontium than latent herpesvirus infections.

Herpesviruses existing in combination in periodontal pockets may act in synergy against host tissues. Co-existence of HSV-1 and EBV was found most frequently in both chronic periodontitis (78.9%) and aggressive periodontitis (28.5%) patients in the present study.

Genetic predisposition of the patient for disease susceptibility and severity should also be contemplated when considering viral etiopathogenesis. A patient may be genetically predisposed to severe periodontal breakdown, as shown in cases with genetic polymorphism of numerous interleukins (29-31). Therefore, studies that detect or quantify herpesviruses should also analyze the genetic polymorphism to better understand the role of herpesviruses in periodontitis.

In a study conducted by Ting et al, it was hypothesized that active HCMV infection could be associated with the initiation and progression of localized juvenile periodontitis (9). In another study conducted by Contreras and Slots, results suggested that active HCMV replication could

occur in periodontal sites (32), but it remained unclear if HCMV reactivation was related to the initiation or the progression of destructive periodontal disease.

Despite circumstantial evidence of a role of herpesviruses in destructive periodontal disease, a cause-and-effect relationship remains to be established. Questions remain as to whether active periodontal herpesvirus infection gives rise to destructive periodontal disease or whether destructive periodontal disease reactivates a latent viral infection. Thus, more studies on viral pathogenesis from different parts of the world are essential to establish herpesviruses as an etiologic or co-etiologic agent of periodontal diseases.

In conclusion, we found that herpesviruses (HSV-1 and 2, EBV, and HCMV) are associated more with chronic periodontitis than aggressive periodontitis, and this finding may be related to the age range of the patients, limited sample size, and methodology used to detect the viruses in this study. Also, prevalence of viruses in periodontal pockets may vary according to ethnicity, type of periodontal disease, immune status, and genetic predisposition of patients. The present understanding of the potential role of herpesviruses in etiopathogenesis of destructive periodontal disease is credible but not decisive. Additional studies in other populations are required to better understand the association of viruses in periodontal diseases.

References

1. Lang N, Bartold PM, Cullinan M, Jeffcoat M, Mombelli A, Murakami S, Page R, Papapanou P, Tonetti M, Van Dyke T (1999) Consensus report: aggressive periodontitis. Ann Periodontol 4, 53.
2. Goodson J, Tanner AC, Haffajee AD, Sornberger GC, Socransky SS (1982) Patterns of progression and regression of advanced destructive periodontal disease. J Clin Periodontol 9, 472-481.
3. Lindhe J, Ranney R, Ira Lamster, Charles A, Chung CP, Flemmig T, Kinane D, Listgarten M, Löe H, Schoor R, Seymour G, Somerman M (1999) Consensus report: chronic periodontitis. Ann Periodontol 4, 38.
4. Papapanou PN (1996) Periodontal diseases: epidemiology. Ann Periodontol 1, 1-36.
5. Parra B, Slots J (1996) Detection of human viruses in periodontal pockets using polymerase chain reaction. Oral Microbiol Immunol 11, 289-293.
6. Contreras A, Slots J (1996) Mammalian viruses in human periodontitis. Oral Microbiol Immunol 11, 381-386.
7. Contreras A, Nowzari H, Slots J (2000) Herpesviruses in periodontal pocket and gingival tissue specimens. Oral Microbiol Immunol 15, 15-18.
8. Kubar A, Saygun I, Ozdemir A, Yapar M, Slots J (2005) Real-time polymerase chain reaction quantification of human cytomegalovirus and Epstein-Barr virus in periodontal pockets and the adjacent gingiva of periodontitis lesions. J Periodont Res 40, 97-104.
9. Ting M, Contreras A, Slots J (2000) Herpesvirus in localized juvenile periodontitis. J Periodont Res 35, 17-25.
10. Saygun I, Kubar A, Ozdemir A, Yapar M, Slots J (2004) Herpesviral-bacterial interrelationships in aggressive periodontitis. J Periodont Res 39, 207-212.
11. Slots J, Kamma JJ, Sugar C (2003) The herpesvirus – *Porphyromonas gingivalis* – periodontitis axis. J Periodont Res 38, 318-323.
12. Slots J, Sugar C, Kamma JJ (2002) Cytomegalovirus periodontal presence is associated with subgingival Dialister pneumosintes and alveolar bone loss. Oral Microbiol Immunol 17, 369-374.
13. Saygun I, Kubar A, Sahin S, Sener K, Slots J (2008) Quantitative analysis of association between herpesviruses and bacterial pathogens in periodontitis. J Periodont Res 43, 352-359.
14. Fleming DT, McQuillan GM, Johnson RE, Nahmias AJ, Aral SO, Lee FK, St Louis ME (1997) Herpes simplex virus type 2 in the United States, 1976 to 1994. N Engl J Med 337, 1105-1111.
15. Cappuyns I, Gugerli P, Mombelli A (2005) Viruses in periodontal disease ? a review. Oral Dis 11, 219-229.
16. Kornman KS, Page RC, Tonetti MS (1997) The host response to the microbial challenge in periodontitis: assembling the players. Periodontol 2000 14, 33-53.
17. Lobene RR, Weatherford T, Ross NM, Lamm RA, Menaker L (1986) A modified gingival index for use in clinical trials. Clin Prev Dent 8, 3-6.
18. Silness J, Loe H (1964) Periodontal disease in pregnancy. Correlation between oral hygiene and oral condition. Acta Odontol Scand 22, 121-135.
19. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J (1990) Rapid and simple method for purification of nucleic acids. J Clin Microbiol 28, 495-503.
20. Markoulatos P, Georgopoulou A, Siafakas N, Plakokefalos E, Tzanakaki G, Kourea-Kremastinou J (2001) Laboratory diagnosis of common herpesvirus infections of the central nervous system

by a multiplex PCR assay. J Clin Microbiol 39, 4426-4432.
21. García L, Tercero JC, Legido B, Ramos JA, Alemany J, Sanz M (1998) Rapid detection of Actinobacillus actinomycetemcomitans, Prevotella intermedia and Porphyromona gingivalis by multiplex PCR. J Periodont Res 33, 59-64.
22. Wahlfors J, Meurman JH, Väisänen P, Alakuijala, A Korhonen, H Torkko, J Janne (1995) Simultaneous detection of Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis by a rapid PCR method. J Dent Res 74, 1796-1801.
23. Imbronito AV, Grande SR, deFretas NM, Okuda O, Lotufo RFM, Nunes FD (2008) Detection of Epstein-Barr virus and human cytomegalovirus in blood and oral samples: comparison of three sampling methods. J Oral Sci 50, 25-31.
24. Kubar A, Saygun I, Yapar M, Ozdemir A, Slots J (2004) Real-time PCR quantification of cytomegalovirus in aggressive periodontitis lesions using TaqMan Technology. J Periodont Res 39, 81-86.
25. Ling LJ, Ho CC, Wu CY, Chen YT, Hung SL (2004) Association between human herpesviruses and the severity of periodontitis. J Periodontol 75, 1479-1485.
26. Slots J, Contreras A (2000) Herpesviruses: a unifying causative factor in periodontitis? Oral Microbiol Immunol 15, 277-280.
27. Tyler KL, Fields BN (1996) Pathogenesis of viral infections. In Fields virology 1996, 3rd ed, Fields BN, Knipe DM, Howley PM eds, Lippincott-Raven, Philadelphia, 173-218.
28. Contreras A, Zadeh HH, Nowzari H, Slots J (1999) Herpesvirus infection of inflammatory cells in human periodontitis. Oral Microbiol Immunol 14, 206-212.
29. Diehl SR, Wang YF, Brooks CN, Burmeister JA, Califano JV, Wang S, Schenkein HA (1999) Linkage disequilibrium of interleukin-1 genetic polymorphisms with early-onset periodontitis. J Periodontol 70, 418-430.
30. Atilla G, Emingil G, Köse T, Berdeli A (2006) TGF-β1 gene polymorphisms in periodontal diseases. Clin Biochem 39, 929-934.
31. Chen D, Wang Q, Ma ZW, Chen FM, Chen Y, Xie GY, Wang QT, Wu ZF (2007) MMP-2, MMP-9 and TIMP-2 gene polymorphisms in Chinese patients with generalized aggressive periodontitis. J Clin Periodontol 34, 384-389.
32. Contreras A, Slots J (1998) Active cytomegalovirus infection in human periodontitis. Oral Microbiol Immunol 13, 225-230.