# PREVALENCE OF CYTOMEGALOVIRUS, HUMAN HERPESVIRUS-6, AND EPSTEIN-BARR VIRUS IN PERIODONTITIS PATIENTS AND HEALTHY SUBJECTS IN THE THAI POPULATION

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**Abstract.** Fifty periodontitis patients and 30 healthy patients with oral cavities were selected from the Faculty of Dentistry, Mahidol University, Bangkok, Thailand, from March 2001 to November 2002. Their ages varied between 15 and 70 years. Among the periodontitis patients, specimens were collected from both disease and healthy sites. All samples were evaluated for the presence of CMV, HHV-6, and EBV-1 by nested PCR. Among the periodontitis patients, CMV was found in 34%, of which 8% were at the disease sites, 10% were at the healthy sites, and 16% were from both sites. EBV was not found in this group of the patients, while HHV-6 was found in 4%, at the disease sites only. CMV was found in one (3.3%) healthy control while HHV-6 and EBV-1 were not found. The depth of sample sites, various demographic and baseline characteristics *eg* sex, age, occupation and root planning were not associated with the presence of these viruses.

## INTRODUCTION

Periodontal disease is a chronic inflammation of the gums and connective tissue. The causes of infection may included bacterial plaque and herpesvirus (Contreras *et al*, 2000). Viral infections may facilitate the destruction of periodontal tissue by lytic activity against periodontal cells, immune mediated tissue destruction and immune suppression, which increase the susceptibility of the host to bacterial attacks (Banks and Rouse, 1992; Taga *et al*, 1995). Cytomegalovirus (CMV) and Epstein-Barr virus type 1 (EBV-1) assume a particularly close relationship with human periodontitis while herpes simplex virus (HSV), human herpesvirus 6 (HHV-6) and EBV-2 seem to exhibit little or no association with most types of periodontitis disease (Puthavathana *et al*, 1991; Contreras *et al*, 1999; Thawaranantha *et al*, 1999). The association of these viruses with periodontal disease has not been studied in Thailand. The objective of this study was to determine the prevalence of CMV, HHV-6, and EBV-1 and their association with periodontitis among the Thai population.

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## MATERIALS AND METHODS

Subjects were 50 periodontitis patients and 30 healthy persons selected from those who visited the Faculty of Dentistry, Mahidol University, Bangkok, Thailand during March 2001 to November 2002. Their ages varied from 15 to 70 years. The periodontitis patients had no clinical evidence of previous periodontal destruction with at least a 5 mm probing depth at 3 or more sites while the healthy controls had no clinical evidence of periodontal disease and a normal probing depth less

than 3 mm.

**Specimen collection**

In each periodontitis patient, two pooled subgingival samples were taken using the paper point technique from the 3 selected deepest probing sites and 3 selected normal sites. For the healthy controls, only one pooled subgingival sample from 3 selected sites was obtained. Prior to subgingival sampling, supragingival plaque was removed with sterile cotton pellets and the sample sites were isolated with cotton roles. A sterile paper point was inserted to the bottom of the selected pocket or sulcus and retained for 10 seconds. Paper points from deep periodontal pockets and normal sites were placed into separate sterile plastic microtubes containing PBS for the detection of CMV, EBV-1, HSV, and HHV-6 by nested PCR.

**Nested PCR**

A nested PCR method was used to detect viral DNA from CMV, HHV-6, and EBV-1. DNA was extracted from subgingival samples by digesting with proteinase K solution and Dnase-free Rnase. Amplification of DNA was performed with a total volume of 50 μl. The reaction mixture consisted of 10x reaction buffer, $MgCl_2$ at various concentrations, 200 μl of deoxynucleotide triphosphase (dNTP), 25 units of Taq (Thermus aquaticus) DNA polymerase, and various concentrations of inner and outer primer. The optimal conditions for nested PCR of each virus are illustrated in Table 1. The products were analyzed by using 3% agarose gel (with ethidium bromide) and electrophoresis for 30 minutes. The gel was photographed under UV illumination.

Statistical analysis was performed using the chi-square or the Fisher's Exact test for comparing the detection rate of herpesvirus infection in the periodontitis patients and the healthy control group, and their association with various demographic, baseline characteristics and the average depth of sample at the study sites. The median age of the two studied groups was compared by the Mann-Whitney *U* test.

## RESULTS

The demographic profiles of the periodontitis patients and the healthy control group are illustrated in Table 2. There were statistically significant differences for age ($p<0.001$) and occupation ($p=0.008$), while there was no statistically significant difference for sex ($p=0.559$) between the periodontitis patients and the healthy controls.

The clinical characteristics of the periodontitis patients and healthy controls are demon-

Table 1
Optimal conditions for PCR and primers used in nested PCR for detection CMV, HHV-6 and EBV-1.

| | CMV | | | HHV-6 | | | EBV-1 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Temp | Time | Cycle | Temp | Time | Cycle | Temp | Time | Cycle |
| Denaturation | 94ºC | 1 minute | | 94ºC | 2 minutes | | 94ºC | 1 minute | |
| Annealing | 65ºC | 2 minutes | 35 | 62ºC | 2 minutes | 25 | 56ºC | 30 second | 40 |
| Extension | 72ºC | 1 minute | | 72ºC | 1 minute | | 72ºC | 5 minutes | |
| First primer | 5′- GTCCTCTGCCAAGA GAAAGATGGAC-3′ | | | 5′- TTCTCCAGATGTGCCA GGGAAATCC-3′ 5′- CATCATCATTGTTATCG CTTTCACTCTC-3′ | | | 5′- AGACCATGAAATAACA GACAATGGAC -3′ | | |
| Second primer | 5′- TCCTCCTGTATGTGA CCCATGTGCTT-3′ | | | 5′- AGTGACAGATCTGGGC GGCCCTAATAACTT-3′ 5′- AGGTGCTGAGTGATCA GTTTCATAACCAA-3′ | | | 5′- CGGTGTGTTCGTATAT GGAGGTAGTA-3′ | | |

Table 2
Demographic characteristics of the study subjects.

| Characteristics | Periodontitis group (n=50) No. (%) | Healthy control group (n=30) No. (%) | p-value |
|---|---|---|---|
| Age (years) | | | |
| ≤ 19 | 0 (0) | 2 (6.7) | |
| 20-19 | 3 (6.0) | 15 (50.0) | |
| 30-39 | 6 (12.0) | 6 (20.0) | |
| 40-49 | 13 (26.0) | 5 (16.7) | |
| 50-59 | 21 (42.0) | 2 (6.7) | |
| 60-69 | 5 (10.0) | 0 (0) | |
| ≥ 70 | 2 (4.0) | 0 (0) | |
| Median (range) | 50.5 (20-72) | 27.5 (19-54) | <0.001 |
| Sex | | | |
| Male | 23 (46.0) | 11 (36.7) | 0.559 |
| Female | 27 (54.0) | 19 (63.3) | |
| Occopation | | | |
| Officer | 16 (32.0) | 18 (60.0) | [a]0.0008 |
| Merchant | 9 (18.0) | 0 (0) | |
| Laborer | 11 (22.0) | 1 (3.3) | |
| Student | 14 (28.0) | 11 (36.7) | |

[a]Combined officer and student *vs* combined merchant and laborer.

strated in Table 3. There was no statistically significant difference regarding subject smoking habits or underlying disease between periodontitis patients and healthy controls (p=0.338, p=0.288, and p=0.233, respectively).

In Table 4, only one healthy control had CMV infection while in periodontitis patients, CMV was detected 17/50 (34%) of which 4 (8%) were from disease sites, 5 (10%) were from healthy sites and 8 (16%) were from both sites. HHV-6 was found at 2 (4%) disease sites and at 1 (2%) normal site while no EBV-1 infection was found in these groups of patients. The relation between the average depth of the sample sites and the results of positive nested PCR among periodontitis patients is demonstrated in Table 5. Among the periodontitis patients, most of the CMV infections were found at an average depth of 2.0-2.9 mm at healthy sites and 5.0-5.9 mm at disease sites, while HHV-6 was found at an average depth of 3.0-3.9 mm. In healthy controls, CMV was detected at an average depth of 2.0-2.9 mm similar to the healthy sites in the periodontitis patients.

Various demographic and baseline characteristics in relation to positive nested PCR among periodontitis patients are illustrated in Table 6. CMV infection can be found in all age groups among periodontitis patients while HHV-6 infection was found between the ages of 40 and 59 years. Females had higher rate of CMV infection than males in this study, and CMV infection was found more in laborers than in other occupations. There was no association found between sex and occupation and the detection rate of HHV-6 infection.

## DISCUSSION

It is now accepted that herpesviruses besides bacteria can be the causative agents of periodontitis lesions. Periodontal herpesvirus may reduce metabolic abnormalities and suppress the responses of monocytes/macrophages, T and B cells, and other immune cells, rendering these cells less effective in combating infection by periodontopathic bacteria (Contreras *et al*, 1999).

CMV was found in 34% of periodontitis pa-

Table 3
Clinical characteristics and baseline data of the study subjects.

| Characteristics | Periodontitis group | | Healthy control group | | p-value |
|---|---|---|---|---|---|
| | No. | No. (%) | No. | No. (%) | |
| Subject status | | | | | |
| Healthy | 39 | 29 (74.4) | 30 | 26 (86.7) | 0.338 |
| Smoking | 50 | 3 (6.0) | 30 | 0 (0) | 0.288 |
| Underlying disease | 37 | 12 (32.4) | 30 | 5 (16.7) | 0.233 |
| Mild gingivitis | | 0 (0) | | 2 (6.7) | |
| Hyperlipidemia | | 1 (2.7) | | 0 (0) | |
| Hypertension | | 2 (5.4) | | 0 (0) | |
| Diabetes | | 0 (0) | | 1 (3.3) | |
| Gout | | 1 (2.7) | | 0 (0) | |
| Migraine | | 1 (2.7) | | 0 (0) | |
| Allergy | | 1 (2.7) | | 0 (0) | |
| Epilepsy | | 1 (2.7) | | 0 (0) | |
| Hypothyroid | | 0 (0) | | 1 (3.3) | |
| Hepatitis B | | 0 (0) | | 1 (3.3) | |
| Hypertension | | 1 (2.7) | | 0 (0) | |
| Hypertension, diabetes | | 2 (5.4) | | 0 (0) | |
| Hypertension, gout | | 1 (2.7) | | 0 (0) | |
| Hypertension, diabetes | | 1 (2.7) | | 0 (0) | |

Table 4
Occurrence of CMV, EBV-1, and HHV-6 infection in periodontitis patients and healthy group.

| Group | Total number | Sites | CMV (%) | HHV-6 (%) | EBV-1 (%) |
|---|---|---|---|---|---|
| Healthy | 30 | Healthy | 1 (3.3) | 0 (0) | 0 (0) |
| Periodontitis | 50 | Disease | 4 (8.0) | 2 (4.0) | 0 (0) |
| | | Control | 5 (10.0) | 1 (2.0) | 0 (0) |
| | | Both sites | 8 (16.0) | 0 (0) | 0 (0) |

Table 5
Relations between average depth at study sites and the results of positive nested PCR among 50 periodontitis patients.

| Study group | Sites | Average depth at sample sites (mm) | Number | Nested PCR | | |
|---|---|---|---|---|---|---|
| | | | | CMV (%) | HHV-6 (%) | EBV-1 (%) |
| Periodontitis | Healthy | 1.0-1.9 | 9 | 3 (33.0) | 0 | 0 |
| | | 2.0-2.9 | 38 | 10 (26.3) | 0 | 0 |
| | | 3.0-3.9 | 3 | 1 (33.0) | 3 (100) | 0 |
| | Disease | 5.0-5.9 | 24 | 7 (29.2) | 0 | 0 |
| | | 6.0-6.9 | 16 | 3 (18.7) | 0 | 0 |
| | | 7.0-7.9 | 6 | 1 (16.7) | 0 | 0 |
| | | 8.0-8.9 | 1 | 0 (0) | 0 | 0 |
| Healthy controls | | 1.0-1.9 | 1 | 0 (0) | 0 | 0 |
| | | 2.0-2.9 | 29 | 1 (3.3) | 0 | 0 |

Table 6
Various demographic and baseline characteristics in relation to positive nested PCR results among periodontitis patients.

| Various | Periodontitis patients | | |
|---|---|---|---|
| | CMV | HHV-6 | EBV-1 |
| Age (years) | | | |
| 20-29 | 2 | 0 | 0 |
| 30-39 | 2 | 0 | 0 |
| 40-49 | 5 | 1 | 0 |
| 50-59 | 5 | 2 | 0 |
| 60-69 | 2 | 0 | 0 |
| ≥ 70 | 1 | 0 | 0 |
| Sex | | | |
| Males (23) | 6 | 2 | 0 |
| Female (27) | 11 | 1 | 0 |
| Occupation | | | |
| Officer (16) | 4 | 1 | 0 |
| Merchant (9) | 3 | 1 | 0 |
| Laborer (11) | 7 | 1 | 0 |
| Student (14) | 3 | 0 | 0 |
| Root planning | | | |
| Treated | 16 | 1 | 0 |
| Untreated | 1 | 0 | 0 |

tients in this study, which was lower than that reported by Contreras and Slots in 1996 (60%) and Contreras *et al* in 1999 (55%). Such differences may be that the samples obtained in this study did not specify the type of periodontitis, while those obtained in the previous study were in the advanced stage and the types of samples which were obtained in this study were of gingival cervicular fluid, while the periodontitis lesions obtained in the previous study were of gingival tissue. In addition, CMV can infect gingival tissue better than cervicular fluid. Similar reasons may also apply to the lower percentage of CMV infection in both the disease and healthy sites in this study compared to the previous study by Contreras and Slots (2000) who found the infection in 64% at diseased sites and in 86% at healthy sites from patients undergoing periodontal surgery in the juvenile periodontitis group. Contreras and co-workers (2001) also reported that CMV could be detected in 71% of HIV-seropositive periodontitis patients and in 7% of non-HIV periodontal patients at subgingival soft tissue curettage and periodontal flab surgery. It is probable that the high percentage among the HIV-seropositive group may due to the depression of CD4, which can reactivate CMV infection.

HHV-6 was detected in 6% of periodontitis patients and in none of the healthy controls. These results are different from a previous study reported in 1997 (Contreras *et al*, 1997) in which HHV-6 was detected in 10% of malnourished Nigerian children aged 3-14 years. Cassai *et al* (2003) reported HHV-6 in 8% of adult patients affected by chronic periodontitis. The reasons for this may due to (1) a genetic factor which appears to influence susceptibility to periodontal diseases (Gilbert and Sofaer, 1989), (2) oral hygiene, (3) host factors, since good cellular and humoral immune responses can be antimicrobial, and (4) the age which can effect the type of oral microbials (Gilbert and Sofaer, 1989).

EBV-1 was negative in both periodontitis patients and healthy controls. These results are in contrast with a study reported in 1999 (Contreras *et al*, 1999) where they detected a high percentage of EBV-1 in periodontitis lesions. Such differences may due to (1) the different stages of periodontitis while collecting the specimens, (2) the specimens in our study were collected from three sample sites while Contreras and co-workers (2001) collected the specimens from six sites per tooth which would have a better chance to collect more viruses. The third reason is the association between periodontitis and EBV-1, since the presence of herpesviruses in older periodontitis patients may due to virus reactivation where seropositive EBV-1 patients usually secrete low levels of virus. In this study, the median ages of the periodontitis patients and healthy controls were 50.5 and 27.5 years old, respectively. The last reason may due to initial periodontal treatment, which can suppress or reduce the number of the microorganisms.

Various demographic and baseline characteristics in this study *eg* sex, age, and occupation were not associated with herpesvirus infection among periodontitis patients and the healthy control group. From these results, it is probable that the detection rate for herpesvirus may depend on genetics (Michalowicz *et al*, 1991), socioeconomics status and geographic location (Griffiths

and Emery, 1997). The depth of the sample sites were also not associated with the presence of the herpesvirus. Most of the CMV infections in periodontitis patients and the healthy control group were found at an average depth of 5.0-5.9 mm and 2.0-2.9 mm, respectively, while HHV-6 infection was found at an average depth of 3.0-3.9 mm.

## ACKNOWLEDGEMENTS

The authors would like to thank all the patients for their cooperation in collecting specimens. Thanks also go to the Faculty of Tropical Medicine for part of the financial support.

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