

Research Article

# HCMV Antibody Titer in Gingival Crevicular Fluid in Chronic Periodontitis

Vahid Esfahanian<sup>1</sup> • Shirin Zahra Farhad<sup>1</sup> • Mehrnaz Sadighi Shamami<sup>2</sup> • Manoochehr Mesripour<sup>3</sup> •  
Mohamadreza Ghafari<sup>3\*</sup> • Morvarid Mafi<sup>4</sup> • Farnaz Kouchakian<sup>5</sup>

<sup>1</sup> Assistant professor, Department of Periodontology, Dental faculty, Isfahan (Khorasgan) branch - Islamic Azad University, Isfahan, Iran

<sup>2</sup> Assistant professor, Department of Periodontics, Faculty of Dentistry, Tabriz University of Medical Sciences, Tabriz, Iran

<sup>3</sup> Professor of Biochemistry, Department of Biochemistry, Khorasgan Islamic Azad University (Isfahan), Iran

<sup>4</sup> Post-Graduate Student of Periodontics, Department of Periodontics, Khorasgan Islamic Azad Dental School (Isfahan), Iran

<sup>5</sup> Post-Graduate Student of Periodontics, Department of Periodontics, Young Researchers Club, Khorasgan Branch, Islamic Azad Dental School (Isfahan), Iran

<sup>4</sup> Private Practice

\*Corresponding Author; E-mail: Shahabgh1982@gmail.com

Received: 17 May 2012; Accepted: 12 April 2013

J Periodontol Implant Dent 2013;5(2):61–65 | doi: 10.5681/jpid.2013.010

This article is available from: <http://dentistry.tbzmed.ac.ir/jpid>

© 2013 The Authors; Tabriz University of Medical Sciences

This is an Open Access article distributed under the terms of the Creative Commons Attribution License

(<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

---

## Abstract

**Background and aims.** Despite available evidence for the role of herpes viruses in destructive periodontal diseases, no cause-and-effect relationship has been found to date. This study compared the titer of immunoglobulin G (IgG), immunoglobulin M (IgM) against human cytomegalovirus (HCMV) in gingival crevicular fluid between individuals with periodontitis and with healthy periodontium.

**Materials and methods.** Thirty samples of gingival crevicular fluid (ten from infected sites and ten from non-infected sites from ten individuals with periodontitis and ten from ten individuals with healthy periodontium) were provided. The titer of IgG and IgM were evaluated. Data was analyzed by one-way ANOVA, Kruskal-Wallis test, t-test, Duncen's test and Pearson's correlation coefficient.

**Results.** There were no statistically significant differences in the means of IgM titers of the three groups under study ( $P>0.05$ ), but there were significant differences in IgG titers between the three groups ( $P<0.001$ ).

**Conclusion.** Within the limits of the present study, it can be concluded that IgG titers against the human cytomegalovirus can increase in patients with periodontal diseases but the IgM titers do not show a significant increase in these individuals.

**Key words:** Chronic periodontitis, cytomegalovirus, gingival crevicular fluid, immunoglobulin G, immunoglobulin M.

---

## Introduction

Periodontal diseases are the inflammation of supporting structures of the teeth, caused by a complex community of bacterial species that interact with host tissues and cells.<sup>1</sup> Although the key factor in periodontitis is specific infectious agents, it is unlikely that only a single agent or even a small group of pathogens is the only cause or modulator of this heterogeneous disease. Herpes viruses have emerged as pathogens in different types of periodontal diseases since mid-1990s<sup>2,3,4</sup> and recent studies have shown a relationship between herpes viruses, especially Epstein Barr virus-1 (EBV-1) and human cytomegalovirus (HCMV), and periodontitis, especially the severe types.<sup>5,6</sup>

Polymerase chain reaction (PCR) has detected human cytomegalovirus in saliva and periodontal pockets, especially in periodontitis lesions, than in periodontally healthy sites.<sup>8,9</sup> Chalabi et al<sup>5,9</sup> showed a relation between human cytomegalovirus and periodontitis; it has been proven that sites affected by herpes virus-associated periodontitis, harbor elevated levels of periodontopathic bacteria, including *Tannerella forsythia*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Treponema denticola*, *Campylobacter rectus* and *Aggregatibacter actinomycetemcomitans*.<sup>6,10-12</sup> This theory supports the idea that infection with viruses may help bacteria in the development of periodontal diseases by compromising the immune system.<sup>6</sup>

Human cytomegalovirus infects monocytes, macrophages and T lymphocytes of periodontium and Epstein Barr infects B lymphocytes.<sup>13</sup> Saygunet al<sup>14,15</sup> showed a relationship between the presence of human cytomegalovirus in gingival crevicular fluid (GCF) and changes caused by periodontitis. They declared that there is a relationship between the presence of human cytomegalovirus and EBV-1 in the subgingival plaque and gingival attachment loss; therefore, periodontal treatment decreases the amount of human cytomegalovirus and EBV-1 in saliva.<sup>14,15</sup> They also reported that in periodontally compromised patients a correlation between gingival tissues and salivary counts of human cytomegalovirus DNA and Epstein barr DNA can be detected, which suggests that the periodontal pocket may be a reservoir for salivary herpes viruses.

The number and prevalence of herpes viruses in periodontal pockets may vary according to the age, ethnicity, type of periodontal disease, genetic predisposition and immune status of patients.<sup>16-20</sup>

This research was performed to compare the titra-

tion of antibodies against human cytomegalovirus between patients with periodontitis and patients with healthy periodontium to investigate the histimmunologic responses against this virus.

## Materials and Methods

In this observational, uni-center, diagnostic, single-arm study, ten patients with healthy periodontium (control group) and ten patients with chronic periodontitis (test group) were studied. All the patients had at least twenty teeth, no systemic diseases, no history of periodontal therapy in the last year and no history of anti-viral therapy in the last six months. Individuals with chronic periodontitis had at least three sites with 5 mm of probing pocket depth and 3 mm of clinical attachment loss.

The diagnosis of chronic periodontitis was settled by clinical attachment loss (CAL) and probing pocket depth (PPD) measurements in six areas of each tooth. The patients were asked to rinse their mouth for two minutes with water and samples were retrieved from three sites (two sites with periodontitis and one healthy site) by means of a #15 paper point after proper isolation. Each paper point was placed in the sampling site for four minutes and then was placed in test tubes containing diluents of commercially available diagnostic kit for human cytomegalovirus. In each patient, sampling was performed at two sites with periodontitis and the mean of the gained titer served as the titer of immunoglobulin for the diseased sites. Samples of the people that were healthy and had proper conditions for the study were acquired by the same method only from one site and these samples served as the control group.

Three groups were analyzed in this study:

- Group A: diseased sites in individuals with periodontitis
- Group B: healthy sites in individuals with periodontitis
- Group C: healthy sites in individuals with healthy periodontium

The tubes sent to laboratory were prepared for evaluation by means of a Human Cytomegalovirus ELISA kit (Creative Diagnostics, New York, USA). All the preparation steps were followed according to the provider's instructions. The titration of immunoglobulin M (IgM) and immunoglobulin G (IgG) was finally evaluated with a Microplate Reader STAT FAX 2100 (Awareness Technology, Inc. New York, USA) at a wavelength of 450 nm.

One-way ANOVA, Kruskal-Wallis test, *t*-test, Duncen's test and Pearson's correlation coefficient were used to analyze data.

**Results**

Table 1 shows the means of probing pocket depths and clinical attachment loss in patients with periodontal diseases. The mean of the IgG and IgM titers are shown in Figure 1.

One-way ANOVA showed no statistically significant differences in the means of IgM titers of the three study groups ( $P>0.05$ ), but there was a significant difference in IgG titers between the three groups ( $P<0.001$ ). The mean of IgM titers in none-infected areas was lower than the infected areas in individuals with periodontal diseases, but Kruskal-Wallis analysis showed no significant differences ( $P=0.7$ ).

Although the mean of IgM titers was higher in the test group compared to the control group, but t-test analysis showed no significant differences ( $P=0.14$ ). This statistical test also showed that the mean of IgG was significantly higher in individuals with periodontitis (groups A and B) than healthy individuals (group C) ( $P<0.001$ ).

Duncan's test showed a significant difference in the mean of Ig G between the three groups, but the difference between the infected areas and the non-infected sites in individuals with periodontitis was not significant and Kruskal-Wallis analysis confirmed this result as well ( $P<0.001$ ).

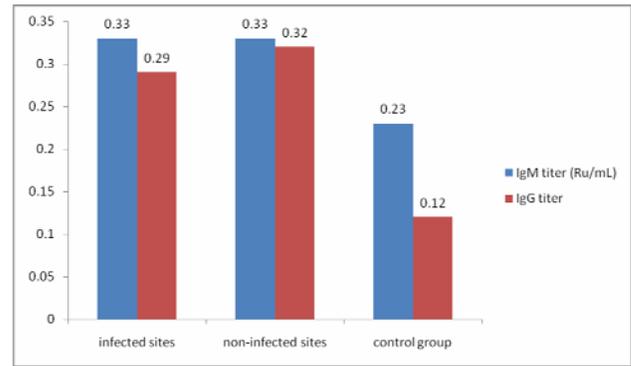
Pearson's correlation coefficient revealed a non-significant inverse relationship between the sum of IgM titers in infected and non-infected sites and full-mouth probing pocket depths ( $P=0.128$ ). The same was observed for the sum of IgG titers in infected and non-infected sites and full-mouth probing pocket depths ( $P=0.113$ ). The other results of this analysis are shown in Table 2. It must be pointed out that all the relations found were non-significant.

**Discussion**

Available knowledge about the conversion of gingivitis sites into periodontitis sites and the reason of laterally symmetrical pattern of tissue breakdown progress in localized periodontitis is very limited.<sup>21,22</sup> The etiology of periodontal disease is a complex process that involves multifactorial interactions between microbial and host factors and a variety of different modulating environmental factors.<sup>23</sup> There

**Table 2. The relation between IgG and IgM titers and probing pocket depths (PPD) and clinical attachment loss (CAL) in patients with periodontal diseases**

	Infected sites		Non-infected sites		Infected sites + non-infected sites	
	PD	CAL	PD	CAL	PD	CAL
<b>IgM</b>	Reverse	Reverse	Direct	Direct	Direct	Direct
<b>IgG</b>	Reverse	Reverse	Direct	Direct	Reverse	Reverse



**Figure 1. The means of IgM and IgG titers in infected and non-infected sites in individuals with periodontitis and individuals with healthy periodontium (control group).**

are some aspects in periodontal diseases that are not interpretable by their bacterial etiology. These aspects include the rapid periodontal tissue breakdown in the presence of minimal plaque, phases of disease activity and quiescence, progression to advanced periodontal destruction that occurs in a fraction of a given population and site specificity in periodontal diseases.<sup>23,24</sup>

It has been estimated that 50–80% of adolescents and adults in developed countries are infected by human cytomegalovirus.<sup>23</sup> Also, this data suggests that this virus is usually present in an inactive state.<sup>23</sup> Reactivation of this virus and other herpes viruses in periodontal sites can comprise an important pathogenic event in the development of periodontal diseases.<sup>23</sup> Contreras et al detected human cytomegalovirus in monocytes, macrophages and T-lymphocytes.<sup>25</sup> Therefore, these infected immune cells may not mount a proper immune response against periodontopathogenic bacteria and predispose the periodontal tissues to microbial superinfection.<sup>23,25</sup>

**Table 1. The means of probing pocket depths (PPD) and clinical attachment loss (CAL) in patients with periodontal diseases**

	Number	Maximum	Minimum	Mean	Standard Deviation
<b>PPD of infected sites</b>	20	4.5	3.1	3.9	0.44
<b>PPD of non-infected sites</b>	10	3.5	1.5	2.6	0.6
<b>Full mouth PPD</b>	10	5	3.5	4.1	0.7
<b>CAL of infected sites</b>	20	4.7	3.7	4.2	0.39
<b>CAL of non-infected sites</b>	10	4	1.5	2.7	0.9

Ting et al<sup>26</sup> hypothesized that active human cytomegalovirus infection can be associated with the initiation and progression of localized aggressive periodontitis. Also Contreras and Slots declared that active human cytomegalovirus replication occurs in periodontal sites but did not conclude whether human cytomegalovirus reactivation is related to the initiation and progression of destructive periodontal diseases.<sup>23,27</sup>

Herpes viruses have developed different strategies to cope with the hostile immune system.<sup>21,28</sup> They downregulate anti-viral host defense to suit their replication needs. They also destroy the components of macrophages' major histocompatibility complex pathways. These pathways are the principal components of antigen presentation in macrophages. Major histocompatibility complexes are also the main components in silent natural killer cells, apoptosis inhibition and diversion of potent cytokine responses.<sup>21,28</sup>

Despite available evidences for the role of herpes viruses in destructive periodontal diseases, no cause-and-effect relationship has been found to date.<sup>23</sup> It must be concluded that human cytomegalovirus can increase the immune system response in periodontal sites and therefore it can be another etiologic factor for periodontal diseases. However, it must be pointed out that prevalence of virus in periodontal pocket may vary due to ethnicity, immune status, type of periodontal disease and genetic predisposing factors.<sup>23</sup> Therefore, it must be noted that the present understanding of the potential role of this virus as an etiopathologic factor in destructive periodontal disease is credible but not decisive and additional studies in other populations are necessary to find the association of this virus in periodontal diseases.

There are studies available that have found an associations between the presence of human cytomegalovirus and periodontal diseases.<sup>5,8,14,15,29-32</sup> The present study found a significant difference in the mean of IgG between individuals with periodontal disease and individuals with healthy periodontium, but there was no significant difference in the means of IgG and IgM titers between the infected sites and non-infected sites in individuals with periodontitis. The means of IgM and IgG titers in individuals with periodontitis was higher than individuals with healthy periodontium. This amount was significant for IgG but non-significant for IgM. In addition, there was a relation between the IgG and IgM titers and probing pocket depths and clinical attachment loss in the test and control groups but all of them were non-significant (more details are available in Table 2). Watanabe et al<sup>21</sup> found no significant relationship

between human cytomegalovirus and periodontitis, consistent with the results of the present study about IgM titers, although it is different from the IgG results gained here.

### Conclusion

Within the limits of the present study, it can be concluded that the titer of IgG against the human cytomegalovirus can increase in patients with periodontal diseases but the titer of IgM does not show a significant increase in these individuals.

### References

1. Socransky S, Haffajee AD. Dental biofilms: difficult therapeutic targets. *Periodontol* 2000;28:12-5.
2. Contreras A, Slots J. Herpesviruses in human periodontal disease. *J Periodontol Res* 2000;35:3-16.
3. Idesawa M, Sugano N, Ikeda K, Oshikawa M, Takane M, Seki K, et al. Detection of Epstein-Barr virus in saliva by real-time PCR. *Oral Microbiol Immunol* 2004;19:230-2.
4. Konstantinidis A, Sakellari D, Papa A, Antoniadis A. Real-time polymerase chain reaction quantification of Epstein-Barr virus in chronic periodontitis patients. *J Periodontol Res* 2005;40:294-8.
5. Chalabi M, Moghim S, Mogharehabet A, Najafi F, Rezaie F. EBV and CMV in chronic periodontitis: a prevalence study. *Arch Virol* 2008;153:1917-9.
6. Wu YM, Yan J, Ojcius DM, Chen LL, Gu ZY, Pan JP. Correlation between infections with different genotypes of human cytomegalovirus and Epstein-Barr virus in subgingival samples and periodontal status of patients. *J Clin Microbiol* 2007;45:3665-70.
7. Boom R, Sol CJ, Salimans MM, Jansen CL, Wertheim-van Dillen PM, van der Noordaa J. Rapid and simple method for purification of nucleic acids. *J Clin Microbiol* 1990;28:495-503.
8. Contreras A, Nowzari H, Slots J. Herpesviruses in periodontal pocket and gingival tissue specimens. *Oral Microbiol Immunol* 2000;15:15-8.
9. Chalabi M, Rezaie F, Moghim S, Mogharehabet A, Rezaei M, Mehraban B. Periodontopathic bacteria and herpesviruses in chronic periodontitis. *Mol Oral Microbiol* 2010;25:236-40.
10. Saygun I, Kubar A, Ozdemir A, Yapar M, Slots J. Herpesviral-bacterial interrelationships in aggressive periodontitis. *J Periodontol Res* 2004;39:207-12.
11. Slots J, Sugar C, Kamma JJ. Cytomegalovirus periodontal presence is associated with subgingival *Dialister pneumosintes* and alveolar bone loss. *Oral Microbiol Immunol* 2002;17:369-74.
12. Slots J, Kamma JJ, Sugar C. The herpesvirus-*Porphyromonas gingivalis*-periodontitis axis. *J Periodontol Res* 2003;38:318-23.
13. Contreras A, Zadeh HH, Nowzari H, Slots J. Herpesvirus infection of inflammatory cells in human periodontitis. *Oral Microbiol Immunol* 1999;14:206-12.
14. Saygun I, Sahin S, Ozdemir A, Kurtis B, Yapar M, Kubar A, et al. Detection of human viruses in patients with chronic periodontitis and the relationship between viruses and clinical parameters. *J Periodontol* 2002;73:1437-43.
15. Saygun I, Kubar A, Ozdemir A, Slots J. Periodontitis lesions

- are a source of salivary cytomegalovirus and Epstein-Barr virus. *J Periodontol Res* 2005;40:187-91.
16. Kubar A, Saygun I, Ozdemir A, Yapar M, Slots J. Real-time polymerase chain reaction quantification of human cytomegalovirus and Epstein-Barr virus in periodontal pockets and the adjacent gingiva of periodontitis lesions. *J Periodontol Res* 2005;40:97-104.
  17. Parra B, Slots J. Detection of human viruses in periodontal pockets using polymerase chain reaction. *Oral Microbiol Immunol* 1996;11:289-93.
  18. Fleming DT, McQuillan GM, Johnson RE, Nahmias AJ, Aral SO, Lee FK, et al. Herpes simplex virus type 2 in the United States, 1976 to 1994. *N Engl J Med* 1997;337:1105-11.
  19. Cappuyns I, Gugerli P, Mombelli A. Viruses in periodontal disease - a review. *Oral Dis* 2005;11:219-29.
  20. Kornman KS, Page RC, Tonetti MS. The host response to the microbial challenge in periodontitis: assembling the players. *Periodontol* 2000;1997;14:33-53.
  21. Watanabe SA, Correia-Silva Jde F, Horta MC, Costa JE, Gomez RS. EBV-1 and HCMV in aggressive periodontitis in Brazilian patients. *Braz Oral Res* 2007;21:336-41.
  22. Sheiham A. Is the chemical prevention of gingivitis necessary to prevent severe periodontitis?. *Periodontol* 2000;1997;15:15-24.
  23. Bilichodmath S, Mangalekar SB, Sharma DC, Prabhakar AK, Reddy SB, Kalburgi NB, et al. Herpesviruses in chronic and aggressive periodontitis patients in an Indian population. *J Oral Si* 2009;51:79-86.
  24. Goodson JM, Tanner AC, Haffajee AD, Sornberger GC, Sorcransky SS. Patterns of progression and regression of advanced destructive periodontal disease. *J Clin Periodontol* 1982;9:472-81.
  25. Contreras A, Zadeh HH, Nowzari H, Slots J. Herpesvirus infection of inflammatory cells in human periodontitis. *Oral Microbiol Immunol* 1999;14:206-12.
  26. Saygun I, Kubar A, Ozdemir A, Yapar M, Slots J. Herpesviral-bacterial interrelationships in aggressive periodontitis. *J Periodontol Res* 2004;39:207-12.
  27. Contreras A, Slots J. Active cytomegalovirus infection in human periodontitis. *Oral Microbiol Immunol* 1998;13:225-30.
  28. Boeckh M, Nichols WG. Immunosuppressive effects of beta-herpesviruses. *Herpes* 2003;10:12-6.
  29. Contreras A, Nowzari H, Slots J. Herpesviruses in periodontal pocket and gingival tissue specimens. *Oral Microbiol Immunol* 2000;15:15-8.
  30. Contreras A, Slots J. Active cytomegalovirus infection in human periodontitis. *Oral Microbiol Immunol* 1998;13:225-30.
  31. Saygun I, Yapar M, Ozdemir A, Kubar A, Slots J. Human cytomegalovirus and Epstein-Barr virus type 1 in periodontal abscesses. *Oral Microbiol Immunol* 2004;19:83-7.
  32. Kubar A, Saygun I, Ozdemir A, Yapar M, Slots J. Real-time polymerase chain reaction quantification of human cytomegalovirus and Epstein-Barr virus in periodontal pockets and the adjacent gingiva of periodontitis lesions. *J Periodontol Res* 2005;40:97-104.