

Association between anti-CMV IgG and salivary levels of IL-6 and TNF- α in chronic periodontitis

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ABSTRACT

Background: Periodontitis is an infection attributable to multiple infectious; it causes an interrelated cellular and humoral host immune responses. Recent reports have indicated that human cytomegalovirus (HCMV) may contribute to pathogenesis of periodontitis. The HCMV can stimulate the release of cytokines from inflammatory and non-inflammatory cells and weaken the periodontal immune defense. This study aimed to reveal the presence of anti-CMV IgG, and determine the levels of IL-6 and TNF- α and to correlate the presence of cytomegalovirus (CMV) with cytokines levels.

Materials and Methods: Forty patients with chronic periodontitis and 40 healthy control subjects (their age and sex were matched with the patients) were involved in this study. Periodontal parameters used in this study included plaque index (PLI), gingival index (GI), probing pocket depth (PPD), clinical attachment level (CAL) and bleeding on probing (BOP). Saliva samples were taken from all subjects. ELISA was carried out to estimate the levels of anti-CMV IgG, IL-6 and TNF- α . Statistical tests used involved t-test, Mann-Whitney, Chi-square, Fisher exact and spearman's correlation test.

Results: This study found a significant difference ($p < 0.05$) in the frequency of anti-CMV IgG in saliva between patients and controls. The number and percentage of patients group who had positive for anti-CMV IgG were 14 (35%), while controls were 5 (12.5%). A significant increase was found in mean of PPD, CAL and BOP among patients with the positive IgG as compared to those patients with the negative IgG. In addition, there was a significant elevation in the salivary levels of IL-6 and TNF- α in patients compared with healthy controls. IL-6 was significantly associated with GI and BOP, whereas TNF- α was significantly associated with PPD and CAL. On the other hand, there was a significant correlation between TNF- α and anti-CMV IgG.

Conclusion: The findings revealed that the significant association between the presence of virus with periodontal parameters and cytokines level in patients group gives additional evidence toward the potential importance of the direct and indirect effects of CMV infection in periodontitis.

Keywords: Periodontitis, Anti- CMV IgG, Cytokines. (Received: 04/04/2020; Accepted: 10/05/2020)

INTRODUCTION

Periodontitis is a chronic infection in tissues backup teeth with various features including the inflammation in gingival tissues, construction of periodontal pockets, connective tissue attachment losing, resorption of alveolar bone, and even losing tooth.⁽¹⁾ It is an advanced, multifactorial disorder related with inflammation. Chronic periodontitis is a multifactorial inflammatory disease correlated with dysbiotic dental plaque biofilms and considered a progressive destruction of the tooth-supportive structures.⁽²⁾

The bacterial pathogenesis theory cannot totally explain the clinical features of periodontal diseases only, and the conventional treatments targeting such bacteria have restricted roles in avoiding periodontal diseases.⁽³⁾ Viruses may also play a role in the pathogenesis of periodontal diseases. The herpes virus has been known to be a pathogenic cause for several periodontal diseases since the 1990.⁽⁴⁾ In addition, herpes viruses have been associated with periodontal diseases, especially *Epstein-Barr virus* (EBV), *Human cytomegalovirus* (HCMV).⁽⁴⁻⁶⁾ Periodontal herpes viral infections can rise and change inflammatory mediator and cytokine responses,

which can up regulate IL-1 β and TNF- α gene appearance in monocytes and macrophages.^(3,4,7) These host mediators directly or indirectly participate in periodontal tissue damage and specifically in bone resorption.⁽⁸⁾

Interleukin-6 is well-known as one of the key cytokines of host response to inflammation and tissue damage such as that seen in chronic periodontitis and stimulates bone resorption by itself and in conjunction with other bone-resorbing causes.⁽⁹⁾ The TNF- α is very important pro-inflammatory cytokine released at the site of periodontitis that plays a prominent function in the pathogenesis of periodontitis.⁽¹⁰⁾ The hypothesis of the present study was that herpes virus infection initiates periodontal tissue breakdown and that host immune responses against the herpes virus infection are an important component of the etiopathogenesis of the disease.

This study was performed to detect the association between Anti-CMV IgG and salivary levels of IL-6 and TNF- α in chronic periodontitis.

MATERIALS AND METHODS

Overall of 40 patients with chronic periodontitis (CP) (age range from 30-55 years) were studied, in parallel with 40 actually healthy volunteers (control) of a similar age range. The diagnosis of CP was done according to the criteria of American Academy of Periodontology (AAP).⁽¹¹⁾ Clinical periodontal examination was performed for all subjects by the same examiner. Periodontal

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parameters used in this study were PLI, GI, PPD, CAL and BOP.⁽¹²⁻¹⁴⁾

Four surfaces of each tooth were assessed. Saliva samples were collected from CP patients and healthy control groups to evaluate salivary level of anti-CMV IgG, IL-6 and TNF- α (Bioactive Diagnostica-Germany, Diclon-France, Komabiotech-Korea) respectively. All participants were instructed not to eat or drink (except having water) at least 1 hour prior to donation of saliva, the subject should sit in a relaxed position and samples containing blood should be discarded. Saliva was collected between 9-12 am. After the subject rinses his mouth several times by sterilized water and then waits for 1-2 minutes for water clearance, 5ml of whole unstimulated saliva was collected into polyethylene tubes. Three ml of saliva was centrifuged at (3000 rpm) for 10 minutes and the resulting supernatant was stored at -40°C in eppendorf tubes until assayed.

Each of the subjects obtained detailed information concerning the nature of the study and the procedures included, and their informed consent was acquired on a form approved by ethical committee of College of Dentistry in University of Baghdad.

Statistical analysis was accomplished using computerized statistical analysis SPSS software version 24 (statistical package for social science). Semirnov-Kolmogorov test was used to test the normality of distribution of the data. Student t-test was used for parametric data, and Mann-Whitney test for non-parametric data to test the statistical mean difference between two groups.

Association between the different parameters was calculated by the Spearman test and p-values of $p < 0.05$ were considered significant.

RESULTS

The demographic data of subjects (patients and controls) included in this study are demonstrated in table (1). The mean age of patients was (45.00 ± 1.29) years, and (42.65 ± 1.15) years for control group. The male:female number of the patients group was (19/21) and in the control group was (11/29). There were no significant differences ($p > 0.05$) in the age and gender among the two study groups. The variances in periodontal parameters between patients and controls are presented in table (2).

Table 1: Demographic Characteristics of Patients and Controls.

Characteristics		Patients group n=40	Control group n=40	T-test p-value	
Age	Range	30-55	30-50	0.170 ^{NS}	
	Mean	45.00	42.65		
	SE	1.29	1.15		
	SD	8.19	7.30		
Gender	Male	No.	19	0.646 ^{NS}	
		%	47.5%		27.5 %
	Female	No.	21		29
		%	52.5%		72.5 %

NS: Non-Significant, SD: Standard Deviation, SE: Standard Error, No. : Number, %: Percentage

Table 2: Clinical Periodontal Parameters in Patients and Controls.

Parameters Mean \pm SE	Patients group n=40	Control group n=40	T-test p-value
PLI	1.24 ± 0.07	0.98 ± 0.07	0.01**
GI	1.31 ± 0.06	0.47 ± 0.07	0.001**
PPD (mm)	4.50 ± 0.57	0.0	0.0001**
CAL(mm)	3.66 ± 0.19	0.0	0.0001**
BOP (%)	87.5%	37.5%	0.0001**

Highly significant;** : PLI: Plaque Index; GI: Gingival Index; PPD: Probing Pocket Depth; CAL: Clinical Attachment Level; BOP: Bleeding on probing; SE: Standard Error

As seen in table (3) the number and percentage of patients group who had positive anti-CMV IgG was 14 (35%), while for controls group was 5 (12.5%). Regarding the correlation between the presence of anti-CMV IgG and periodontal parameters in patients group, the current results

revealed that there is significant increase in mean levels of PPD, CAL and BOP among patients with the positive IgG (5.13 ± 0.42 , 4.092 ± 0.320 and 74.928 ± 11.391) as compared to those patients with the negative IgG (4.25 ± 0.47 , 3.067 ± 0.285 and 45.538 ± 7.819), table (4).

Table 3: Prevalence of Anti-CMV IgG Antibodies in Saliva of Patients and Controls.

Anti-CMV-IgG	Patients group n=40		Control group n=40		T-test p-value
	Frequency	Percentage	Frequency	Percentage	
Positive	14	35%	5	12.5%	0.033*
Negative	26	65%	35	87.5%	

Table 4: Association between the Presence of Anti-CMV IgG Iu/ml and Clinical Periodontal Parameters.

Periodontal parameters	anti-CMV IgG		T-test p-value
	CMV Positive n=14	CMV Negative n=26	
	Mean ± SE	Mean ± SE	
PLI	1.207±0.1269	1.273±0.094	0.343 ^{NS}
GI	1.435±0.131	1.294±0.079	0.167 ^{NS}
PPD (mm)	5.13 ± 0.42	4.25± 0.47	0.005**
CAL	4.092 ±0.320	3.067±0.285	0.016*
BOP (%)	74.928 ±11.391	45.538 ±7.819	0.0181*

The current results found that there was a significant elevation in the mean rank salivary levels of IL-6 and TNF-α among patients with chronic periodontitis when matched to healthy controls, (P< 0.01), table (5). As observed in table (6), there is a significant positive association

between IL-6 and each of GI and BOP (r=0.418, p=0.024 and r=0.334, p=0.034) respectively, otherwise, there is a significant positive association between TNF-α and each of PPD and CAL (r=0.402, p=0.029 and r=0.398, p=0.031).

Table 5: Salivary Levels of IL-6 and TNF-α (pg/ml) in Patient and Control Groups.

Salivary IL-6	Patients group n=40	Control group n=40	Mann-Whitney p-value
Minimum	2.84	0.0	0.0001**
Maximum	80.66	44.12	
Mean Rank	47.86	25.31	
Median	9.37	4.41	
Salivary TNF-α			
Minimum	7.8	0.0	0.017**
Maximum	250	150	
Mean Rank	42.96	30.04	
Median	62	31	

Table 6: Spearman’s Correlation between Salivary IL-6 and TNF-α Levels and Clinical Periodontal Parameters in Patients.

Periodontal parameters	Salivary IL-6	
	Patients group n=40	
	Correlation r	Mann-Whitney p-value
PLI	0.113	0.485
GI	0.418	0.024*
PPD(mm)	0.016	0.918
CAL	0.010	0.949
BOP	0.334	0.034*
Salivary TNF-α		

	Correlation (r)	Mann-Whitney p-value
PLI	0.163	0.313
GI	0.042	0.793
PPD	0.402	0.029*
CAL	0.398	0.031*
BOP	0.072	0.655

On the other hand, there is non-significant increase ($P>0.05$) in the mean rank of IL-6 in patients with the positive IgG (18.86pg/ml) than that in patients with the negative IgG (21.38 pg/ml), also non-significant increase ($P>0.05$) in mean rank of TNF- α was found patients with the positive IgG (22.36pg/ml) than that in patients with the negative IgG (19.5pg/ml), table (7).

Table 7: Comparison between Positive and Negative IgG Patients for IL-6 and TNF- α Levels

Salivary IL-6 level	Anti-CMV IgG		Mann-Whitney p-value
	CMV Positive n=14	CMV Negative n=26	
Minimum	3.497	2.848	0.136 ^{NS}
Maximum	27.454	80.669	
Mean Rank	18.86	21.38	
Median	11.35	8.72	
Salivary TNF- α level			
Minimum	12.586	7.8	0.122 ^{NS}
Maximum	250	250	
Mean Rank	22.36	19.5	
Median	62	62	

DISCUSSION

Human CMV has frequently been associated with periodontal disease. The virus affects periodontal monocytes/macrophages and T-lymphocytes, and reactivation of CMV in periodontitis lesions tends to be correlated with progressive periodontal disease.⁽¹⁵⁾ Several studies have reported associations between the presence of HCMV and periodontal diseases.^(5,16,17) In this study the frequency of anti-CMV IgG was 14 (35%) in patients, and 5 (12.5%) for healthy controls with significant differences between patients and controls. Other results reported by Esfahanian and colleagues also showed that there were significant differences in mean IgG between the two groups.⁽¹⁸⁾

Similarly, a previous Iraqi study conducted by Al-Alousi in (2013)⁽¹⁹⁾ to investigate the frequency of anti-CMV IgG in saliva by ELISA in 35 periodontitis patients and 18 healthy controls revealed that the frequency of CMV in chronic periodontitis patients was significantly higher when compared to healthy control group, and found that the mean salivary level of HCMV IgG

was significantly higher in patients with periodontitis as compared to those of healthy control group. So the study concluded that the frequency of HCMV in saliva of chronic periodontitis patients could have a crucial role in the development of this disease.

In contrast with this result, a previous study conducted by Watanabe *et al.* (2007)⁽²⁰⁾ showed no statistical correlation between HCMV and periodontitis. This variation in the frequency detection of CMV may be attributed to sample size, type of sample and selection of the subjects.

The results of the present study revealed that there is a significant correlation between clinical parameters (PPD, CAL and BOP) and the presence of CMV. There is significant increase in mean levels of PPD, CAL and BOP among patients with the positive IgG as compared to those patients with the negative IgG. This result is in accordance with the observations of the previous study conducted by Gaekwad and Gujjari (2012)⁽²¹⁾ who found that the presence of virus was correlated with the measurements of PPD and the CAL. The higher PPD in CMV positive sites indicates that this virus might have helped specific bacterial colonization leading to greater disease severity.⁽²²⁾

Furthermore, some studies^(16, 23, 24) also compared clinical parameters with virus isolation and found statistically significant association in terms of PI and GI. However, Chalabi *et al.*, 2008⁽¹⁷⁾ and Chalabi *et al.*, 2010⁽²⁵⁾ showed a relation between human CMV and periodontitis. Conversely Ling *et al.*, (2004)⁽²⁶⁾ compared the disease severity in terms of clinical parameters (GI, PI, CAL and PD) with virus isolation and revealed that there was no statistically significant association between the presence of CMV and any of the clinical parameters. Similarly, a study done by Rupali *et al.*, (2012)⁽²²⁾ showed no significant association between the presence of virus and CAL.

Interleukin-6 is one of the important mediators of the inflammatory response in several inflammatory diseases, including periodontitis.⁽²⁷⁾ Results of the present study showed that the salivary IL-6 level was significantly elevated in chronic periodontitis subjects as compared to controls. These data are compatible with previous findings⁽²⁸⁻³⁰⁾ that showed significant elevation in level of salivary IL-6 among patients as compared to healthy controls. In addition, McCauley and colleagues

pointed out that elevated levels of IL-6 have been shown to be induced by periodontal pathogens and are correlated with the continuous tissue destruction observed in periodontitis.⁽³¹⁾ In consistent with this result, Husniah Batool et al. (2018)⁽³²⁾ found that the level of salivary IL-6 was significantly elevated in calculus associated chronic periodontitis patients as compared to healthy controls and these levels elevated with the progression of chronic periodontitis. So they concluded that salivary level of IL-6 may assist in the sub-categorization of chronic periodontitis.

On the other hand, these findings disagree with those of Dhruva et al., (2009)⁽³³⁾ who demonstrated no significant differences between chronic periodontitis and control group according to salivary levels of IL-6. In addition, Teles et al., (2014)⁽³⁴⁾ demonstrated in their study that the levels of IL-6 were higher in patients than the healthy individuals but statistically not significant and stated that the range of IL-6 concentration in saliva was often quite variable.

Concerning the correlation between IL-6 level and periodontal parameters, this study found significant correlation between salivary IL-6 and each of GI and BOP. This result was consistent with Javed et al., (2014)⁽²⁹⁾ who proved that there is a significant correlation between the level of IL-6 in saliva and the clinical parameters such as PPD, CAL and BOP, they found an increase in the salivary IL-6 levels as the severity of the periodontal disease increased. Likewise, Hussein (2017)⁽³⁵⁾ who used serum sample showed that there was a significant strong positive correlation between serum IL-6 levels with PLI, GI, PPD and CAL. Furthermore, Noh et al., (2013)⁽³⁶⁾ indicated that IL-6 may promote the degeneration of inflamed periodontal tissues.

TNF- α is a pro-inflammatory cytokine that has an effect in the activation of inflammatory leukocytes, modification of vascular permeability and induction of bone resorption.⁽³⁷⁾

The current study revealed high level of salivary TNF- α in patients group than that in controls group. This agrees with the study conducted by Varghese et al., (2015)⁽³⁷⁾ and Ehsan et al., (2017)⁽³⁸⁾ who noticed that TNF- α value in chronic periodontitis patients was significantly higher than in control subjects. Further, another study assessed the salivary activity rates of TNF- α and stated that the level of this cytokine was higher in patients than healthy individuals, and suggested that TNF- α level can be used as biomarkers to diagnose disease.⁽³⁹⁾

In contrast, these results were at variance with other studies^(40,41) that showed no significant difference in level of TNF- α between the patients

and controls. Teles et al., (2014)⁽³⁴⁾ reported a lack of association between the levels of salivary biomarkers, including TNF- α and periodontal disease status. They attributed their result to the inhibition of cytokines in the saliva by the putative inhibitors present in the whole saliva.

Another interesting finding in this study was the significant positive association of salivary TNF- α with each of PPD and CAL. Correspondingly, a previous Iraqi study revealed a significant positive association of TNF- α level with GI, PPD and CAL.⁽⁴²⁾ Besides, Kurtis et al., 2005⁽⁴³⁾ also reported a positive correlation between salivary TNF- α levels and clinical parameters such as PPD, CAL, PI and GI in samples of patients with chronic and aggressive periodontitis. Unlike the current result, Varghese and his colleagues found non-significant correlation between salivary TNF- α level and the clinical parameters possibly due to the extensive dilution of these markers in the saliva, thereby failing to reflect the minor variations in the clinical parameters.⁽³⁷⁾ Interestingly, positive correlation between the levels of the pro-inflammatory cytokines (IL-6 and TNF- α) and the disease severity in the present study was mightily confirm the hypothesis that these cytokines are likely to be involved in the pathogenesis of periodontitis.

Kurtis et al. (43) also reported a positive correlation between salivary TNF- α levels and clinical parameters such as PD, CAL, PI, and GI in GCF samples of patients with chronic and aggressive periodontitis.

The findings of the present study found that there is no association between IL-6 and TNF- α level with the presence of CMV. This was in agreement with another study of Jakovljevic et al., (2018)⁽⁴⁴⁾ who stated that only 4 out of 54 HCMV (13.5%) patients showed increased viral copy numbers and there was no significant correlation between the levels of pro-inflammatory cytokines (IL-1 β , IL-6 and TNF- α) and viral copy numbers. Therefore they suggested that low viral loads point to a relatively rare occurrence of active HCMV infection in his samples and latent HCMV infection does not enhance the production of investigated pro-inflammatory cytokines.

This result disagrees with Botero et al., (2008) who disclose that HCMV infection in gingival fibroblasts up-regulated the production of pro-inflammatory-related cytokines and chemokines. Also the expression of IL-1 β and TNF- α was increased both in vitro and in specimens from HCMV-positive subjects with periodontitis. Thus it was concluded that overproduction of pro-inflammatory cytokines as a result of viral infection should be considered an important

pathogenic mechanism linking HCMV to periodontitis in vivo.⁽⁴⁵⁾ It is worthy to mention that CMV infects periodontal macrophages and T-cells and elicits a release of pro-inflammatory cytokines which play an important role in the host defense against the virus, but they also have the potential to induce alveolar bone resorption and loss of periodontal ligament. Over-production of pro-inflammatory cytokines occurs due to chronic stimulation of TLR9 by herpes virus DNA which may lead to tissue destruction.⁽⁶⁾

A limitation of the current study is the study of only one microorganism (virus), as multiple microorganisms are involved in the pathogenesis of periodontitis. Moreover, other pro-inflammatory and anti-inflammatory cytokines need to be investigated for an association with CMV in periodontitis.

CONCLUSIONS

The presence of HCMV was documented through detection of HCMV-specific antibodies. The significant correlation between the presence of virus with periodontal parameters (PPD, CAL and BOP) gives additional evidence toward the potential importance of the direct and indirect effects of CMV infection in periodontitis. More clinical and longitudinal analyses with larger samples are required to evaluate the role of CMV in different periodontal diseases. Further investigations using other different samples such as gingival tissues and GCF are needful.

Conflict of interest: None.

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الخلاصة

الخلفية: التهاب اللثة هو مرض يعزى إلى أسباب معدية متعددة والاستجابات المناعية الخلوية والخلطية المترابطة. أشارت التقارير الأخيرة إلى أن الفيروس المضخم للخلايا البشرية (HCMV) قد يساهم في التسبب في التهاب اللثة. يمكن لـ HCMV تحفيز إطلاق السيتوكينات من الخلايا الالتهابية وغير الالتهابية وإضعاف الدفاع المناعي اللثوي. هدفت هذه الدراسة إلى الكشف عن وجود مضاد لـ CMV IgG ، وتحديد مستويات IL-6 و TNF- α وربط وجود الفيروس المضخم للخلايا (CMV) بمستويات السيتوكينات. المواد والطرق: شارك في هذه الدراسة أربعون مريضاً مصاباً بالتهاب اللثة المزمن و 40 شخصاً متطوعاً من الأصحاء (تم مطابقة أعمارهم وجنسهم مع المرضى). تشمل مؤشرات التهاب اللثة المستخدمة في البحث الحالي مؤشر البلاك (PLI) ، ومؤشر اللثة (GI) ، وعمق جيب التحقيق (PPD) ، ومستوى فقدان الأنسجة الرابطة (CAL) والنزيف عند الفحص (BOP). تم أخذ عينات اللعاب من جميع الأشخاص. تم إجراء الفحص الناعي المرتبط بالانزيم ELISA لتقدير مستويات مضاد CMV IgG و الانترلوكين-6 و عامل نخر الورم الفا. شملت الاختبارات الإحصائية المستخدمة اختبار t ، و Man-Whitney ، و Chi-square ، و Fisher بالضبط ، واختبار ارتباط spearman.

النتائج: وجدت هذه الدراسة أن هناك فرقا معنويا ($p < 0.05$) في تواتر مضاد CMV IgG في اللعاب بين المرضى والأصحاء. كان عدد ونسبة مجموعة المرضى الذين لديهم إيجابية لمضاد الفيروس المضخم للخلايا (35 IgG 14%) ، بينما كانت لمجموعة الأصحاء 5 (12.5%). وجدت هذه الدراسة زيادة كبيرة في متوسط PPD و CAL و BOP بين المرضى الذين يعانون من IgG الإيجابي مقارنة مع المرضى الذين يعانون من IgG السلبي. بالإضافة إلى ذلك ، هناك ارتفاع كبير في مستويات اللعاب من IL-6 و TNF- α في المرضى مقارنة بالأشخاص الأصحاء. يرتبط IL-6 بشكل كبير بالمؤشر GI و BOP ، في حين ارتبط TNF- α بشكل كبير بـ PPD و CAL. من ناحية أخرى ، هناك ارتباط كبير بين TNF- α ومضاد لـ CMV IgG.

الاستنتاجات: كشفت النتائج أن الارتباط الهام بين وجود الفيروس مع مؤشرات التهاب اللثة ومستوى السيتوكينات في مجموعة المرضى يعطي أدلة إضافية على الأهمية المحتملة للآثار المباشرة وغير المباشرة لعدوى الفيروس المضخم للخلايا في التهاب اللثة.